

4-15-2013

# Chemical Warfare Agents in Plants: Biodefensive Terpenes from Sagebrush

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# Chemical Warfare Agents: Bio-defensive Terpenes from Sagebrush

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STUDENT RESEARCH INITIATIVE PROGRAM

## Abstract

To prevent being eaten, some plants create compounds that are toxic to herbivores, and herbivores respond by creating new ways to metabolize these toxins. For example, sagebrush produces bio-defensive terpenes to deter foraging by sage grouse. A recent investigation to characterize plant secondary metabolites (PSM's) in sagebrush resulted in a correlation between terpene concentration and nutritional content in local sagebrush to the habitat selection of sage grouse. This study identified many of the terpenes in sagebrush, but not all of them. A highly volatile and elusive terpene is suspected to be an important PSM that significantly affects sage grouse foraging. The purpose of the current work is to identify the structure of this PSM, as well as other unknown PSM's in sagebrush, to better understand their role as chemical warfare agents to defray foraging. Sagebrush extracts will be analyzed and components identified using Gas Chromatography-Mass Spectrometry (GC-MS).

## Introduction

Plants produce primary and secondary metabolites that perform an extensive array of functions. The importance of plant secondary metabolites (PSMs) in nature is grossly understated. These compounds provide many benefits for the plant including the ability to be used as a deterrent.<sup>(1)</sup> Sagebrush contains relatively high concentrations of PSMs; the key class of these defensive molecules are terpenes.<sup>(2)</sup> Terpenes are the largest class of compounds in the biological volatile organic compound (BVOC) classification.<sup>(3)</sup> Isoprene, a C<sub>5</sub>H<sub>8</sub> hydrocarbon, is the basic building block of terpenes. While these compounds do have a pleasant aroma, as seen with limonene in citrus fruits, and pinene in conifers, terpenes are also toxic.<sup>(1)</sup> In the ongoing warfare between plants and herbivores, defense mechanisms are constantly evolving.<sup>(4)</sup> This is observed in the phenomenon referred to as specialization. One such example can be seen in the interaction between sagebrush and sage grouse (Figure 1).<sup>(5)</sup>

## Background



Figure 1. Sage grouse chick.

Recent work has attempted to characterize the importance of terpene concentration and nutritional content in local sagebrush on the habitat selection of sage grouse.<sup>(5)</sup> Though terpenes have been well researched as a whole, this study has come across an important volatile compound produced by sagebrush that significantly effects sage grouse foraging (unknown #1). The purpose of our research is to identify the structure of unknown #1, in addition to other unidentified PSMs in sagebrush, in order to elucidate the dynamics of chemical defense mechanisms on foraging.

## Methods

Gas Chromatography-Mass Spectrometry (GC-MS) is used because of its accuracy in identifying volatile compounds using headspace methods (Figures 2; 3). A representative headspace vial is shown in Figure 2.<sup>(2,6)</sup> Traditionally a GC-MS sample is prepared by dissolving the sample in a solvent (usually methylene chloride or hexane) and injecting the solvent into the GC-MS for analysis. This method works well for non-volatile low molecular weight compounds (<400 amu), however many terpenes are BVOCs, which requires a method to isolate both volatile and the non-volatile components. A headspace sample is prepared for volatile terpenes, in a vial containing the sample in native form (solid or liquid). Volatile components from complex sample mixtures can be separated from non-volatile sample components and isolated in the headspace or gas portion of a sample vial. A sample of the gas in the headspace is then directly injected into a GC-MS for component identification.



