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ABSTRACT

Hemp (*Cannabis* sp.) has been a fundamental plant for the development of human societies. Its fibers have long been used for textiles and rope making, which requires prior stem retting. This process is essential for extracting fibers from the stem of the plant, but can adversely affect the quality of surface waters. The history of human activities related to hemp (its domestication, spread, and processing) is frequently reconstructed from seeds and pollen detected in archaeological sites or in sedimentary archives, but this method does not always make it possible to ascertain whether retting took place. Hemp is also known to contain phytocannabinoids, a type of chemicals that is specific to the plant. Here we report on the detection of one of these chemicals, cannabinol (CBN), preserved in a sediment record from a lake in the French Massif Central covering the past 1800 yr. The presence of this molecule in the sedimentary record is related to retting. Analysis of the evolution of CBN concentrations shows that hemp retting was a significant activity in the area until ca. A.D. 1850. These findings, supported by pollen analyses and historical data, show that this novel sedimentary tracer can help to better constrain past impacts of human activities on the environment.

INTRODUCTION

Hemp is one of the earliest cultivated plants (Russo, 2007). Its high adaptability (Raman, 1998) allowed it to spread worldwide, perhaps through a co-evolution with mankind (McPartland and Guy, 2004). Hemp can therefore be considered as a fundamental plant in human history (Raman, 1998). Indeed the development of all civilizations has relied on its many uses: e.g., as foodstuff (the seeds), medicine and intoxication (the resin), and overwhelmingly for making ropes, textile, and paper (the fibers). The fibers are separated from the stems after the retting process, usually consisting of submerging the stems in water (Wills, 1998). Though retting is required for extracting fibers and is thus used worldwide, this traditional process has been known for centuries to dramatically damage water quality, causing massive fish death and making water undrinkable for cattle and humans (Anonymous, 1772). Tracing this ancient pollution is of major interest, because it can provide clues to past interactions between human societies and environments, the understanding of which is crucial to anticipate the consequences of future global changes (Dearing, 2006). To date, pollen, seeds, or textile fragments are the only indicators currently exploitable in archaeological studies to detect the use of retting (e.g., Schofield and Waller, 2005), and thus to assess the extent of the induced pollution.

Recording a continuous history of hemp retting can be achieved by using a set of archaeological sites that are chronologically continuous and where seeds and pollen are preserved, and implies morphometric analyses of pollen due to the resemblance between *Cannabis* and *Humulus* (hop genus) pollen grains (Mercuri et al., 2002; Whittington and Gordon, 1987). Moreover, pollen and seeds may be absent in archaeological sites (Wills, 1998). Monitoring tracers of human activity preserved in a natural archive can help overcome these difficulties. The analysis of the molecular biomarker content of lacustrine sediments (which continuously record environmental changes) and soils can provide information on past environments, but only in a very few cases can these be unequivocally related to human activity (e.g., Bull et al., 2002; Jacob et al., 2005, 2008; Zocatelli et al., 2010; Lavrieux et al., 2011; Le Milbeau et al., 2013). Up to now, no study has revealed the occurrence of any hemp biomarker in natural archives, although the plant contains phytocannabinoids, a group of chemical compounds unique to this plant (Russo, 2007). We here report on the detection of cannabinol in a sediment core drilled in Lake Aydat (Auvergne region, France) that covers the past 1800 yr.

METHODS AND STUDY SITE

Sedimentary Core

Lake Aydat (45°39.809′N, 2°59.106′E) is located in the northern part of the French Massif Central (Fig. 1), a volcanic region located in the center of France. A high-resolution continuous sediment sequence covering the past 6700 yr retrieved under 14.5 m water depth was dated (accelerator mass spectrometry [AMS] radiocarbon dates, ^{137}Cs measurements, and detection of historical flood deposits) and extensively described in a previous study (Lavrieux et al., 2013). The present study focuses on the upper part of the core, consisting of dark and faintly laminated sediment interrupted in many places by flood deposits. Samples were selected in the background sediment, i.e., after the flood events were removed. Their position is displayed together with the depth-age model in Figure 1.

