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Stable isotope models to predict geographic origin and cultivation conditions of marijuana

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ABSTRACT

Here we describe stable isotope based models using hydrogen and carbon isotope ratios to predict geographic region-of-origin and growth environment for marijuana, with the intent of applying these models to analyses of marijuana trafficking in the USA. The models were developed on the basis of eradication specimens and border specimens seized throughout the USA. We tested reliability of the geographic region-of-origin and growth environment models with a "blind" set of 60 marijuana eradication specimens obtained from counties throughout the USA. The two geographic region-of-origin model predictions were 60–67% reliable and cultivation environment model predictions were 86% accurate for the blind specimens. We demonstrate here that stable isotope ratio analysis of marijuana seizures can significantly improve our understanding of marijuana distribution networks and it is for that purpose that these models were developed.

1. Introduction

The use and production of marijuana (*Cannabis sativa* L.) poses significant public health and safety threats in the United States [1–4]. However, current understanding of the geographic sources and distribution patterns of marijuana in the United States remains relatively poor. Stable isotope ratio analysis has the potential to significantly improve our understanding of marijuana production and trafficking because stable isotopes function as natural recorders, revealing aspects of a plant's geographic origin and growth environment [5]. Expanding on previous stable isotope studies of marijuana [6–11], we have conducted an extensive survey of USA marijuana seizures, demonstrating stable isotope ratio data have the potential to provide links between seized specimens, information about growth environment and geographic origin, information on the variety of sources supplying individual areas, and information on temporal changes in distribution and cultivation practices [12–16]. Here we introduce and test two models to predict geographic region-of-origin and one model to predict growth environment of marijuana seized throughout the USA.

Stable isotope ratio analysis of precipitation reveals, on average, a global geographic pattern that can be described by models relating latitude and altitude to the stable isotope ratio values of precipitation. In turn, these spatial models can be used to generate global maps of precipitation isotope landscapes, or isoscapes [17,18]. Because the hydrogen atoms of plant source water are incorporated into organic molecules during biosynthesis, plant tissues from different geographic locations should record isotopic variations in source water. Geographic variations in the hydrogen isotopes of water used during cultivation should therefore be reflected in the hydrogen isotopes of marijuana tissues [12].

Carbon isotope ratios also record aspects of a plant's growth environment. Carbon in plant material reflects the isotopes of CO_2 incorporated during photosynthesis and the stomatal responsiveness to humidity in the growth environment. In general, enzymatic fixation of CO_2 during photosynthesis discriminates against the heavier isotope of carbon (^{13}C), resulting in plant tissues with lower carbon isotope ratios than atmospheric CO_2. The CO_2 of indoor growth environments will likely have lower carbon isotope ratios than outdoor conditions because respired CO_2 in the indoor environments does not mix completely with the atmosphere. Well-ventilated indoor growth environments, however, could yield plants with carbon isotope ratios similar to outdoor-grown plants. In addition, indoor-grown marijuana is often cultivated with supplemental bottled CO_2 to increase crop productivity. While the carbon isotope ratio of outdoor atmospheric CO_2 averages approximately −8‰, the source of CO_2 in bottled tanks is either derived from fossil fuels or other biogenic sources. In either case, the carbon isotope ratio of bottled CO_2 is significantly lower, ranging from −37‰ to −28‰ [19] resulting in plants with even lower carbon isotope ratio values. Thus, carbon isotope ratios have the potential to indicate whether marijuana was indoor- or outdoor-grown; whether plants were grown in shade or

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sun; and whether plants were grown indoors with or without bottled CO_2 [14].

2. Methods

2.1. Model development

We developed and assessed two region-of-origin models and a single cultivation conditions model for predicting geographic region-of-origin and growth environment for seized marijuana. The approaches used in the two region-of-origin models differed. However, in both models, a region-averaged hydrogen isotope ratio approach was used based on observations of eradication specimens of known origin. The observed value of a specimen of unknown origin was then compared to the region-specific values in order to determine that region closest in value to the observed specimen and thus most likely to have been the source region. Model I predictions were built around 17 distinct geographic regions based on zones of the modeled source water precipitation hydrogen isotope ratios across landscapes as described by Bowen and colleagues [17,18]. In contrast, Model II predictions were built on categorization of broad geographic divisions of the USA, Canada, and Mexico into five regions and then calculating region-specific average hydrogen isotope ratios of marijuana for those regions. The two model approaches provide different specificity of geolocation information that will be useful to law enforcement investigations and to policy makers. The cultivation model established thresholds for indoor and outdoor growth environments (see Fig. 5) and is described in detail elsewhere [14].

2.1.1. Region-of-origin Model I

Region-of-origin Model I is based on combining continental-scale patterns in the predicted hydrogen isotope ratio values (δ^2H) of precipitation [17,18] and county-level observations of δ^2H values on marijuana eradication specimens [12,13]. For this model, the United States was divided into regions of equal 10‰ ranges of δ^2H precipitation values; county was used as the fundamental spatial unit in this approach. The result was 17 isotopically-distinct regions that differed in average hydrogen isotope ratio value; all 50 states of the USA were included in this modelling approach (Fig. 1). Observed marijuana leaf δ^2H values within each of these regions were averaged and compared to the region-wide mean precipitation δ^2H value. In this regression-based approach, little insight is provided into the mechanistic basis for observed variations in marijuana leaf δ^2H values, although it is clear from mechanism-based biochemical models that these two parameters are tightly associated [20]. Over 500 marijuana eradication specimens from known USA counties of origin were obtained from 13 of these regions and analyzed for δ^2H values [12,13].

