Report of Japanese beetle late 2016-January 2017- non-exchangeable hydrogen isotope values from CDFA interceptions in California.

Rebecca Hood-Nowotny and Andrea Watzinger. Environmental Resources & Technology, AIT Tulln, Austria.

Greg Simmons, USDA-APHIS-PPQ-CPHST.

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Methods

Fourteen Japanese beetle samples from CDFA detections in California and a reference beetle from Alabama were sent by CPHST California in Station to APHIS co-operator Rebecca Hood-Nowotny, an isotope scientist at the Austrian Institute of Technology working with APHIS to develop insect forensic tools for regulatory program analysis (table 1).

All beetle samples were collected in small plastic vials or Eppendorf tubes and dried at 60 °C or samples were stored in laboratory grade alcohol. The samples were then shipped to Tulln Austria, for isotope analysis. Samples stored in alcohol were repeatedly (X3) washed with distilled water, and dried at 60°C for at least 48 hours after washing. A single leg was sampled for the analysis using similar sample sizes, and identical appendages (e.g. a piece of front right leg) were always analysed for accurate comparison. The rationale was to take the simplest approach to sample preparation while overcoming possible contamination and accounting for the minute sample size (one leg). Quadruplicate 0.2 mg samples were cut from the same leg for hydrogen isotope analysis and were weighed into silver cups and equilibrated in a desiccator for at least forty eight hours with comparable casein standards in order to allow for the calculation of the non-exchangeable hydrogen isotope values. Samples and standards were prepared for introduction into the mass spectrometer using a high temperature elemental analyser system (HT/EA) (Thermo Fisher Bremen, Germany) with a glassy carbon combustion furnace, to achieve high temperature pyrolysis at 1430 °C. The carrier gas was helium and peaks were introduced in continuous flow mode into the isotope ratio mass spectrometer (Delta Plus, Thermo Finnigan Bremen, Germany). A full complement of internal secondary standards (in-house water standards measured against VSMOW and VSLAP) and external primary international standards (NBS22 and an EU-casein) were ran with the samples to calculate delta values and the non-exchangeable hydrogen.

The isotope values were expressed as parts per thousand or per-mil (‰) or δ deviation from the internationally recognized standards, V-SMOW (Vienna- Standard Mean OceanWater) (Gröning, 2004) and non-exchangeable hydrogen was reported on a V-SMOW scale based on casein corrected values. This comparative equilibration method was used to normalize all results. In all organic materials, there is a fixed proportion of the hydrogen ions that are exchangeable with ambient hydrogen. To account for this background and to calculate the non-exchangeable hydrogen, we used an internationally accepted standard casein for correction which has an internationally agreed value and known proportion of exchangeable hydrogen. The standard deviation of the repeated analyses of the internal standard was consistently < 3.0 ‰.

We used a regression analysis based on Hungate et al., 2016 to extrapolate back the predicted precipitation hydrogen isotope value based on the non-exchangeable hydrogen values of the beetles we collected. We compared these values with long term mean annual hydrogen isotope values of tap water from the Global Network of Isotopes in Precipitation (GNIP http://www-naweb.iaea.org/napc/ih/IHS_resources_gnip.html).

We assumed that the chitin dominated structural material in the lower leg had lower rates of turnover than that of the whole body as was verified by the data of Hungate et al., (2016). Based on this information, we could be fairly confident that the signatures were reflective of the natal origin of the beetles if the beetles were fairly newly emerged. This assumption was verified based on the Alabama beetle data; the extrapolated precipitation data matched the signature for that Eastern region.

Results.

Table 1. California collected Japanese beetle samples shipped to AIT isotope laboratory for analysis. Field collected in California by CDFA (ID by A. Cline).