Figure 3. GC-MS instrument used to quantitate and qualitate terpenes

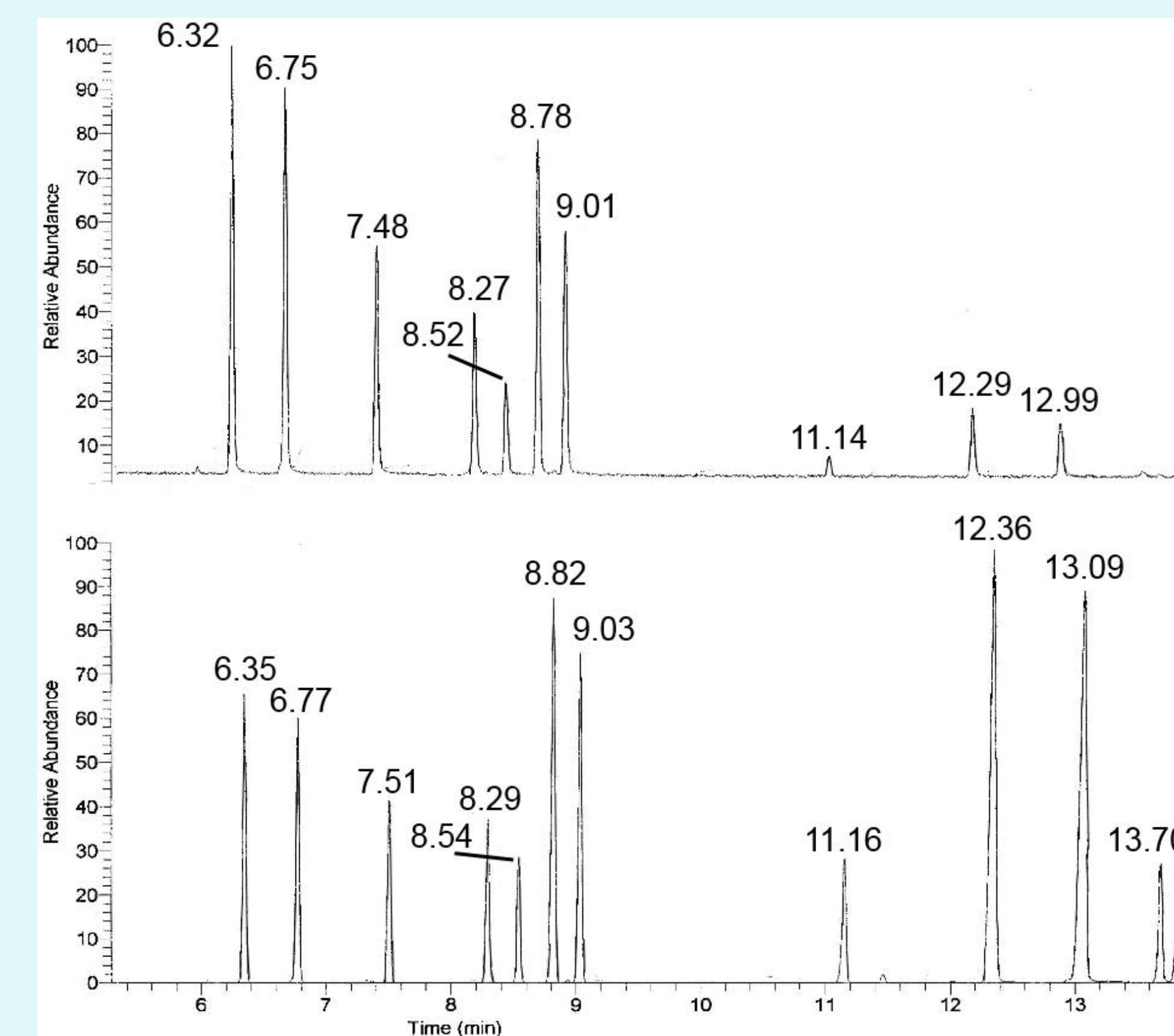


Figure 4. Gas Chromatograph results of terpene standards for headspace (top) and traditional (bottom) methods.