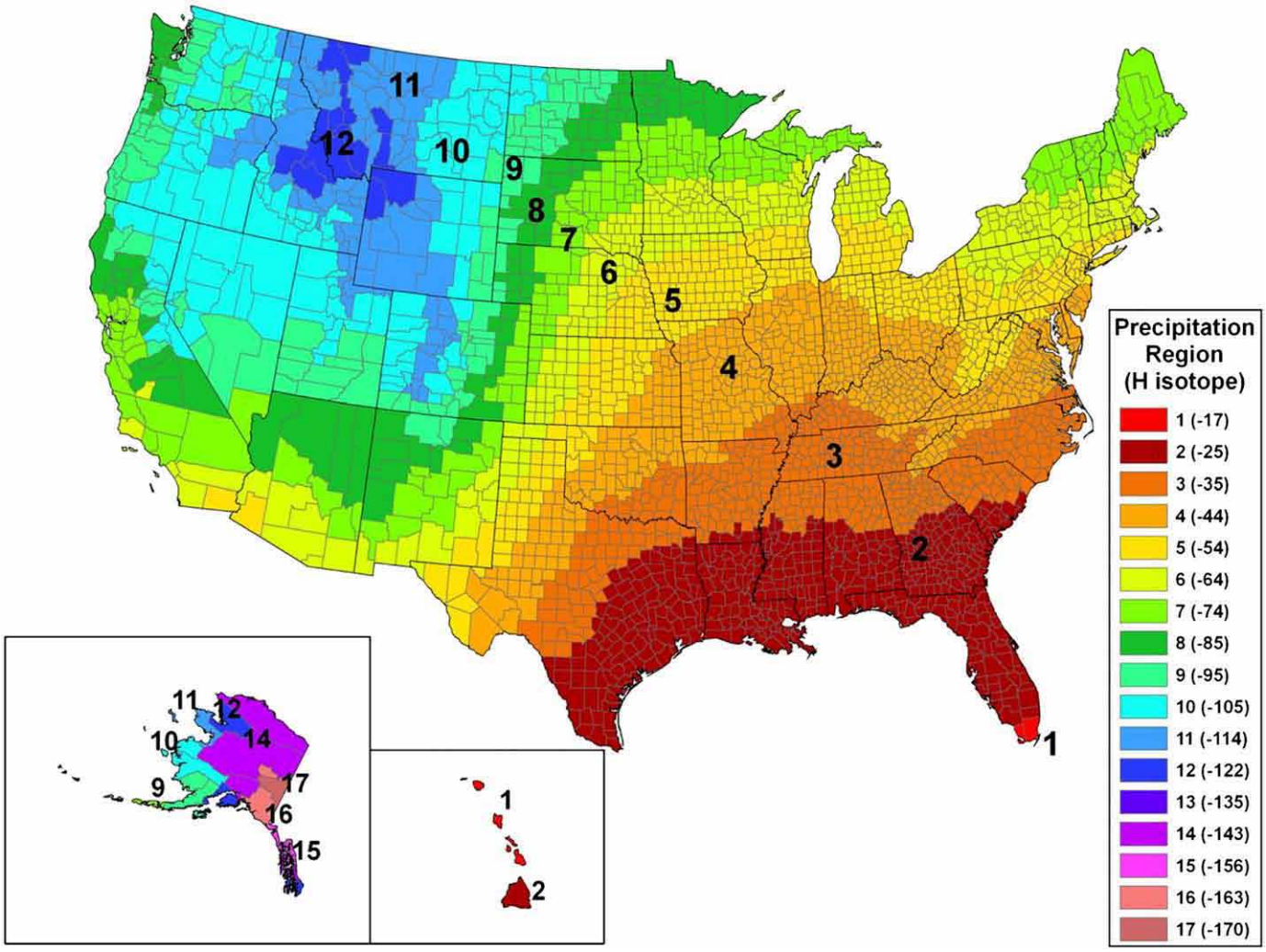

Fig. 1. Classification of counties in the United States into distinct regions based on expected mean annual hydrogen isotope ratio (δ^2H) values of precipitation (from http://www.waterisotopes.org) for those counties. Each region encompasses 10 units of precipitation hydrogen isotope ratios (δ^2H, ‰). Note that region 13 does not occur within United States borders.

The resulting mean marijuana leaf δ^2H values and standard deviations for each of these regions are shown in Fig. 2.

