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Author(s): Bacon, K. L., Brown, C., Boom, A. & F.L. Gill

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Investigating chemical preservation in herbarium specimens of Humulus lupulus Linnaeus 1753

Karen L. Bacon[1,2*], Clare Brown[3], Arnoud Boom[4] and Fiona L. Gill[5]

[1]School of Geography, University of Leeds

[2]Botany & Plant Sciences, National University of Ireland, Galway

[3]*Leeds Museums and Galleries*

[4]*Department of Geography, University of Leicester*

[5]*School of Earth and Environment, University of Leeds*

*Corresponding author: karen.bacon@nuigalway.ie

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Abstract

This study investigates if leaves from the hops plant (*Hummulus lupulus* Linnaeus 1753) retain their chemical signature with increasing time in herbarium storage. We used pyrolysis-gas chromatography-mass spectrometry to investigate the chemical composition of four *H. lupulus* leaves, two modern and two herbarium samples (1919 and 1882). The older samples showed far fewer chemical compounds than the modern leaves. This suggests that herbarium samples do not retain most chemical compounds found in living plants for this species. This has implications for storage of leaves depending on whether chemical composition is something that is considered desirable or not to be preserved for specific plant species.

Key words: herbaria, chemical preservation, plants, *Hummulus lupulus*

Introduction

Herbaria are of major scientific and social importance with most new species of plants being identified through samples examined in herbaria (Bebber *et al.*, 2010; Penn et al., 2017) and are of major importance to our understanding of global plant diversity and plant responses to climate change. For example, Guerin et al., (2012) identified a significant decrease in leaf width of Australian *Dodonaea viscosa* subsp. *angustissima* over 127 years that they determined was a response to climate change. Herbaria were instrumental in identifying the now commonly applied inverse relationship between stomatal numbers and atmospheric CO_2 levels (Woodward, 1987) and are commonly utilised to form baselines for palaeobiological studies (e.g. Beerling and Chaloner, 1993; McElwain *et al.*, 1995 ; Penuelas and Matamala, 1990; Wang *et al.*, 2018).

In terms of societal importance, herbaria record not just our plants but also our attitudes to them. Drawing plants was one of the few scientific endeavours deemed suitable for women in the 1700-1900s (e.g. Shteir, 1981) and many herbaria collections preserve the work of female botanists that would otherwise have been lost. Plants also contain a wide variety of chemical compounds,

Today the variety of chemical compounds in plants are important for everything from medicine to clothing (e.g. Lovkova *et al.*, 2001) and many also contain psychoactive compounds (e.g. Graziano *et al.*, 2017). It is this last component of plant chemistry that can become problematic for herbaria or universities and botanic gardens as psychoactive compounds that are now illegal or closely monitored by governments and law enforcement were not always illegal (e.g. the recent suspension of academics at Miami University due to Iboga in a botanical collection; Flaherty, 2019). Curators of herbaria need to consider how legal changes to the status of various chemical compounds, such as cannabinoids, may impact on the legality of their collections and even how holding such collections may lead to increased risk of violence towards the museums that hold them (e.g. sharp increase in thefts of rhino horns e.g. BBC, 2011; Daley, 2017).

Cannabinoids in the UK are an interesting example because it is the compound, tetrahydrocannabinol (THC), that is illegal and the plants (*Cannabis sativa*) cannot be legally owned or sold without the correct licence. The licence to hold the plant is expensive (> £3000), beyond the budgets of most museums or universities where herbaria are usually housed. The legislation mentions 'cannabis' but the levels of psychoactive drugs in the plants are not considered, meaning that this may unfairly target herbaria collected for scientific purposes – particularly as we currently do not know if these chemicals preserve for the length of time that plants tend to be held in storage. In order to begin to investigate this, we sampled four leaves from *Humlus lupulus* (Hops), which shares many similar chemical compounds to those found in cannabis but is not a restricted species and therefore is easily accessible for study. Ultimately, our aim would be to provide evidence to whether these chemicals and similar ones in *C. sativa* actually remain preserved over decades and centuries because such information could be useful if the drugs licences were ever to be revisited.

Methods

Two herbaria samples (1919 and 1882) and two modern leaf samples (one immature and one mature leaf, grown locally in Leeds in 2018) were analysed by pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) for this pilot investigation to determine if they retain the same chemical profile in storage. Two small hole punches were taken of the leaf material from each leaf, which was then ground to a fine powder and placed in glass vials for pyrolysis. The herbaria material was collected from the Leeds Museum and Galleries herbarium held in the Discovery Centre in June 2018.

Py-GC/MS was performed using a CDS1000 pyroprobe interfaced with a Perkin Elmer Clarus 500 GC/MS system. Powdered leaf was encapsulated in a quartz tube and then pyrolysed at 610 °C for 15 seconds in the presence of tetramethylammonium hydroxide (TMAH; Challinor, 2001). Pyrolysis, with TMAH, is a process whereby macromolecular organic matter is thermally broken down into smaller gas chromatography amenable methylated monomeric structures that can then be identified. Gas chromatography was carried out using a CP-Sil 5CB MS column (30 m × 0.25 mm × 0.32 μm). The GC temperature programme began at 40 °C (1.8 min), was ramped to a final temperature of 310 °C at 4 °C minute−1 and held for a further 20 minutes. Compounds within the pyrograms were identified based on their mass spectra and retention times.

