Millersville University SEIZE THE OPPORTUNITY

Department of Biology & Chemistry

The baiting and supplemental feeding of white-tailed deer has been used to help with nutrient deficiencies that may occur during the winter season in temperate areas (Lambert and Demarais 2001) and to help increase hunting success (Brown and Cooper 2006). However, the baiting and supplemental feeding of whitetailed deer has been shown to increase the spread of disease such as Chronic Wasting Disease, Bovine brucellosis and Bovine Tuberculosis (Brown and Cooper 2006, Dunkley and Cattet 2003, The Wildlife Society 2006). Also, baiting and supplemental feeding can cause deer herds to become overpopulated in certain areas which may reduce herd health, reduce the quality of an area's vegetation, increase the number of deer predators and adversely impact local ecosystems (Brown and Cooper 2006, Dunkley and Cattet 2003, The Wildlife Society 2006).

Based on the philosophy of fair chase, and the potential biological and ecological impacts of baiting and supplemental feeding as cited above, 25 of the United States have outlawed hunting over bait and 5 states have certain restrictions on baiting (The Wildlife Society 2006) (Figure 1). However, the use of illegal baiting has increased in the United States. For example, according to an informal questionnaire conducted by Conservation Officers in the Iowa Department of Natural Resources, the number of issues in regards to illegal baiting increased from 2008 to 2010 (Figure 2). At the same time commercial baits to attract white-tailed deer (e.g., Deer Cane®) have become readily available to consumers at common retail sporting goods stores. This is becoming a national, and with growing international markets, a potentially international issue.

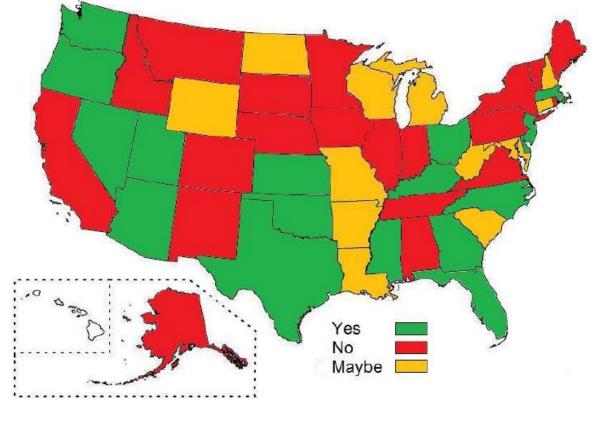


FIGURE 1. Map of states that allow (Yes), do not allow (No) or have special regulations on hunting deer over bait.

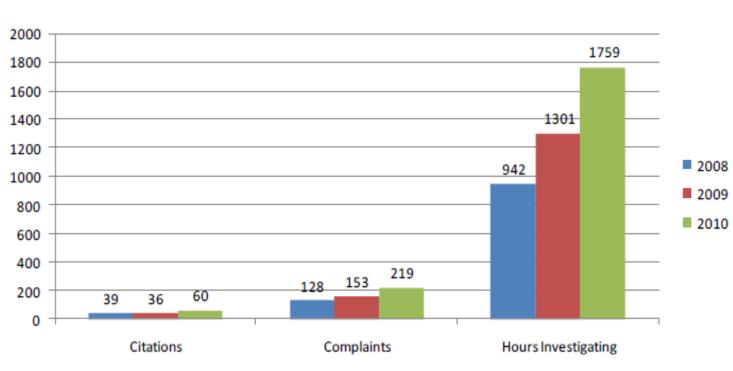


FIGURE 2. Results from a statewide survey of Iowa Conservation Officers regarding 400 baiting issues

Many of these commercial baits (e.g., Deer Cane®) contain distinct ingredients which include small organic molecules such as sugars and amino acids as well as inorganic salts containing ions such as chloride, phosphorous, magnesium, calcium, etc. (Shaw et al. 2007). The objective of this study was to conduct chemical analysis of white-tailed deer feces exposed to commercial baits in comparison to white-tailed deer that are not. The goal will be to determine if chemical signatures are left by commercial baits in the feces of white-tailed deer. Having the ability to identify baited sites based on feces testing could aid conservation officers in their ability to identify areas and hopefully individuals who illegally hunt over bait.





Detection of Human Baiting Activity for White-Tailed Deer (Odocoileus virginianus) Using Fecal Analysis

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Analysis of Monosodium Glutamate Presence in Deer Cane via Thin Layer <u>Chromatography</u>

In an attempt to determine the presence or absence of monosodium glutamate in Deer Cane®, Thin Layer Chromatography (TLC) was performed with samples of Deer Cane and compared to glutamic acid. A phosphate buffer was prepared and added to the Deer Cane® sample to minimize changes in pH. The buffer consisted of 0.13g of KH2PO4 and 5.081g of NaHPO4 dissolved in 100 mL of distilled water. It had a molar concentration of 0.2M and a pH of 8.0. One mL of this phosphate buffer was added to the Deer Cane in 3 separate trials. The mobile phase for this experiment was a 70:30 mixture of n-propanol:water. The spotting volume for each trial was 1 µL.