To assess domestic marijuana seizures of unknown origin using region-of-origin Model I, only regions occurring in the coterminous USA are included, that is Regions 1 through 12. This is because Hawaii is unlikely to be a significant marijuana source to the coterminous USA, while Alaska is largely indistinguishable from much of Canada given current data-availability and so is lumped in with assignments of Canadian origin. Furthermore, since Region 1 is represented only by two counties located in extreme southern Florida and all but the southern-most island in the Hawaiian chain, we simplified our approach by subsuming Region 1 into Region 2. Model I predicts the region-of-origin of an unknown specimen using a progression of three steps that determine the region-of-origin assignment. First, the geographic region with a mean δ^2H value that is closest to that of the unknown marijuana specimen δ^2H value is identified. The predicted region-of-origin will include this specific region. Second, the predicted region-of-origin is then broadened to include up to four additional regions through inclusion of the two regions that are immediately higher and the two regions that are immediately lower in number from that of the closest region identified in Step 1. This expansion of the predicted region-of-origin for a specimen of unknown origin is based on the observed δ^2H variability of eradication specimens seized within each region that potentially overlap with adjacent regions (see Fig. 2). Only regions in the coterminous USA are selected in Step 2. If the region identified in Step 1 is lower than Region 4 or higher than Region 10, the total number of regions included in the predicted regions-of-origin may be as low as three. Third, we determine if the unknown specimen could have originated from outside the USA but within North America. If the specimen's δ^2H value is 1.2 standard deviations lower than the mean for Region 12 or 1.2 standard higher than the mean for Region 1, then the unknown specimen is predicted to have originated from north of the coterminous USA border (Canada) or south of the coterminous USA border (Mexico), respectively. The predicted region-of-origin for an unknown specimen then becomes those geographic regions predicted at the end of Step 2 (if predicted to be of USA origin) or Step 3 (if predicted to be originating from Canada or Mexico). Note that an origin of Alaska would be identified as having originated from Canada, so Canada assignments are functionally Canada plus Alaska.

2.1.2. Region-of-origin Model II

A less specific model (called Region-of-origin Model II) was also developed to provide policy makers with more general predictions of marijuana source areas. Model II predicts five broad geographic regions from which a marijuana specimen could have been grown: western Canada, eastern Canada, Mexico, western USA, and eastern USA. The partition of the USA into two regions is essentially a bisection of the coterminous USA, the western border of the eastern USA including Texas, Oklahoma, Kansas, eastern Nebraska, southern Minnesota, southern Wisconsin and southern Michigan; the eastern border of the western USA includes northern Minnesota, northern Wisconsin, the Upper Peninsula of Michigan and western Nebraska (Fig. 3). Statistical analyses of USA domestic eradication and border-seizure specimens (summarized in Table 1, and reported in [13]) provide the basis for this model. The mean δ^2H values for marijuana grown within these 5 regions (or presumed to have been grown, in the case of Canada and Mexico, based on USA border seizures) can be used as filters to constrain predictions of region-of-origin for a marijuana specimen. The model relies on both an individual measurement of the δ^2H value of a marijuana specimen and the seizure location of that specimen to make a region-of-origin prediction (Fig. 4). As with Model I, the use of δ^2H values in Model II capitalizes on the known geographic gradients in δ^2H values of source water that are recorded into the organic molecules of marijuana via an as-yet-undefined mechanistic way.

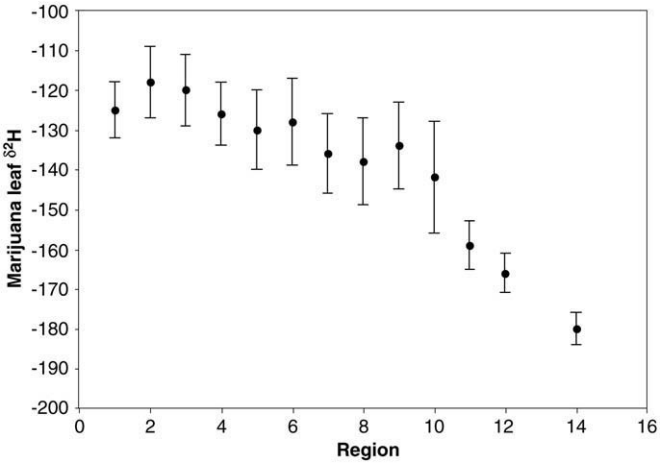

Fig. 2. The relationship between the different regions from Fig. 1 based on differences in mean modeled hydrogen isotope ratio (δ^2H) values for different USA counties, and the mean marijuana leaf δ^2H values of eradication specimens from known counties-of-origin within those regions. Shown are regional means with error lines indicating one standard deviation.

With the region-of-origin Model II approach, knowledge of the seizure location is required because there are two pairs of geographic regions that are indistinguishable on the basis of the region-wide average marijuana δ^2H values alone: (1) δ^2H values of marijuana grown in the western USA are statistically indistinguishable from δ^2H values of marijuana seized along the north eastern USA border and presumed to have been grown in eastern Canada, and (2) δ^2H values of marijuana grown in the eastern USA are statistically indistinguishable from δ^2H values of marijuana seized along the southwest USA border and presumed to have been grown in Mexico [13]. To accommodate this challenge, we assume that when the isotope signature of a seized marijuana specimen matches two geographic areas, the closer area is more likely to be the source. That is, since eastern Canada is closer to eastern states than western states of the USA, a marijuana specimen seized in the eastern USA is more likely to have originated in eastern Canada if it has a δ^2H value consistent with both eastern Canada and the western USA. This is especially evident when, according to DEA eradication statistics, most marijuana grown in the western USA is grown in the far west—California, Washington, and Arizona [21]. Similarly, because Mexico is closer to many western states than most eastern states of the USA, if a marijuana specimen is seized in the western USA it is more likely to have originated in Mexico if it has a δ^2H value consistent with both Mexico and the eastern USA. Again, DEA eradication statistics indicate that most eradicated eastern USA marijuana originates from Kentucky and Tennessee [21]. One consequence of this Model II approach is the exclusion of two possibilities: (1) marijuana seized in the eastern USA that in fact originated from Mexico will not be identified as such and (2) marijuana seized in the western USA that in fact originated from eastern Canada will not be identified as such. As models and data-availability improve, this limitation may be alleviated. However, it is expected that only a small percentage of seizure samples would fall into these two categories and therefore cause potential difficulties in correctly assigning a region-of-origin.

