

Quantifying Ethanol in Bumble Bee Honey and Colorado Rocky Mountain Wildflowers

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Abstract

Ethanol is a natural byproduct of yeast fermentation of plant sugars. In flowering plants, yeasts often form mutualistic relationships by inhabiting nectar, where they benefit from access to sugar and confer antimicrobial protection through the production of ethanol. Ethanol in nectar not only shapes microbial communities but may also influence pollinator behavior. Prior studies have shown that pollinators such as honey bees and Oriental hornets are attracted to or tolerant of ethanol despite its toxicity. However, little is known about the actual concentrations of ethanol naturally present in the dietary sources of sugar for bees, namely their honey (in the case of eusocial bees) and foraged floral nectar.

This study quantified ethanol levels in the honey of eight commercial bumble bee colonies and the floral nectar of five native plant species in the Colorado Rocky Mountains: *Mertensia ciliata*, *Ipomopsis aggregata*, *Corydalis caseana*, *Delphinium barbeyi*, and *Aconitum columbianum*. By providing quantitative data on natural ethanol levels, this study offers insight into the ecological significance of yeast-produced ethanol in pollination systems.

Introduction

Ethanol is naturally produced by yeasts through the fermentation of plant sugars (Fenner et al., 2022). These single-celled eukaryotic fungi often inhabit floral nectar, where they enter mutualistic relationships with angiosperms (flowering plants): yeasts gain access to sugars, while plants benefit from the antimicrobial effects of ethanol, which inhibit the growth of mold and bacteria (Ädel et al., 2005).

Ethanol and yeast activity can alter nectar chemistry and floral scent, thereby affecting pollinator behavior and preferences (Herrera et al., 2008). Studies have shown that honey bees (*Apis mellifera*) are drawn to ethanol in sucrose solutions, even though ethanol provides no caloric value and is inherently toxic (Mustard et al., 2019). Similarly, Oriental hornets exhibit high ethanol tolerance, likely due to alcohol dehydrogenase (ADH) genes that enhance survival in ethanol-rich environments (Bouchebti et al., 2024). These findings suggest ethanol can play a significant role in pollinator ecology and niche expansion.

Despite growing interest in the behavioral effects of ethanol on pollinators, very little is known about the actual ethanol concentrations naturally present in floral nectar and the stored nectar (honey) of eusocial bees. Understanding the quantities of ethanol in bee honey and floral nectar may shed light on pollinator foraging patterns (e.g., overvisitation or intoxication), microbial ecology, and environmental influences on nectar composition. This study focuses on native wildflowers of the Colorado Rocky Mountains—*Mertensia ciliata*, *Ipomopsis aggregata*, *Corydalis caseana*, *Delphinium barbeyi*, and *Aconitum columbianum* to help fill that knowledge gap. We tested the hypothesis that bumble bee honey and floral nectar from bumble bee-visited flowers

contain ethanol due to the frequently observed dispersal of fermentative wild yeasts by bumble bees.

Methods

Wildflower nectar collection

Fieldwork was conducted from July 2-21 at six field locations, including three sites in the East River valley near Gothic, Colorado: Judd Falls (38.964506, -106.986279), the Research Meadow (38.957099, -106.981763), and Deer Creek (38.946317, -106.977206), and three sites along Washington Gulch (38.940290, -107.023079; 38.949488, -107.028717; 38.969705, -107.045739). Long-term bumble bee-plant interaction surveys have been conducted at and near our selected research sites, allowing for targeted sampling of plants that receive high visitation by bumble bees.

Nectar was collected from several plant species: *Mertensia ciliata*, *Ipomopsis aggregata*, *Corydalis caseana*, *Delphinium barbeyi*, and *Aconitum columbianum*. For each species at each site, between 16-32 nectar samples were collected. Flowers that had been open for 2-3 days, allowing for visitation and yeast dispersal by bees, were isolated via bagging to prevent nectar depletion by floral visitors before sampling.

Nectar was extracted using 10 μ L microcapillary pipettes (Drummond Scientific) and deposited into pre-weighed 0.6 mL microcentrifuge tubes containing 20 μ L of qPCR-grade water. Tubes were sealed with parafilm in the field and transported to the wet lab at the Rocky Mountain Biological Laboratory. Tubes were reweighed to estimate nectar mass and then stored at -80°C until analysis.

Bumble bee honey collection

Eight commercial bumble bee colonies (Koppert, Howell, MI, USA) were maintained in the bumble bee rearing room at the USDA-ARS Pollinating Insects Research Unit from March 19 until May 23. Honey was collected using a 200 μ L pipette and deposited into pre-weighed 1.5 mL microcentrifuge tubes containing 200 μ L of qPCR-grade water.

