# Excerpt 1A

Adapted from Vesely et al. *J. Agric. Food Chem.* **2003,** *51,* 6941

## MATERIALS AND METHODS

**Chemicals.** Carbonyl compound standards, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, pentanal, hexanal, furfural, methional, phenylacetaldehyde, and (*E*)-2-nonenal, were purchased from Sigma-Aldrich (Milwaukee, WI). A stock solution containing a mixture of the standard compounds (100 ppb each) in ethanol was prepared. Stock standard solution was prepared daily. An aqueous solution of the derivatization agent *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA) (Sigma-Aldrich, Milwaukee, WI) was prepared (6 g/L) every 3 months and kept refrigerated.

**Beer Samples.** American lager beer samples used for the aldehyde analysis were stored at 30 °C for 4, 8, or 12 weeks. Control samples were stored for 12 weeks at 0 °C. We measured the $SO_2$ level of the fresh beer (3.4 ppm), which is important because $SO_2$ complexes with aldehydes and only "free" aldehydes are measured by the method presented here.

**SPME Fiber and Derivatization.** A 65-*μ*m poly(dimethylsiloxane)/divinyl benzene (PDMS/DVB) fiber coating (Supelco, Bellefonte, PA) was selected for its ability to retain the derivatizing agent and for its affinity for the PFBOA-aldehyde oxime (*7*). PFBOA solution (100 μL) and deionized water (10 mL) were placed in a 20-mL glass vial and sealed with a magnetic crimp cap. The SPME fiber was exposed to the headspace of the PFBOA solution for 10 min at 50 °C. The SPME fiber loaded with PFBOA was then exposed to the headspace of 10 mL of beer placed in a 20-mL glass vial. Different derivatization times and temperatures, as well as salt addition, were tested for optimization. To ensure the reproducibility of the method, an automated process using an MPS2 autosampler (Gerstel, Baltimore, MD) was employed.

# Excerpt 1B

Adapted from Jean et al. *Plant Ecol.* **2018,** *219,* 837

**Methods**

**$^{15}N_2$ incubation assays**

The sampling design differed slightly every year from 2013 to 2015 (Table 1). In 2013, five monospecific *Pleurozium* or *Hylocomium* moss patches were selected in the spruce and birch stands from sites A and B. BNF rates were measured in late July on the top 5–8 cm of 20 *Pleurozium* shoots or 10 *Hylocomium* shoots per patch. In 2014, 12 monospecific moss patches (n = 12 patches per species per stand) were selected along a 60-m transect in each stand of sites A, B, and C. BNF rates were measured in site A in June, July, August, and September 2014, and in sites B and C in August. In August 2015, BNF rates were measured in five *Hylocomium*-dominated patches that also contained *Pleurozium* in sites A and B (n = 5 patches per stand); therefore, both moss species were sampled within the same patch (Jean 2017). In 2014 and 2015, shoots were collected at a length of 5 cm from the apical meristem, and BNF rates were measured on 10 *Pleurozium* or *Hylocomium* shoots per patch.

During incubation trials, we watered moss patches with rainwater 24 h and immediately before the start of the incubation to ensure minimum water availability. Control mosses were collected to measure natural $^{15}N$ abundance from 2 to 10 shoots per moss species from each patch in each sampling period. Enriched moss samples were incubated 24 h on their patch of origin in 60-ml translucent polycarbonate syringes, each filled with 10 ml of air and 10 ml of $^{15}N_2$ (98% enriched, Cambridge Isotope Laboratories Inc., U.S.A.). After incubation, enriched and control samples were weighed wet, dried for 72 h at 60 °C, reweighed, and finely ground. N and C concentrations and $^{15}N$ and $^{13}C$ at % values were determined using an Elemental Analyser coupled to a Continuous-flow Isotope Ratio Mass Spectrometer (University of Florida in 2013 and Northern Arizona University in 2014–2015). BNF was calculated by comparing the $\delta^{15}N$ values from enriched and control samples. In July 2014, we tested if watering biased estimates of BNF rates by incubating both dry and wet mosses (pooled data from both moss species and forest types in site A; n = 24 for each). Average moss moisture was 40.6% ± 5.4 (mean ± SE) and 76.9% ± 3.8 in the non-water and watered treatments, respectively, but did not affect BNF rates (paired t test: F1,23 = - 1.736, P = 0.096).

# Excerpt 2

Adapted from Jean et al. *Plant Ecol.* **2018,** *219,* 837 and Vesely et al. *J. Agric. Food Chem.* **2003,** *51,* 6941

1. Reproducibility of the method was determined by repeatedly analyzing one beer sample 10 times. **Table 1** shows that the method provided very good reproducibility, with coefficients of variations for monitored aldehydes below 5.5%, except for (*E*)-2-nonenal. The higher coefficient of variation for (*E*)-2-nonenal may be due to extremely low levels of this aldehyde in the analyzed beer.