2.1.3. Cultivation model

The cultivation model uses carbon isotope ratio measurements to predict whether a marijuana seizure was from an indoor-, shade- or indoor-, or outdoor-grown crop, and is described in detail elsewhere [14]. Theoretical and published empirical observations of plant carbon isotope ratios serve to define the thresholds illustrated in Fig. 5, and are supported by our observations of eradicated marijuana specimens and law enforcement assignments of whether they were indoor- or outdoor-grown [14].

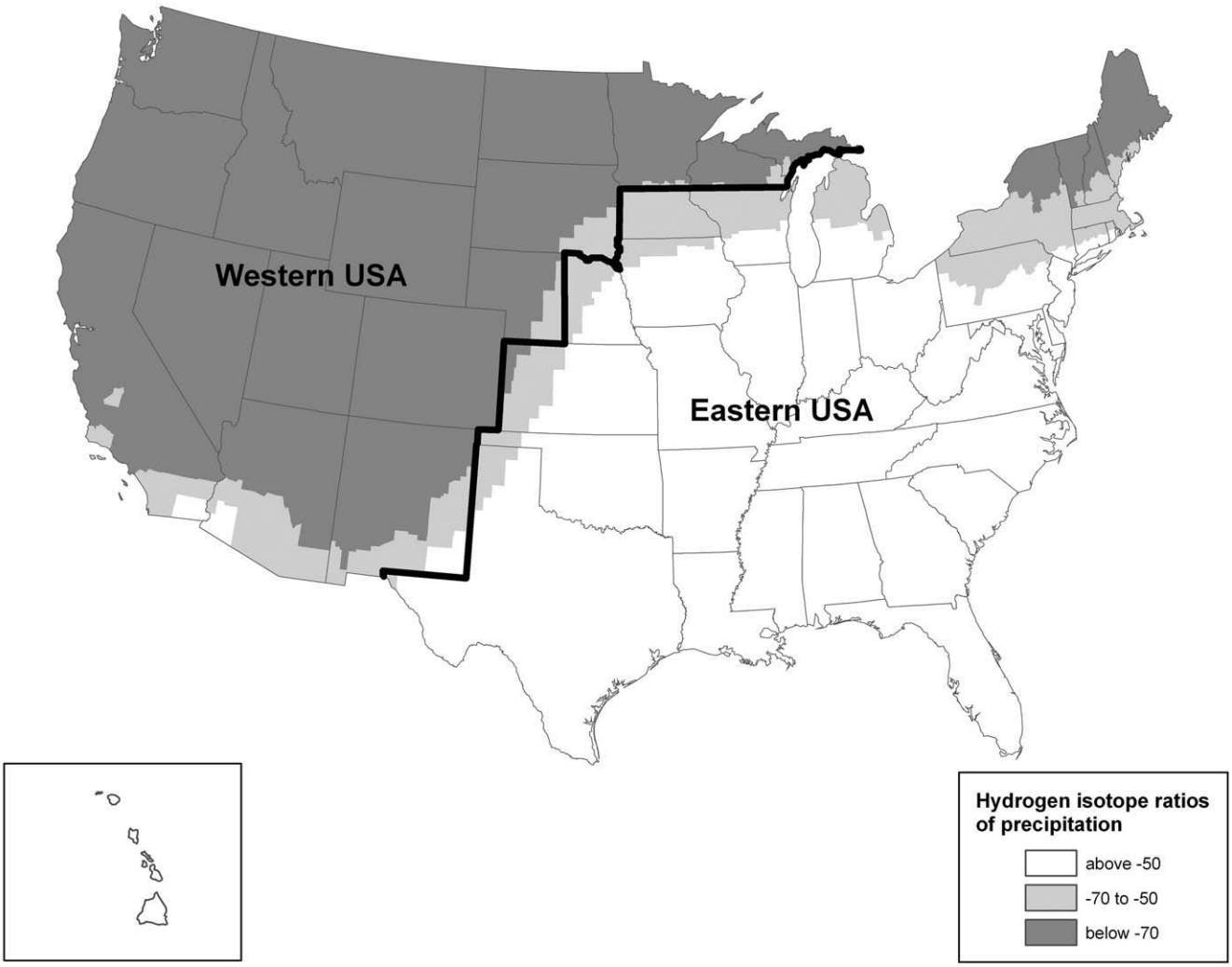

Fig. 3. Partition of the coterminous United States into Western USA and Eastern USA for region-of-origin Model II. The black demarcation line considers state boundaries, useful to policy makers, and modeled patterns of precipitation hydrogen isotope ratio (δ^2H) values that are reflected in marijuana leaf δ^2H values. For the purposes of this model, Hawaii is included in the Eastern USA and Alaska is indistinguishable from Canada.