Tubes with honey samples were sealed with parafilm and transported to the lab at Utah State University. Tubes were reweighed to estimate honey mass and then stored at -80°C until analysis.

Ethanol and total sugar quantification

Total sugar concentration was measured using a handheld refractometer. Ethanol concentrations were quantified using an enzyme-based ethanol assay kit (Megazyme, Neogen, UK) in clear 96-well plates, read on a plate reader (Bio-Rad, Hercules, CA, USA) at Western University in Gunnison, Colorado. Mean and range values of sugar and ethanol content were calculated for each bumble bee colony and for each wildflower species and study site.

Statistical Analysis

Differences in ethanol concentrations among bumble bee colonies, plant species, and study sites were evaluated using analysis of variance (ANOVA) in R.

Results

*Commercial *Bombus impatiens* honey ethanol content*

Table 1. Ethanol concentration summary for each commercial *Bombus impatiens* colony.

Colony	Samples Quantified (n)	Maximum Ethanol Detected (ABV)	Proportion of Samples with Detectable Ethanol
C1	23	0.60%	56.5%
C2	24	0.18%	25.0%
C3	25	1.25%	56.0%
C4	20	0.28%	30.0%
C5	24	0.75%	41.7%
C6	23	0.40%	56.5%
C7	25	2.33%	52.0%
C8	24	0.44%	70.8%
Control (water only)	7	0%	0

Across all bumble bee honey samples, ethanol concentrations ranged from 0% to 2.33% ABV, with an average concentration of 0.08% ABV. Ethanol was detected in nectar from all eight colonies (Table 1). Maximum concentrations detected in honey from each bumble bee colony ranged from 0.18% ABV in C2 to 2.33% ABV in C7. The proportion of samples with detectable ethanol varied from 25.0% in C2 to 70.8% in C8.

188 samples from eight bumble bee colonies were analyzed for ethanol content. Across all colonies,

- Averages for corydalis, ipo, mert, heli, etc..

- Table with all averages

Bumble Bee Honey Ethanol Concentration

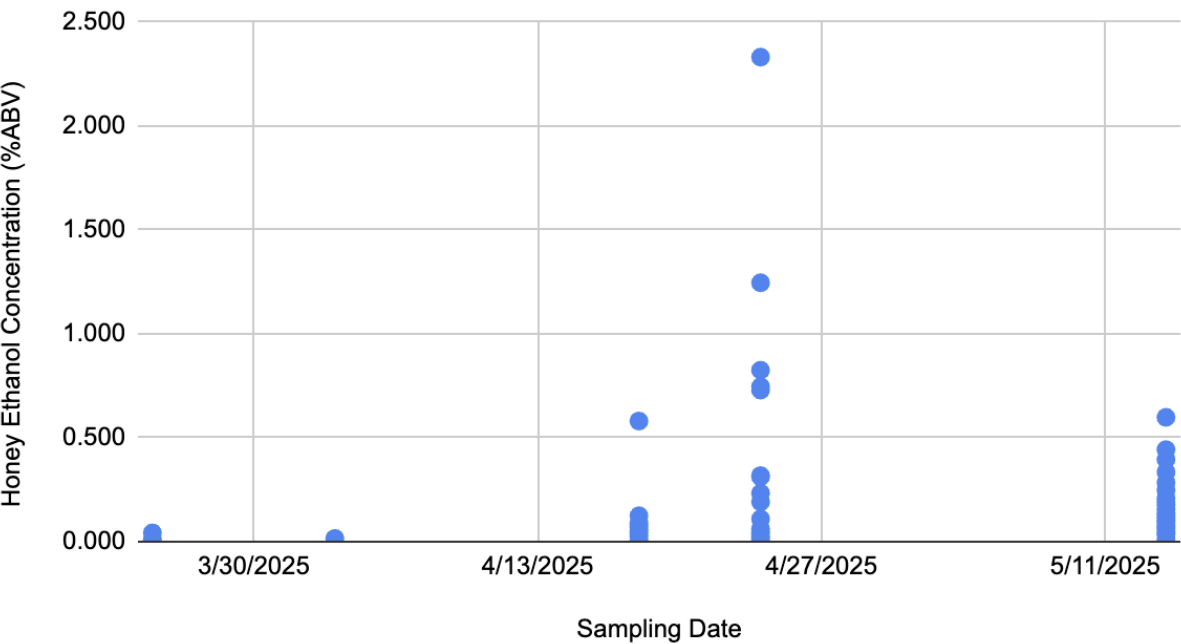


Figure 1: Ethanol concentrations (% ABV) of *Bombus impatiens* honey from eight commercial colonies across five sampling dates.