In Trial 1, 0.011g of Deer Cane® was dissolved in 1 mL of phosphate buffer and spotted on a spot plate. Adjacent to the Deer Cane spot was also a spot of glutamic acid and a spot of Deer Cane + glutamic acid. The spot plate was placed into a developing chamber with the mobile phase and allowed to develop until the solvent had reached about 1 cm away from the top of the plate. The plate was then allowed to dry and then dipped into isatin for color development. The plate was then heated on a hot plate and a picture was taken at the time of color development.

In Trial 2, the same procedure was repeated with 1.0 g of Deer Cane dissolved in 1 mL of phosphate buffer. In trial 3, the mixture of Deer Cane and phosphate buffer was neutralized to a pH of 8.0 before the plate was placed into the developing chamber. Seventy drops (about 3.5mL) of 6M HCl was needed to neutralize 1.0 g of Deer Cane. Pictures were taken for all trials at the time of color development. Our first step to this experiment was to run the reaction with pure Deer Cane® to determine if any color changes appeared upon heating.

We found that there was a very minimal, if any, color change in response to the Pure Deer Cane (Figure 3). Our results did not produce a visible color change because there was not a high enough concentration of glutamic acid within the Deer Cane®. From these results we decided not to perform further experiments involving feces extractions.



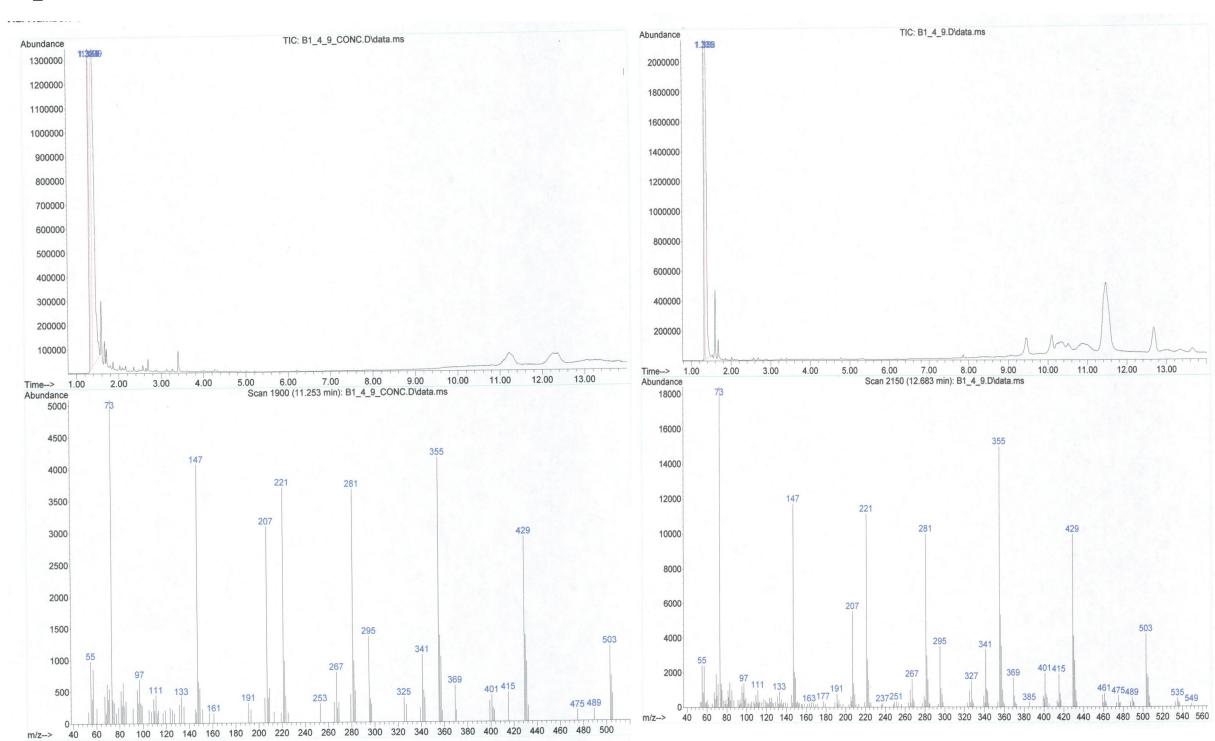
FIGURE 3. Thin Layer Chromatography plates depicting three spots per plate. The spot on the left contains pure Deer Cane, the spot on the right contains pure glutamic acid, and the spot in the middle contains both Deer Cane and monosodium glutamic acid.