2.2. Model verification

To evaluate the two region-of-origin models and the cultivation model, 60 marijuana eradication specimens were analyzed (provided by M. ElSohly, University of Mississippi, average specimen size 15 g), for which geographic origin and growth environment initially were not revealed to analysts. Model testing against these specimens that were not used to produce the models, but had known geographic origins and growth environments, provided an initial evaluation of the efficacy of the models in making these assignments. These "blind" specimens were eradication specimens seized between October 2005 and September 2007 from 49 counties across the coterminous USA and two counties in Hawaii.

Table 1
Mean hydrogen isotope ratio (δ^2H) and mean carbon isotope ratio (δ^{13}C) values, for border seizures and domestically eradicated marijuana. Standard deviations and number of specimen observations in a geographic group (n) are also given.

Geographic group	n	δ^2H (‰)	δ^{13}C (‰)
Northwest Border (Western Canada)	30	-160 ± 16	-35.1 ± 6.1
Northeast Border (Eastern Canada)	79	-141 ± 17	-31.2 ± 5.5
Southwest Border (Mexico)	86	-129 ± 12	-28.2 ± 1.3
Western USA	209	-137 ± 14	-28.6 ± 3.4
Eastern USA	360	-125 ± 10	-29.1 ± 2.9

2.2.1. Stable isotope measurements

Stable isotope abundances are represented in delta notation (δ), in which the stable isotope abundance is expressed relative to an internationally recognized standard in parts per thousand (denoted as ‰), i.e., Eq. (1):

$$\delta = \left(\frac{R\text{sample}}{R\text{standard}} - 1\right) \times 1000 \quad (1)$$

where R is the molar ratio of the heavy to light isotopes, e.g., Eq. (2):

$$R = \frac{^2H}{^1H} or \frac{^{13}C}{^{12}C} \quad (2)$$

Stable isotopic compositions of internationally recognized standards have a δ value of 0‰.

2.2.2. Sample analysis

For all 60 specimens we isolated leaf material and analyzed leaf-only fractions. Generally, we pulverized approximately 50–200 mg of dried marijuana sample with mortar and pestle, filtering and grinding residual large particles by passing ground material through 250 μm stainless steel sieves until the complete sample was ground and homogenized. Because a percentage of hydrogen atoms (6 to 13%,

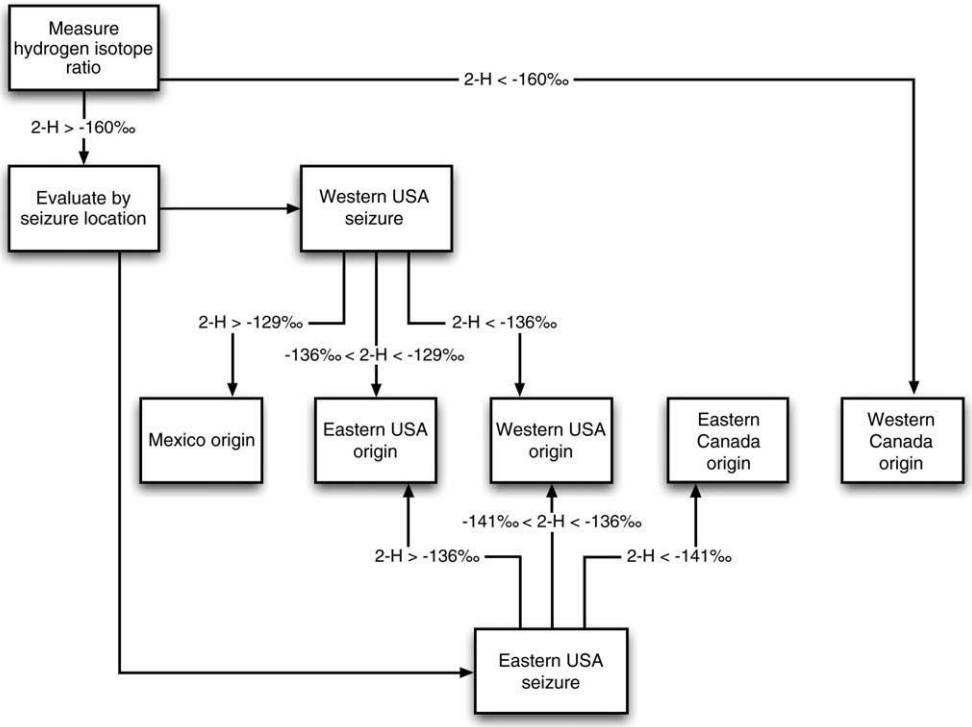

Fig. 4. Flowchart for region-of-origin Model II based on both a hydrogen isotope ratio (δ^2H) value assessment and a seizure location assessment to identify a marijuana specimen as having been grown in one of five different geographic regions in North America. The arrows reveal the δ^2H values used to filter a marijuana specimen into one of the five regions.