Results

The total ion chromatograms obtained for the four samples are shown in Figure 1. Two main groups of compounds were identified: lignin derived compounds and fatty acid methyl esters. The mature and immature modern leaves have very similar chromatograms but when they are compared to the herbarium sample chromatograms there is a clear decrease in the relative abundance and diversity of compounds present, particularly in terms of the lignin-derived compounds. For example, both herbarium samples and modern samples contain a range of fatty acid methy esters, with hexadecanoic acid methyl ester (identified by the black circle in Figure 1) found in all samples but dimethoxybenzene (a lignin derived compound, identified by the asterix in Figure 1) not found in the older samples. Surprisingly, only the oldest sample showed the presence of farnesol (identified by the star in Figure 1), a compound commonly associated with hops. Figure 1 shows a clear decline in chemical compound preservation with the older samples having far fewer compounds and at a lower relative abundance than the modern leaves.

Discussion

Humulus lupulus has a well-documented chemistry, in part due to its use in the brewing industry and potential medical applications (Gulz *et al.*, 1993; Stevens *et al.*, 1997; Zanoli and Zavatti, 2008; Yan *et al.*, 2019). However, how these compounds degrade over time has received little attention. Although some plant compounds, such as n-alkanes (Lockheart *et al.*, 2000) and even

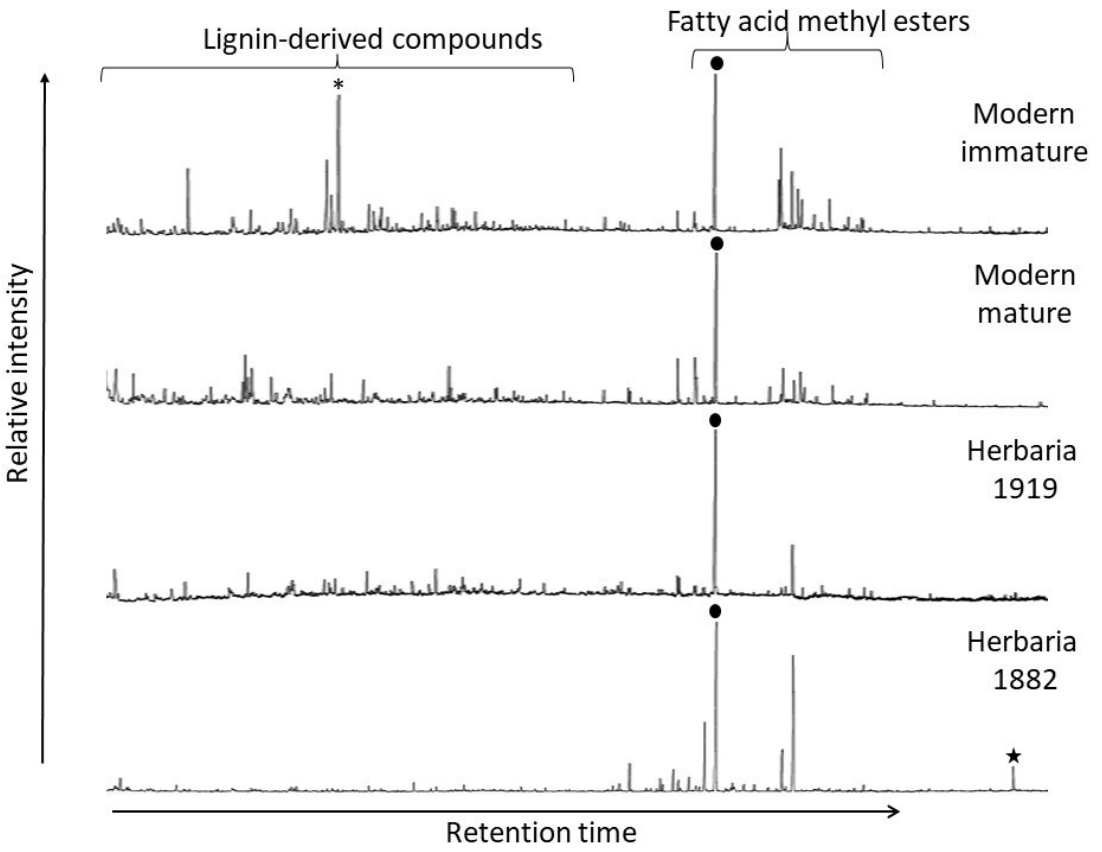

Figure 1: Total ion chromatogram for each of the four samples analysed. Some key compounds identified are: dimethoxybenzene (*), hexadecanoic acid methyl ester(•) and farnesol (⋆)

biopolymers (Logan *et al.*, 1993) are known to preserve in the fossil record for millions of years, far less is known about psychoactive compounds such as those commonly found in plants such as Hops and cannabis. Russo *et al.*, (2008) describe well-preserved cannabis in a 2,500-year-old tomb in China with good chemical preservation but little evidence for psychoactive compounds and no THC detected. Figure 1 shows a clear degradation in the chemical compounds in terms of presence and abundance with the oldest leaf sample having the fewest peaks. The results, while preliminary and requiring further investigation and sampling, provide a clear suggestion that the vast majority of chemical compounds in *H. lupulus* degrade over time and may suggest that concerns over the holding of botanical samples with psychoactive compounds in herbaria are not particularly founded. Contrastingly, were the compounds to be of interest or benefit to study, then the process of storage may need to be reconsidered; however, this again requires much further work.

This study shows that the majority of chemical compounds present in herbarium leaves of *H. lupulus* degrade with increased time in storage since they were collected. This suggests that herbaria and museums with botanical specimens that are known to contain illegal or controlled substances when alive should not necessarily be assumed to be actively holding such compounds when the specimens are over 100 years old. Additionally, it is likely that more detailed work will reduce this timeframe considerably. It would also be beneficial to study cannabis directly, but this presents licencing and legal difficulties at present in the UK.

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