2. BNF rates were generally higher in stands dominated by black spruce than in stands of Alaska paper birch for both moss species, although they were low in all stand types in 2015. Environmental factors specific to each forest type are likely important drivers of variation in BNF rates by feather moss-cyanobacteria associations.

3. During long-term storage at elevated temperatures, American-style beers develop a stale flavor (*10*). Analyzed beer samples were stored at 30 °C for 4, 8, and 12 weeks. Levels of all aldehydes increased during beer storage compared to the control sample (**Table 2**). Although the increase after 12 weeks at 30 °C was significant (16-fold increase for furfural, 7-fold increase for 2-methylpropanal), none of the analyzed aldehydes exceeded their flavor threshold in beer (*11*). We hypothesize that additive or synergistic effects are likely occurring that result in low-level aldehydes contributing to the stale flavor of aged beer *(12)*.

4. Variation in BNF rates between sites and forest types was smaller than the inter- and intra-annual variation, suggesting that factors that vary annually, such as weather, are more important in controlling BNF rates than factors that vary in space.

# Excerpt 3

Adapted from Vesely et al. *J. Agric. Food Chem.* **2003,** *51,* 6941

## INTRODUCTION

Carbonyl compounds, particularly aldehydes, are considered to play an important role in the deterioration of beer flavor and aroma during storage. Strecker degradation of amino acids, melanoidin-mediated oxidation of higher alcohols, and oxidative degradation of lipids are mechanisms implicated in their formation (*1*). The levels of aldehydes in beer are usually very low, presenting an analytical challenge for brewing chemists.

Several analytical methods for the determination of aldehydes in beer have been developed, and good results have been obtained using liquid-liquid extraction (*2*), distillation (*3*), or sorbent extraction (*4*). However, these methods are rather complicated and not highly selective.

A simple way to increase the selectivity of extraction techniques is to derivatize the carbonyl compounds. *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine (PFBOA) is commonly used as a derivatization agent in gas chromatography (*5*). This technique has been applied to the analysis of carbonyl compounds in water and also in beer (*6*). Although these methods provide good reproducibility, they are time-consuming and require multiple isolation steps and the use of expensive and toxic solvents.

Martos and Pawliszyn developed an original extraction technique based on PFBOA on-fiber derivatization of gaseous formaldehyde followed by gas chromatography with flame ionization detection (*7*) that could be adapted to aldehyde analysis in beer.

In this work, we adapted and optimized a method for the analysis of beer aldehydes using solid-phase microextraction (SPME) with on-fiber derivatization. We demonstrate the application of this method to aldehyde level changes during packaged beer storage. Our method does not require solvents, consists of a one-step sample preparation procedure, and provides high sensitivity and reproducibility.

# Excerpt 4

Adapted from Jean et al. *Plant Ecol.* **2018,** *219,* 837

**Introduction** (with citations removed for visual clarity)

Boreal forests of the world are important carbon (C) sinks, making the productivity of these ecosystems of global importance in regulating atmospheric carbon dioxide ($CO_2$) concentrations.

Productivity in boreal ecosystems is often limited by nitrogen (N) availability. Biological $N_2$-fixation (BNF) by various bacteria, such as free-living and symbiotic cyanobacteria, is a major source of N in many ecosystems.

Associations between mosses (e.g., *P. schreberi*) and $N_2$-fixing cyanobacteria (e.g., *H. splendens*) have been identified as a source of ecosystem N inputs in mature Scandinavian boreal ecosystems. There, they contribute up to 2–4 kg N $ha^{-1}$ $year^{-1}$, a source of N that is comparable to atmospheric N deposition. Most of this newly fixed N is thought to remain within the moss layer but may become available to vascular plants through mycorrhizae.

The contribution of *P. schreberi's* cyanobacteria communities to N fluxes and pools in Scandinavian boreal forests is well established and the contribution of cyanobacteria on *H. splendens* is increasingly recognized as substantial.

Most of the work on moss-associated BNF in boreal forests has focused on Fennoscandia's coniferous boreal forests with an extensive moss cover. However, boreal forests are composed of both coniferous and broadleaf deciduous stands.

Strong spatial (among sites and forest types) and temporal (among years or months) variability in BNF is to be expected, but how much this variability may affect the overall contribution of moss-cyanobacteria to stand-level N pools is unclear.

Our objective was to characterize the stand-level N contribution of BNF associated with *P. schreberi* and *H. splendens* in mature Alaska paper birch (*Betula neoalaskana* Sarg.) and black spruce stands in interior Alaska. Measurements across multiple years and replicate stands provided estimates of how moss-associated BNF rates varied over space and time.

Results of this study will give updated estimates of how feather moss-associated BNF contributes to N cycling among deciduous and coniferous boreal forests of interior Alaska.

# 1. Provide foundation/context for your research

## 2. Establish motivation for your study

## 3. Establish significance of your study