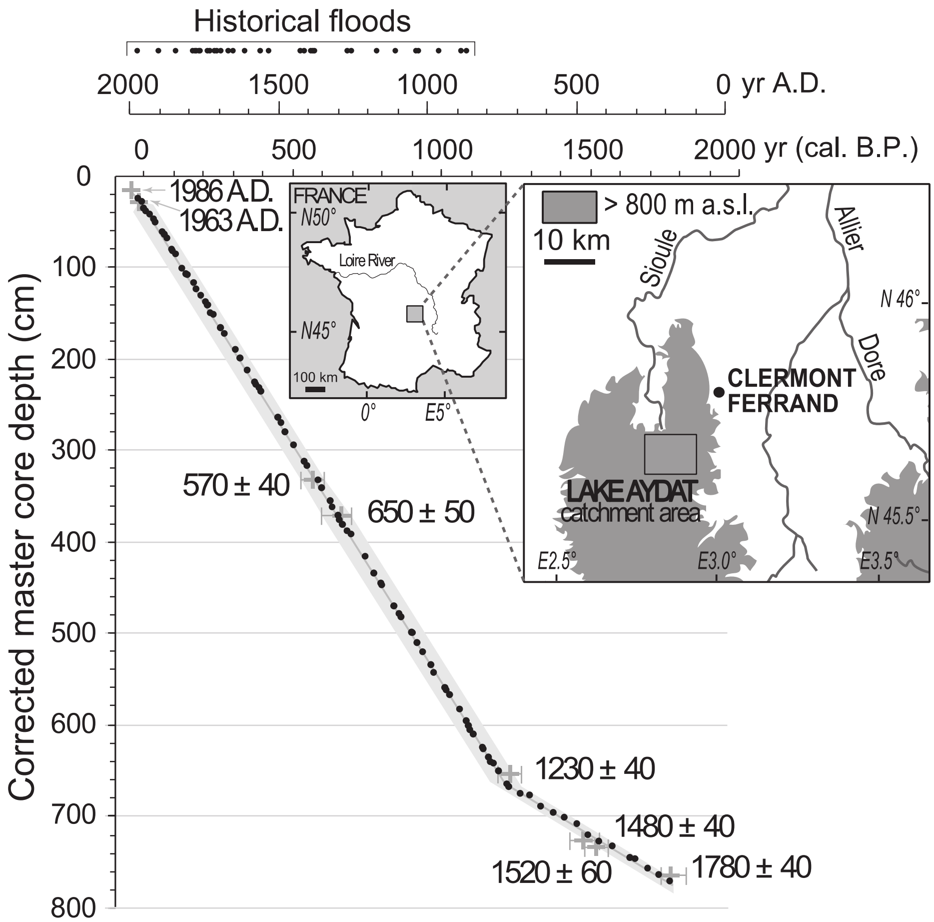

Figure 1. Location of study site in France, depth-age model (Lavrieux et al., 2013), and position of sediment samples along sedimentary record. Corrected master core depth means that flood deposits were removed. Accelerator mass spectrometry radiocarbon dates are reported as calibrated years B.P. (before 1950) and shown as gray crosses and error bars. Gray crosses without error bars are ^{137}Cs dates. Historical floods are shown above the graphics. Reconstructed depth-age model is shown with a dark gray line (light gray-margin of error). Sediment samples are symbolized with black dots. a.s.l.-above sea level.

Pollen Analyses

Pollen analyses were performed on 50 samples, spaced 2.5 cm apart, covering the past 1800 yr (intervals of 35 yr). Samples were prepared using standard procedure (Faegri and Iversen, 1989) at the Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale (UMR 7263/CNRS, France). Minimum counts of 500 dry land pollen grains per sample were made. Pollen rates were calculated as a percentage of total land pollen excluding hygrophytes, aquatic plants, and fern spores. Morphometric analyses of pollen grains were carried out according to Mercuri et al. (2002). The *Cannabis-Humulus* pollen curve presented here combines the values of *Cannabis-Humulus* pollen type (pollen diameter 25–28 μm) and those of *Cannabis* pollen type (diameter >28 μm). The frequencies of *Humulus* pollen type (diameter <25 μm) are excluded. The detailed pollen counts are provided in the GSA Data Repository[1].