unpublished data) in a marijuana specimen are potentially exchangeable with hydrogen atoms in atmospheric water vapor, we allowed powdered marijuana samples to equilibrate to local atmosphere for three days before analysis of hydrogen isotopes. Any resulting exchange effect should then be consistent across specimens [22]. After equilibration, we loaded 170 ± 17 μg of ground material into silver capsules (Costech, 3.5×5 mm, pre-combusted at 500 °C for 15 or more minutes), and then analyzed the marijuana samples in duplicate alongside dry cellulose reference material (also exposed to the same atmosphere and exchange conditions of the marijuana samples) on a thermal conversion elemental analyzer coupled to an isotope ratio mass spectrometer (TC/EA-IRMS, Finnigan Delta Plus XL or Finnigan Delta V) for hydrogen isotope analysis. Precision of the isotope ratio mass spectrometers, based on multiple analyses of the cellulose reference material, was ± 2‰ for δ^2H values. We re-analyzed marijuana specimens with replicate δ^2H standard deviations of >5‰ (those greater than approximately the 99th percentile of all standard deviations of duplicate runs). For all specimens analyzed more than twice, values greater than one standard deviation from the mean of all specimen replicates were omitted from the reported mean specimen value.

For analysis of carbon isotope ratios, we loaded 1.5 ± 0.15 mg of sample into tin capsules (Costech, 3.5×5 mm), and then analyzed the marijuana samples in duplicate on an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Finnigan Delta Plus).

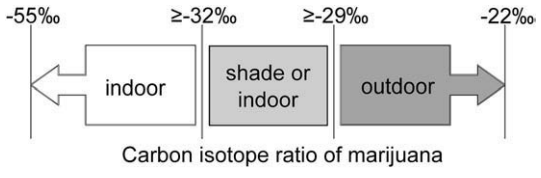

Fig. 5. Assignment domains based on the carbon isotope ratio (δ^{13}C) values of marijuana to indicate indoor and outdoor growth environment conditions. The threshold δ^{13}C values are determined from theoretical expectations, published plant observations, and empirical evidence from marijuana eradication specimens [14].

Samples were corrected to an internal reference material (yeast) calibrated to the PDB standard (belemnite carbonate standard from the PeeDee Formation, SC). Overall precision based on multiple analyses of the yeast reference material was ± 0.1‰ for δ^{13}C values. We re-analyzed marijuana specimens with replicate δ^{13}C standard deviations >0.4‰ (those greater than approximately the 99th percentile of all standard deviations of duplicate runs). For all specimens analyzed more than twice, values greater than one standard deviation from the mean of all specimen replicates were omitted from the reported mean specimen value.

3. Results

3.1. Region-of-origin Model I

Region-of-origin Model I correctly predicted regions-of-origin for 36 of the 60 (60%) of the blind-eradication specimens. Incorrect assignments were made for 24 of the 60 (40%) blind specimens. Incorrect assignments are summarized in Table 2. Four specimens (7% of the total blind specimens) were incorrectly assigned to regions with δ^2H values lower than the mean δ^2H values from the actual regions-of-origin, while 14 specimens (23% of the total blind specimens) were incorrectly assigned to regions with δ^2H values higher

Table 2
Categories of incorrect region-of-origin assignments of blind specimens using Model I. The percentage of total blind specimens falling into each category is provided.

	Percentage
Incorrect assignment because δ^2H values in US region overlap completely with δ^2H values in adjacent Canada region	5%
Incorrect assignment because δ^2H values in US region overlap completely with δ^2H values in adjacent Mexico region	5%
Incorrect assignment with δ^2H value lower than predicted for source region	7%
Incorrect assignment with δ^2H value higher than predicted for source region	23%

than the mean δ^2H values from the actual regions-of-origin. Three specimens (5% of the total blind specimens) identified as originating from north-of-the-border were actually from Regions 10, 11 and 12 all in the northern border regions of Montana, which exhibit source water δ^2H values indistinguishable from those of areas within western Canada. Three specimens (5% of the total blind specimens) identified as south-of-the-border were actually from areas of Regions 2, 3 and 4, all of which extend into Mexico. Thus, although all blind specimens were of domestic origin, 10% of them were misallocated to Canada or Mexico, which share similar precipitation δ^2H values with adjacent areas in the USA. If we were to have precluded these cross-border assignments and rather assigned these specimens to the USA regions with mean marijuana leaf δ^2H values closest to those for the specimens plus the adjacent 2 regions, 42 specimens (70%) would have been correctly assigned. Therefore, the model could be more accurate if used in combination with additional information on likely origins.

3.2. Region-of-origin Model II

Region-of-origin Model II correctly predicted the regional origins of 40 (67%) of the blind specimens. The model predicted that 28 of 36 specimens (78%) actually from the eastern USA would have come from that geographic region. Similarly, the model predicted 12 of 24 specimens (50%) actually from the western USA would have come from that geographic region. Incorrect assignments for 20 of the blind specimens are summarized in Table 3. For the eight blind specimens from the eastern USA that were misidentified as having originated elsewhere, two (3% of the total blind specimens) were misidentified as western USA specimens. In contrast, of the 12 western USA specimens that were incorrectly categorized, three (5% of the total blind specimens) were categorized as having originated from the eastern USA. All of the western specimens misidentified as originating from the eastern USA or Mexico originated from coastal counties adjacent to the Pacific Ocean. Ten of the misallocated specimens misidentified as Canadian-derived (17% of the total blind specimens) were from regions of the USA that have δ^2H values identical to adjacent areas in Canada. Five of the misallocated specimens identified as Mexican-derived (8% of the total blind specimens) were from regions in the USA that have δ^2H values identical to adjacent areas Mexico. Given that all of the blind specimens were domestically grown, if we were to ignore the Canadian and Mexican categories, we would have identified 24 specimens (40%) as originating in the western USA and 36 (60%) as originating in the eastern USA based on the −136‰ filter between eastern and western USA regions (see Fig. 4). If it were known that all specimens were cultivated within the coterminous USA, then assignment to Mexico or Canada would have been precluded and 44 specimens (73% of the total blind specimens) would have been correctly assigned. This clearly indicates that the model is most powerful if additional information about likely sources can be obtained.