Wildflower nectar ethanol content

Table 2. Summary of ethanol concentrations in the nectar of Colorado Rocky Mountain wildflower species.

Species	Site	Number of Samples Quantified	Maximum ethanol detected	Proportion of samples with detectable ethanol
<i>Mertensia ciliata</i>	Research Meadow			
	WG 403			

	Trailhead			
<i>Corydalis caseana</i>	Lower WG			
	WG 403 Trailhead			
<i>Ipomopsis aggregata</i>	Deer Creek			
<i>Delphinium barbeyi</i>	Deer Creek			
	Judd Falls			
<i>Aconitum columbianum</i>	Deer Creek			
	Judd Falls			
	Research Meadow			

Discussion (+ Add results in this later)

We detected ethanol in the honey and wildflower nectar consumed by bumble bees, reflecting the presence of ethanol due to the fermentation of sugars by honey- and nectar-inhabiting yeasts (Souto-Vilaros *et al.*, *in review*). The variation in floral nectar ethanol concentrations observed in this study may reflect differences in sugar content, yeast colonization, and nectar retention time across plant species. Floral morphology also likely contributes to ethanol levels. For example, *Helianthella quinquenervis*, with its open floral structure, may allow for greater evaporation of ethanol from nectar. In

contrast, the more enclosed floral morphology of *Corydalis caseana* may promote conditions favorable for ethanol accumulation.

Prior research has shown that ethanol can influence pollinator behavior, and this has been particularly well-studied in *Apis mellifera* (Western honey bees). Chronic exposure to ethanol concentrations as low as 0.1–1% has been shown to significantly reduce bee lifespan, disrupt social interactions, impair motor coordination, and negatively affect foraging behavior (Hernández-Tobías et al., 2023). While honey bees (and presumably bumble bees) do possess alcohol dehydrogenase (ADH) enzymes, these enzymes operate relatively slowly, with studies indicating that ethanol can remain in the bees' hemolymph at least 8 hours after ingestion, even from quantities as low as 10 uL of 1.25% ethanol (Božič et al., 2007). These findings suggest that even low concentrations of ethanol in honey and nectar could have behavioral and physiological impacts on pollinators, depending on species-specific metabolic capabilities and foraging patterns. We demonstrate here that bumble bees are frequently exposed to biologically relevant quantities of dietary ethanol both from fresh wildflower nectar and from stored honey.

If ethanol presence is widespread among Colorado wildflowers, then it could serve as a selective filter for pollinators, influencing plant fitness, as pollination effectiveness can vary depending on nectar ethanol concentrations and pollinator tolerance. Previous studies show pollinators such as *A. mellifera* actively seek out ethanol in nectar, even when the ethanol concentration is relatively high (e.g., 5% ABV) (reviewed in Abramson et al., 2025). However, *Calypte anna* (Anna's Hummingbird) foragers actively avoid nectars with 2% (v/v) ethanol concentration, though they were not deterred by nectar containing 1% ethanol (Choi et al., 2023). Moreover, with increasing temperatures due

to climate change, yeast activity may increase, potentially elevating nectar ethanol concentrations across seasons or years, leading to altered foraging behaviors, disrupted plant-pollinator timing, or possible changes in pollinator community compositions.

While this study provides foundational data on ethanol quantities consumed by bumble bees during foraging bouts and while in the nest, several limitations remain. Specific yeasts within the bumble bee honey and *Aconitum columbianum* samples are not currently known, though the yeast communities of the other four wildflower species have been surveyed in previous and current studies. Additionally, nectar was collected over a limited time window. So, potential temporal changes in ethanol across environmental conditions remain unexplored. Moreover, while prior research outlines ethanol influence on honey bee behavior, more work is needed to understand the effects of ethanol on bumble bees, who encounter much higher concentrations of ethanol in their honey than honey bees. Future research could include microbial sequencing to identify specific yeast taxa present within nectar microbial communities as it relates to ethanol concentration as well as field ethanol manipulations to measure pollinator behavioral changes. More longitudinal studies could measure ethanol concentration fluctuations across seasons or under different climate conditions. Such studies could help explain whether ethanol is simply a microbial by-product or if it is an ecologically significant trait influencing plant-pollinator interactions.

- though see my advisor's (Robert Schaeffer's) work on *Delphinium barbeyi*, the yeast species were identified there (I believe), and we have an upcoming paper focused on our microbe survey of *Mertensia*, *Corydalis*, and *Ipomopsis* (and *Linaria*).