Date Received by CPHST	PDR Number:	contents:	Notes:	Location	Year Collected
March 2016	340P06128531	beetle	Dry	Carmichael	2014
March 2016	CE0P06379288	leg	Dry	LAX	2015
March 2016	MV1P06113557	leg	Alcohol	Carmichael	2015
March 2016	MV1P06039432	leg	Dry	Carmichael	2015
March 2016	MV1P06039433	beetle	Dry, 9433 #1	Carmichael	2015
March 2016	MV1P06039433	leg	Alcohol, 9433 #2	Carmichael	2015
March 2016	MV1P06039434	leg	Alcohol	Carmichael	2015
March 2016	MV1P06039435	leg	Dry	Carmichael	2015
March 2016	MV1P06108353	leg	Dry	Fair Oaks	2014
June 2016	MV1P06039436	leg	Alcohol	Carmichael	2016
December 2016	190P06060180	leg	Dry	Hawaiian Gardens	Aug 2016
December 2016	AH0P06298347	leg	Dry	La Palma	Sept 2016
December 2016	CE2P06185586	leg	Dry	Cerritos	July 2016
direct to AIT	Alabama	beetle	Dry	Decatur, Alabama	July 2016

Table 2. Exchangeable hydrogen value casein corrected (n=4), bracketed value =standard deviation for three samples from Southern California

Sample	**^{2}H** per-mil (‰)
CE2P06185586 (n=3), Cerritos	-57.8 (3.0)
AH0P06298347, La Palma	-65.7 (3.9)
190P0606180, Hawaiian Gardens	-66.6 (3.8)
Alabama beetle, Decatur, July 2016	-33.78 (3.4)
LAX-CEOP6379288	-48.4 (3.7)

The Cerritos sample (CE2P06185586) at -57.8 (3.02) is statistically significantly different from the La Palma and Hawaiian Gardens beetles , but it does not look like it is from Alabama (Table 2, Figure 3). The Cerritos beetle sample has a non-exchangeable hydrogen isotope values that is quite similar to another beetle from Southern California, LAX (CEOP6379288) collected in 2015 (Table 2, Figure 3).

We would need to analyze more beetles from other eastern USA states in order to categorically say this, as we are currently basing our decision on one cohort of beetles, However the Alabama beetle values are consistent with what is expected based on water from the Global Network of Isotopes in Precipitation, which shows that we were able to accurately measure the true ^{2}H signature.

So it appears the Cerritos sample from 2016 does not match with the Alabama USA reference beetles we had for analysis, but it could be from another non-California location where that signature is present. We can say it is probably not from the same place that the beetles collected from La Palma and Hawaiian Gardens. However, this does not rule out that it might have been a beetle that arrived earlier or later in the season as we know that length of time since arrival and different rainfall events can lead to differences in H isotope signatures (Hungate et al. 2016). We recommend further isotope analysis to verify and monitor the situation, and to make more comparisons with beetles from other likely eastern sources.