Lipid Analyses

Sixty (60) lacustrine sediment samples covering the past 1800 yr were dried, crushed in a mortar, and sieved at 2 mm. An internal standard was added to ~1 g of sediment, which was solvent extracted by automatic solvent extraction (Dionex Accelerated Solvent Extractor) using a mixture of CH_2Cl_2:MeOH (9:1 vol/vol). After removal of the solvent under N_2, the extract was separated into neutral, acidic, and polar fractions on aminopropylbonded silica. The neutral fraction was further separated into aliphatics, aromatics, ethers and esters, ketones and acetates, and alcohols by flash chromatography on a Pasteur pipette filled with activated silica, using a sequence of solvents of increasing polarity. Alcohol fractions were then trimethylsilylated with N,O-bis (trimethylsilyl) trifluoroacetamide and pyridine (2:1 vol/vol; 60 °C, 60 min), and these fractions were injected into a gas chromatography– mass spectrometry (GC-MS) system. The operating conditions are detailed in Lavrieux et al. (2011). Cannabinol (CBN) was identified by comparison with an authentic standard (also trimethylsilylated before injection) and its concentration was estimated by measuring the area of its peak on an m/z 367 + 382 ion-specific chromatogram. After calculating a correction factor between the peak area on the ion-specific chromatogram and the peak area on the total ion current (TIC) chromatogram, the TIC area of the compounds was compared to that of the standard (5α-cholestane) and to the mass of the sample extracted. The detailed CBN concentrations are provided in the Data Repository.

RESULTS

CBN was identified in several alcohol fractions of the free lipids extracted from Lake Aydat sediment samples. Its typical mass spectrum, molecular structure, and fragmentation pattern (as trimethylsilylated derivative) are shown in Figure 2.

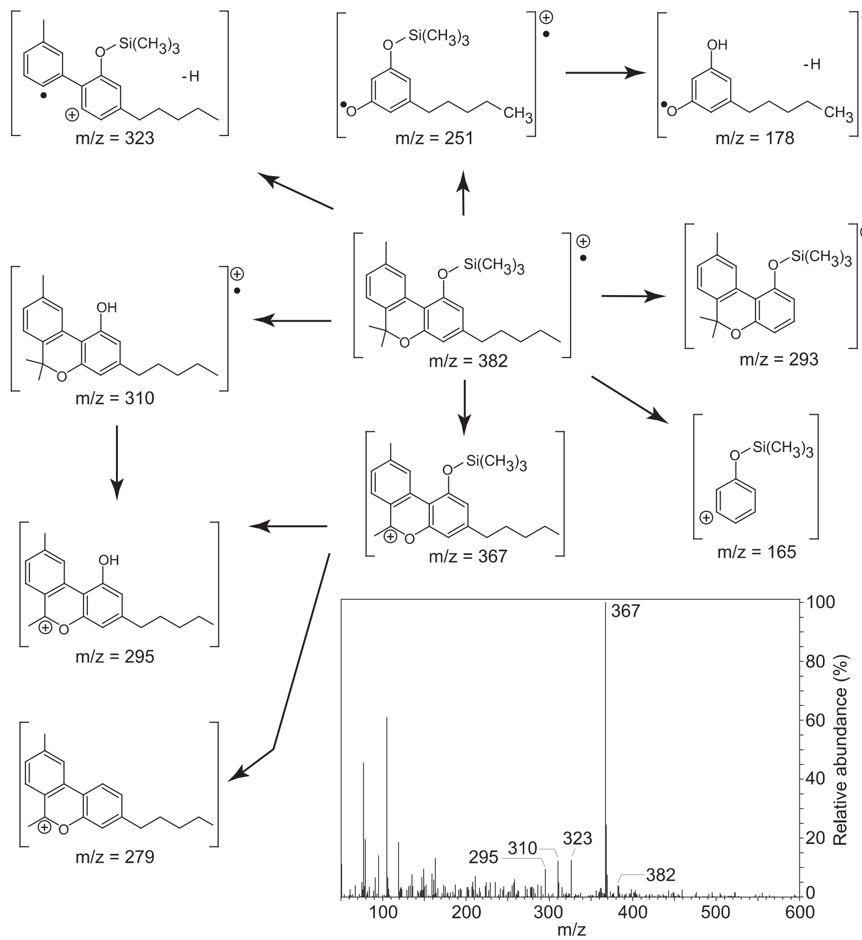

Figure 2. Mass spectrum and molecular structure of cannabinol (trimethylsilylated derivative) from selected sediment sample (A.D. 1322).