Analysis of the Ethyl Acetate Layer of the Fecal Extraction via Gas <u>Chromatography-Mass Spectrometry</u>

The ethyl acetate layer from the initial extraction of fecal matter was purified before analysis with the Gas Chromatographer-Mass Spectrometer using glass wool and silica gel. Steel wool was placed into the bottom of a 9 inch glass pipette and covered with about 1 inch of silica gel. The ethyl acetate layer that was collected from the general extraction was poured through the glass pipette and the filtrate was collected in a test tube. Pure ethyl acetate was used to rinse the column after the ethyl acetate layer had been filtered through the column. The purified ethyl acetate layer was then injected into the Gas Chromatographer-Mass Spectrometer for analysis. The resulting printouts of peaks, each one representing a different chemical structure, was analyzed to determine the variety of chemical compounds present within the samples. The printouts for the feces from the baited site were then compared to the printouts for the feces from the nonbaited site to determine if there are any differences in chemical composition between the two experimental groups.



Once printouts of the two experimental groups were obtained, they were then compared to one another to determine differences in chemical composition. It was determined that there were no clear peaks within the spectrum that indicated the presence of monosodium glutamate. Figure 4 shows printouts for the baited (experimental) group and Figure 5 shows printouts for the control (non-baited) group.



number two from test day April 9th, 2013.

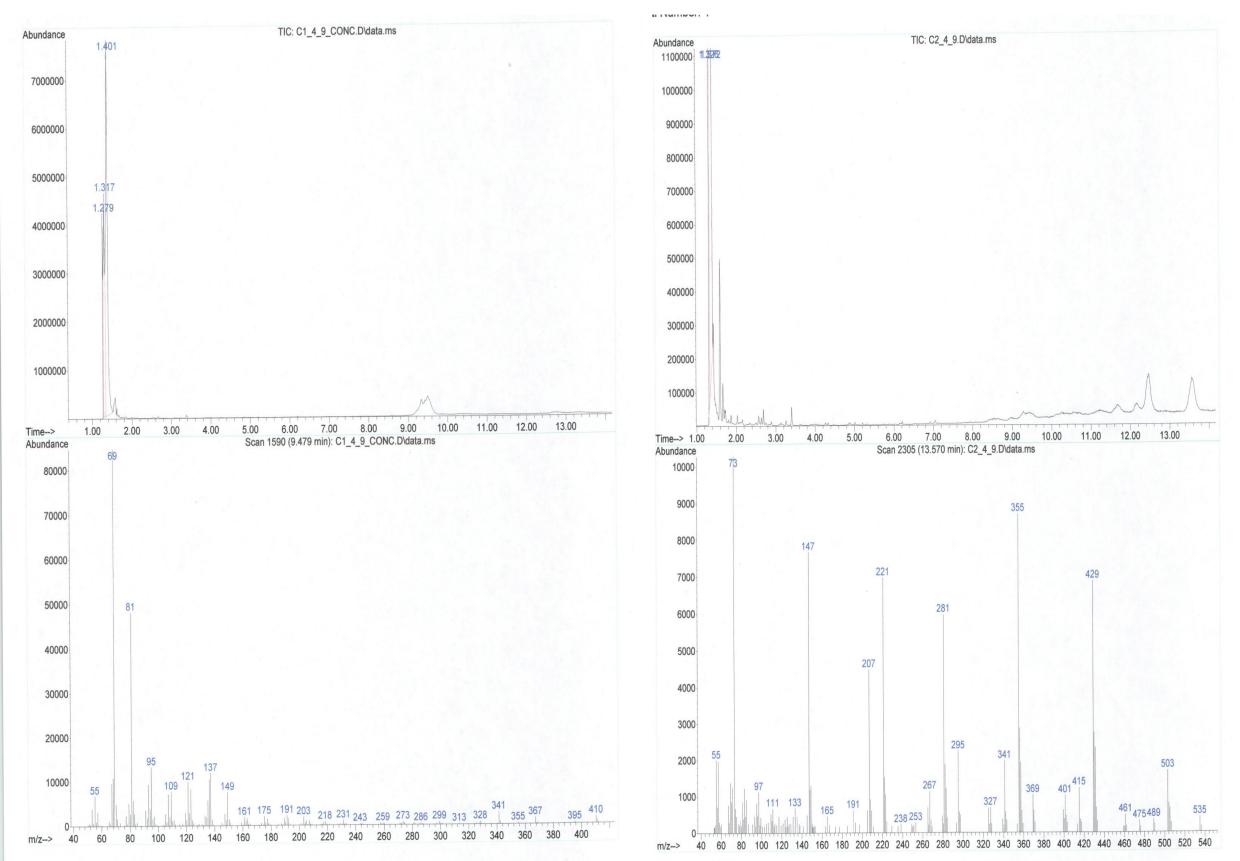


FIGURE 5: Gas Chromatography-Mass Spectrometry printouts of the ethyl acetate layer of feces extraction. These printouts are from the nonbaited (control) population of white-tailed deer. The printouts on the left are of sample number one from test day April 9th, 2013. The printouts on the right are of sample number two from test day April 9th, 2013.

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FIGURE 4: Gas Chromatography-Mass Spectrometry printouts of the ethyl acetate layer of feces extraction. These printouts are from the baited population of white-tailed deer. The printouts on the left are of sample number one from test day April 9th, 2013. The printouts on the right are of sample

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