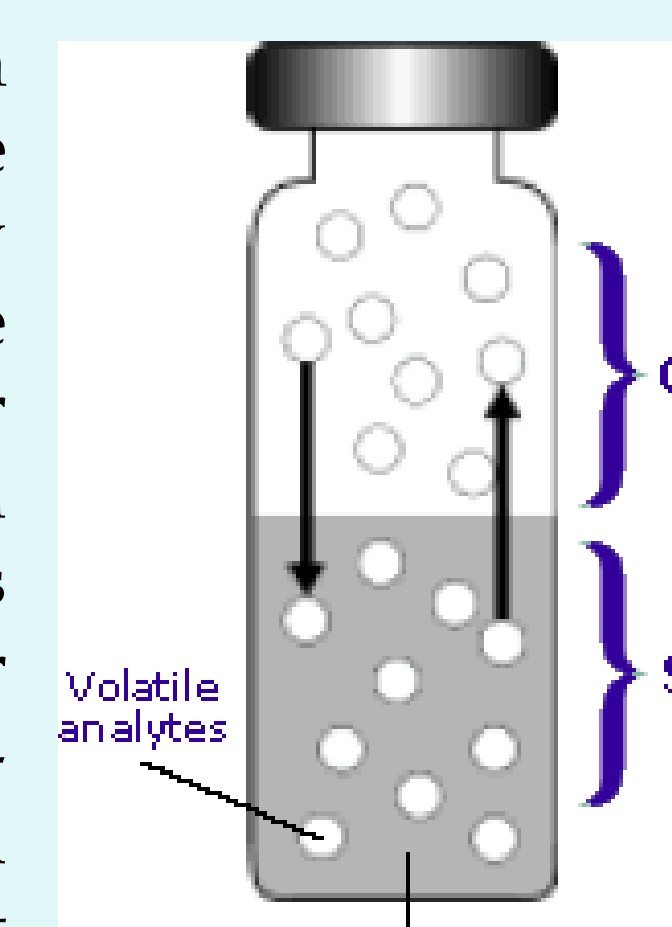


Figure 2. A diagram demonstrating headspace of a vial.

### Procedure:<sup>(6)</sup>

- 1) GC-MS analysis of terpene standards.
- 2) GC-MS Method:  
Start temperature: 40°C,  
ramp 1 - 40°C-125°C at 5°C/min,  
ramp 2 - 125°C-250°C at 30°C/min.
- 3) Compare headspace to solvent extraction methods to identify terpenes (Table 1; Figure 4).
- 4) Extract terpenes from sagebrush and identify them by GC-MS.

Table 1. A list of standard terpenes, GC retention times for headspace vs. solvent extraction methods.

| Terpene        | Retention time<br>Headspace<br>(min) | Retention time<br>Traditional<br>(min) |
|----------------|--------------------------------------|--|
| a-pinene       | 6.35                                 | 6.32                                   |
| camphene       | 6.77                                 | 6.75                                   |
| b-pinene       | 7.51                                 | 7.48                                   |
| a-phellandrene | 8.29                                 | 8.27                                   |
| 1,4-cineole    | 8.54                                 | 8.52                                   |
| p-cymene       | 8.82                                 | 8.78                                   |
| 1,8-cineole    | 9.03                                 | 9.01                                   |
| thujone        | 11.16                                | 11.14                                  |
| camphor        | 12.36                                | 12.29                                  |
| borneol        | 13.09                                | 12.99                                  |
| terpineol      | 13.70                                | NA                                     |

## Conclusion

- Headspace was superior for loading and identifying volatile terpenes by GC-MS, but it was less accurate for identifying non-volatile terpenes (longer GC retention times) (Figure 4).
- Solvent extraction methods will be required for non-volatile PSM's.

## Future Work

Figure 5A: First, Sagebrush is harvested, then stored in -20°C freezer until ready for use.

Figure 5B: Biomass was prepared by flash freezing with liquid N<sub>2</sub>.

Figure 5C: Pulverizing to a powder by mortar and pestle.

Figure 5D: Analyzed by both headspace and solvent extraction by GC-MS.



## Acknowledgements

- Funding provided by Boise State SRI Program
- Jon Scaggs, Boise State Chemistry Department, technical support

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