This compound is the fully aromatized metabolic by-product of Δ^9-tetrahydrocannabinol (Δ^9-THC; ElSohly and Slade, 2005), a psychoactive compound whose concentration in the plant differs depending on the variety of hemp considered (<0.3% in textile hemp versus >1% for intoxicating hemp; Raman, 1998).

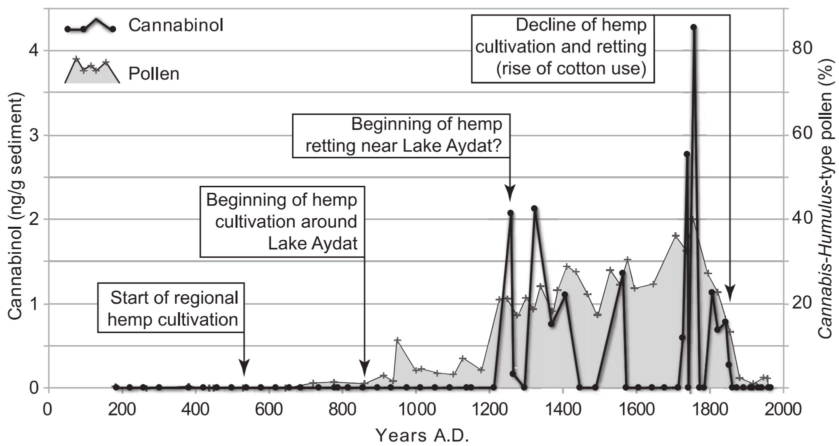

Figure 3. Evolution of cannabinol relative abundance (ng/g sediment) and of *Cannabis*-type and *Cannabis-Humulus*–type pollen (expressed as a percentage of the total of terrestrial pollen) through time in sediment samples. Main historical phases of hemp uses in the area are indicated in boxes.

The concentration of CBN ranged from 0 to 4.27 ng/g sediment in our sediment samples (Fig. 3). Starting from the base of the studied section, *Cannabis*-type and *Cannabis-Humulus*–type pollen was weakly present from A.D. 470, while CBN was detected for the first time in sediments dated to ca. A.D. 1260, at concentration levels of ~2 ng/g sediment. From then on, CBN concentrations and pollen frequency varied strongly, and differently from each other, all along the record. CBN was not detected in two samples dated from ca. A.D. 1445–1490 and five samples dated from ca. A.D. 1570–1720, and maximized at A.D. 1757. Pollen frequency followed a more continuous trend, remaining under ~5% until ca. A.D. 1200, when *Cannabis*-pollen-type values rose to almost 10%, and then increased abruptly to over 20%. Overall, it remained between ~20% and 30% - with a higher proportion of *Cannabis* pollen type - until the end of the 18th century, when it maximized at

~40%. From the beginning of the 19^{th} century, both CBN and pollen strongly decreased: CBN was totally absent in sediments younger than ca. A.D. 1860, while pollen was still weakly present (~2%) during the 20^{th} century. CBN was also absent in 35 soil samples analyzed in the lake catchment (Lavrieux, 2011).

DISCUSSION

Fiber hemp was extensively cultivated in the Auvergne, France, region during historical times (Peuchet, 1800), and was produced in small plots on the outskirts of every village (Charbonnier, 1980). As in other regions, the main reason for producing hemp fibers was for textile making (de Ballainvilliers, 1846), but all other parts of the plant, except the roots, were used: oil (extracted from seeds) was used as lighting fuel and peeled stems were used for heating (Poitrineau, 1965). Seeds were probably also consumed by local populations and cattle, and leaves were probably used for animal bedding (Brown, 1998). Careful retting was needed to produce a high-quality and reputed hemp fiber such as that used by the French Royal Rope Factory for the marine arsenal, where the longest ropes in Europe (200 m all in one piece) were made during the 17^{th} century (Peuchet, 1800). Hemp was also exported for the paper industry (Peuchet, 1800). Thus, the numerous uses of hemp as well as its widespread culture in the region explain the occurrence of one of its molecular biomarkers in Lake Aydat sediments. Sedimentary CBN could originate either directly from the plant or from hemp remains that were mixed in soils and subsequently eroded to the lake. Because the whole hemp plant was used, most of the material likely to contain CBN was exported. Only roots could constitute a potential contributor of soil CBN, but hemp roots have not been shown to contain more than small amounts of cannabinoids (De Pasquale et al., 1974;