Table 3
Categories of incorrect region-of-origin assignments of blind specimens using Model II. The percentage of total blind specimens falling into each category is provided.

	Percentage
Incorrect assignment because δ^2H values in western USA region overlap completely with δ^2H values in adjacent Canada region	7%
Incorrect assignment with eastern USA seizure identified as having western USA origin	3%
Incorrect assignment with eastern USA seizure identified as having eastern Canada origin	10%
Incorrect assignment with western USA seizure identified as having eastern USA origin	5%
Incorrect assignment with coastal western USA seizure identified as having Mexico origin	8%

3.3. Cultivation model

Assignments of likely indoor-, likely shade- or indoor-grown, or likely outdoor-grown were made for all blind specimens based on carbon stable isotope ratio measurements and the thresholds shown in Fig. 5. Nine of the 60 blind specimens had carbon isotope ratios (δ^{13}C) consistent with indoor cultivation, 13 had δ^{13}C values consistent with either shade growth or indoor cultivation, and the remaining specimens had values that indicated outdoor growth environments. For three of the 60 blind specimens, growth environment was unknown. For the remaining 57 specimens, growth environment assessments were 86% correct based on DEA-recorded growth environment as either indoor- or outdoor-grown. Table 4 shows the basis of incorrect assignments. Eight specimens (14% of the total blind specimens for which growth environment was known) were assessed incorrectly in terms of indoor or outdoor growth, and all but one of these specimens (12% of the total blind specimens for which growth environment was known) were reportedly indoor-grown plants with δ^{13}C values above −29‰ assessed as outdoor-grown. The one outdoor-grown specimen (2% of the total blind specimens for which growth environment was known) identified as indoor-grown fell just below the shade- or indoor-grown threshold of −32‰.

4. Discussion

4.1. Marijuana Region-of-origin Modelling

Previous efforts by Shibuya and colleagues [9–11] showed that it was possible to partially differentiate among regions-of-origin for marijuana grown in Brazil based on observed differences in carbon and nitrogen isotope ratios. In these Brazilian studies, three of the five major production regions of Brazil exhibited different realms in a carbon–nitrogen isotope ratio plot [9,10]. The inclusion of elemental abundances did not further clarify regions-of-origin beyond the patterns detected by carbon and nitrogen stable isotope ratio analyses [11]. In contrast, Ehleringer et al. [12] were unable to distinguish region-of-origin for marijuana eradication seizures across the USA based on carbon and nitrogen isotope ratios, because the variation in carbon isotope ratios associated with contrasting growth regimes (sun, shade, indoors, bottled-CO_2 indoors) in any one region overwhelmed any subtle differences in carbon isotope ratio to be expected with differences in humidity gradients across the USA. In addition, δ^{15}N values of marijuana cultivated in the USA reflect a preponderance of synthetic nitrogen fertilizers resulting in no clear geographic pattern [12]. In contrast, the apparent reliance on available soil nitrogen in Brazilian marijuana production systems results in a region-specific nitrogen isotope ratio pattern [9].

Our approach to predicting marijuana regions-of-origin was to instead capitalize on naturally occurring gradients of hydrogen isotopes in source water across landscapes [5,17,18]. We expected regional differences in hydrogen isotope ratios to be reflected in the marijuana specimens and built two contrasting models that related δ^2H values in marijuana specimens to geographic regions-of-origin. Assignment of individual specimens selected randomly from our

Table 4
Categories of incorrect growth environment assignments of blind specimens. The percentage of total blind specimens with known growth environments falling into each category is provided.

	Percentage
Incorrect assignment of indoor-grown specimen identified as outdoor-grown	12%
Incorrect assignment of outdoor-grown specimen identified as indoor-grown	2%

marijuana eradication dataset yielded estimated reliabilities of 61–69%, suggesting potential strength in this modelling approach [13]. The blind verification tests reported here support this initial conclusion, with the region-of-origin models correctly assigning the true region-of-origin for 60–73% of the blind specimens. While the Model I approach de-emphasizes cross-border trafficking and the Model II approach places an increased emphasis on foreign sourcing of marijuana seized within the USA, both models had equivalent predictabilities with the 60 blind specimens analyzed if we assume that the specimens could have originated from any location within North America.