Russo, 2007). Because soils are reputed to retain the molecular imprint of their past land uses (Lavrieux et al., 2012), and although soils are the main contributors of terrestrial organic matter to lacustrine sediments through erosion, another source must be invoked to explain the presence of CBN in the lake sediments. The most obvious explanation involves the practice of retting, a process largely used during historical times in Auvergne (Diderot and d'Alembert, 1778; de la Platière, 1784) which consists of submerging the stems in water. This was commonly done in all kinds of aquatic environments such as pits, marshes, ponds, or rivers. The stems were then left in water for a few days to a few weeks in order to facilitate extraction of the hemp fibers.

As stated above, pollen analyses conducted on the sediment core show the continuous cultivation of hemp in the region from at least ca. A.D. 470 and in the catchment from at least A.D. 870, and show a strong increase in pollen frequency between A.D. 1180 and 1860 (Fig. 3). Conversely, CBN concentration shows a more irregular pattern in this time period and is not detected outside of it. Previous studies in the same catchment (Miras et al., 2004; Lavrieux et al., 2013) revealed continuous human occupation associated with a marked anthropic impact on the environment in the area throughout the time span considered. While the absence of CBN before ca. A.D. 1260 could be explained by limited cultivation (as suggested by the low pollen frequencies) and retting, leading to CBN concentrations that are too low to be detected in the older samples, this hypothesis cannot explain the significant differences observed between pollen and molecular signals for later periods (A.D. 1445– 1490 and 1570–1720). Even though hemp pollen, which is disseminated by wind (e.g., Small and Antle, 2003), could come, in part, from outside the catchment (contrary to CBN, which is necessarily autochthonous), the high frequencies observed during this period leave no doubt concerning the reality of retting near Lake Aydat. No relationship between these discrepancy phases and the different sedimentological parameters expanded by Lavrieux et al. (2013) could be highlighted, underlining that the molecular signal is not determined by the sedimentation rate and/or the dilution in the mineral phase of the sediment. So, in the absence of any other tangible evidence, it can be hypothesized that variations in environmental conditions (for example, intensity of exposure to sunlight and/or of insect predation) known to influence phytocannabinoid concentrations (e.g., Pate, 1994) could have diminished the quantity of CBN in the plant and thus, the quantity archived in the sediment. Further studies are required to test such a hypothesis.

Considerable amounts of CBN were still detected throughout the 18th century in our samples, synchronous with maximal values of *Cannabis* pollen rates, despite a royal ordinance dated A.D. 1669 that forbade retting in French rivers in order to preserve water quality, fish stocks, and cattle health (Anonymous, 1772). This observation is in accordance with historical documents, which indicate that this ban was never put into practice and was still being debated 160 yr later (Duvergier, 1830).

While the first occurrence of CBN does not correlate with the first occurrence of *Cannabis* sp. pollen in sediments, the abundance of these tracers both strongly decrease in sediments younger than A.D. 1860. On the worldwide scale, this period corresponds to the development of the cheaper cotton industry (e.g., May and Lege, 1999) (followed by synthetic textiles), which hastened the abandonment of textile hemp cultivation.

CONCLUSIONS

Consistent with pollen analyses, the presence of CBN in Lake Aydat sediment samples during the period of hemp cultivation in the region strongly suggests that this compound—an unequivocal molecular biomarker of *Cannabis* sp.—can be used in sediments as a sedimentary tracer of anthropogenic activity and pollution for archaeological and paleoecological studies. In addition to pollen studies reflecting the cultivation of hemp, our results indicate that CBN tracks more specifically the subsequent retting process, reputed to significantly alter water quality.

Although further work is necessary to better evaluate the stability of CBN in older sediments, this compound can be tracked in natural archives to reconstruct hemp retting history and its induced pollution in a continuous time frame, as opposed to archaeological studies classically performed on archaeological sites that are more constrained in space and time, and can give reliable information about past impacts of human activities on the environment.

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[1] GSA Data Repository item 2013209, Table DR1 (*Cannabis*-type and *Cannabis-Humulus*–type pollen counts), is available online at www.geosociety.org/pubs/ft2013.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.