The strengths of these region-of-origin models are likely based on environmental gradients in hydrogen isotopes of source waters that are reflected in region-specific average marijuana leaf δ^2H values. It is important to note, however, that there are appreciable overlaps in the expected δ^2H values of source water among the regions defined in Models I and II. Thus, the model predictions are statistical in nature and do not reflect absolutely distinct and non-overlapping differences between regions. Neither region-of-origin model provides much insight into the basis for the incorrect assignments, although these incorrect assignments did appear to be related to the overlapping δ^2H values of marijuana leaves among different geographic regions. For example, some of the eastern USA marijuana specimens (20% of blind specimens for Model I, and 13% of blind specimens for Model II) had δ^2H values more similar to specimens originating from eastern Canada or the western USA. Here it appears a single δ^2H value filter for geographic assignment is insufficient to capture the breadth of δ^2H values that appear in eastern USA eradication specimens. On the other hand, incorrect assignments of western USA specimens by both models seem to be concentrated among marijuana specimens having been cultivated along the Pacific coast, an area of concentrated marijuana cultivation and large isotopic variations in source water.

Models are diagnostic and predictive tools that can improve with more and better quality data. The region-of-origin models presented here represent the first available models with sufficient reliability to be used for USA marijuana trafficking studies. Models I and II have quite promising accuracies of 60–73%, which is strong given the reliance on a single stable isotope ratio measurement and the reliance on limited geographic information describing where the specimen was obtained (i.e., USA county of origin). Model II is constrained, because it does not allow every marijuana specimen to have originated from all possible combinations of geographic locations. That is, the model does not allow for the possibility of Mexican-grown marijuana to be marketed in the eastern USA or for eastern Canadian-grown marijuana to be marketed in the western USA. The National Drug Intelligence Center (NDIC) has indicated that Mexican drug trafficking organizations are expanding operations to the southeastern USA with Atlanta as a national-level distribution center for Mexican marijuana [23]. However, Model II cannot be used to test whether Mexican-grown marijuana is present in Atlanta or available in the eastern USA generally. Model II may also over-estimate the amount of Canadian-derived marijuana in interior Midwest areas (such as the St. Louis and Kansas City/Topeka areas), as marijuana seized in these areas with δ^2H values less than −141‰ will be sourced to eastern Canada rather than the western USA, even though the western USA may be a more likely source region.

These shortcomings in model predictability arise from the inability to distinguish between Mexico and the eastern USA and between eastern Canada and the western USA in terms of source water δ^2H values. Model I has the same basis and results in similar shortcomings. For example, using Model I, Mexican-derived marijuana in the Atlanta market would be identified as of eastern USA origin unless its hydrogen isotope ratio was greater than 1.2 standard deviations from the mean of marijuana from Region 1 (see Fig. 1). Based on modeled continental patterns of the δ^2H values of precipitation [17], most of Mexico exhibits values less than those of Region 1 such that marijuana from Mexico most likely would not show δ^2H values greater than the mean from Region 1. Because of this, Model I may underestimate Mexican-grown marijuana seized within the USA. Similarly, the model may underestimate the presence of northeast Canadian-grown marijuana within the USA.

Continued stable isotope analysis of border seizures and domestic eradication seizures would allow for improvements to these region-of-origin models, including distinguishing marijuana from distinct geographic areas with similar source water δ^2H values. Particularly, the availability of more specific geographic data from eradicated marijuana seizures—such as latitude, longitude and elevation measurements—and collecting information on the irrigation methods used would allow fine-tuning of region-of-origin models. In addition, stable isotope ratio analysis of individual compounds within marijuana (such as individual cannabinoids) rather than bulk preparations of the plant material may lead to less variability and thereby improve the predictability of geographic models.

4.2. Discussion of cultivation model

Ehleringer et al. [24] was among the first to quantify the relationships between carbon isotope ratio and sunlit-growth conditions, a prerequisite for distinguishing plant growth under indoor versus outdoor conditions. Denton et al. [7] described variations in δ^{13}C values in marijuana associated with growth conditions and observed patterns consistent with observations by Ehleringer et al. [12]. The Denton et al. dataset provided a foundation for the much more extensive observations by Ehleringer et al. [12] relating growth environment and marijuana eradication specimens in the USA. The cultivation model utilized here [14] defines cut-off limits to distinguish growth regimes and was very reliable in its predictions. Overall, the cultivation model had a reliability of ~86%, which is remarkably high given the lack of any control over sample acquisition and recording of growth environment data by law enforcement agencies. From our blind assessment of the cultivation model, we find that a single isotope ratio measurement can be used to reliably assess the extent to which a marijuana specimen was outdoor- versus indoor-grown.

4.3. Policy implications

Marijuana traffickers consistently respond to law enforcement activities by shifting operations to avoid detection and seizure while capitalizing on market demands for marijuana of higher potency and purity. For example, indoor production of marijuana continues to increase as growers attempt to avoid intensified outdoor eradication efforts and attain higher profits through indoor production of high-potency marijuana [23]. Furthermore, marijuana traffickers are expanding production operations to avoid heightened law enforcement pressure in traditionally high production and trafficking states, as well as to avoid higher scrutiny at USA border crossings [23]. Prior to the development of stable isotope models to predict region-of-origin and growth environment of marijuana, no independent tool existed to understand marijuana trafficking patterns in the USA and to track the effects of various policy or law enforcement actions. The continued development and improvement of stable isotope ratio analysis of marijuana will help enhance and expand its applicability to crop reduction strategies and to tracking marijuana production and distribution.

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