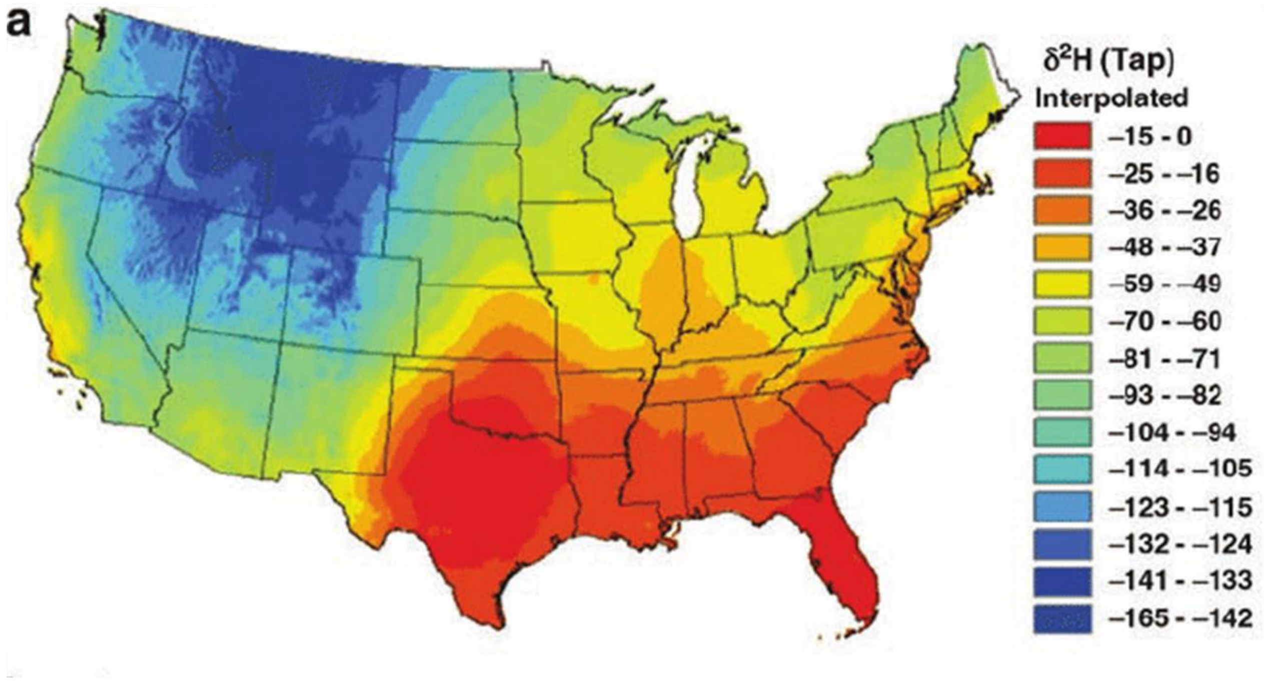


Figure 1. Maps of hydrogen isotope values of tap water which are produced based on Isoscapes (i.e. interpolated values from point sampling of tap water in particular regions).

Table 3. Extrapolated water values based on beetle values measured in Vienna and regression created by Hungate et al., 2016.

MV1P060108353	Fair Oaks	-115.091
CEOP6379288	LAX	-89.0213
MV1P06039432	Carmichael	-109.24
340P06128531	Carmichael	-109.993
MVP06113557	Carmichael	-102.029
MV1P06039433	Carmichael	-105.007
MV1P06039436	Carmichael	-108.759
Alabama	Dacatur	-73.3039
MV1P06039434	Carmichael	-106.519
MV1P06039435	Carmichael	-105.375
202439 - CE2P06185586	**Cerritos**	-99.133
202440 - AH0P06298347	**La Palma**	-107.646
202441 - 190P0606180	**Hawaiian Gardens**	-108.615

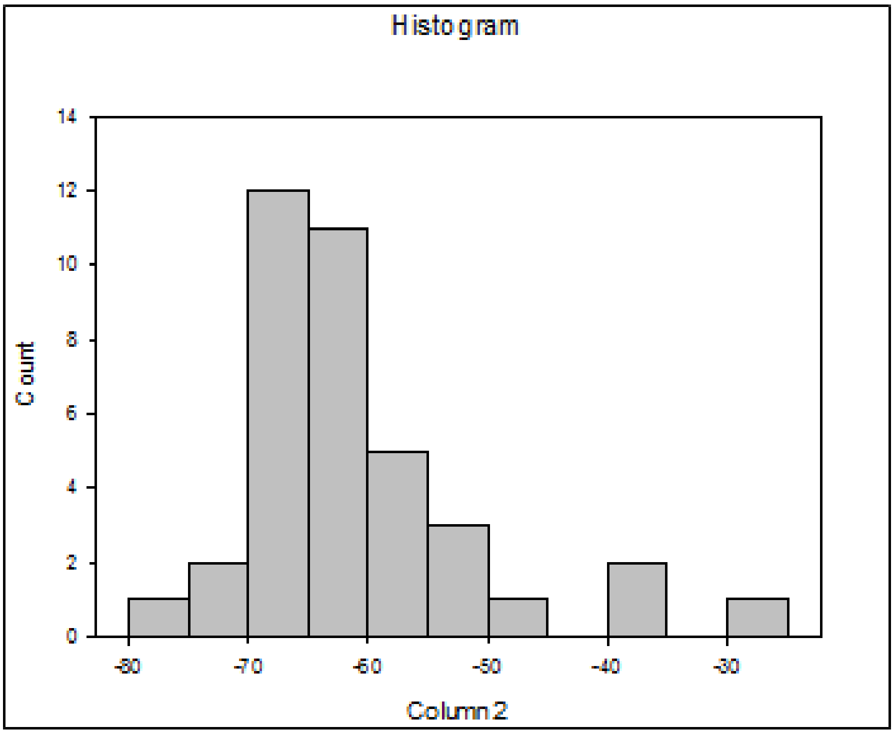
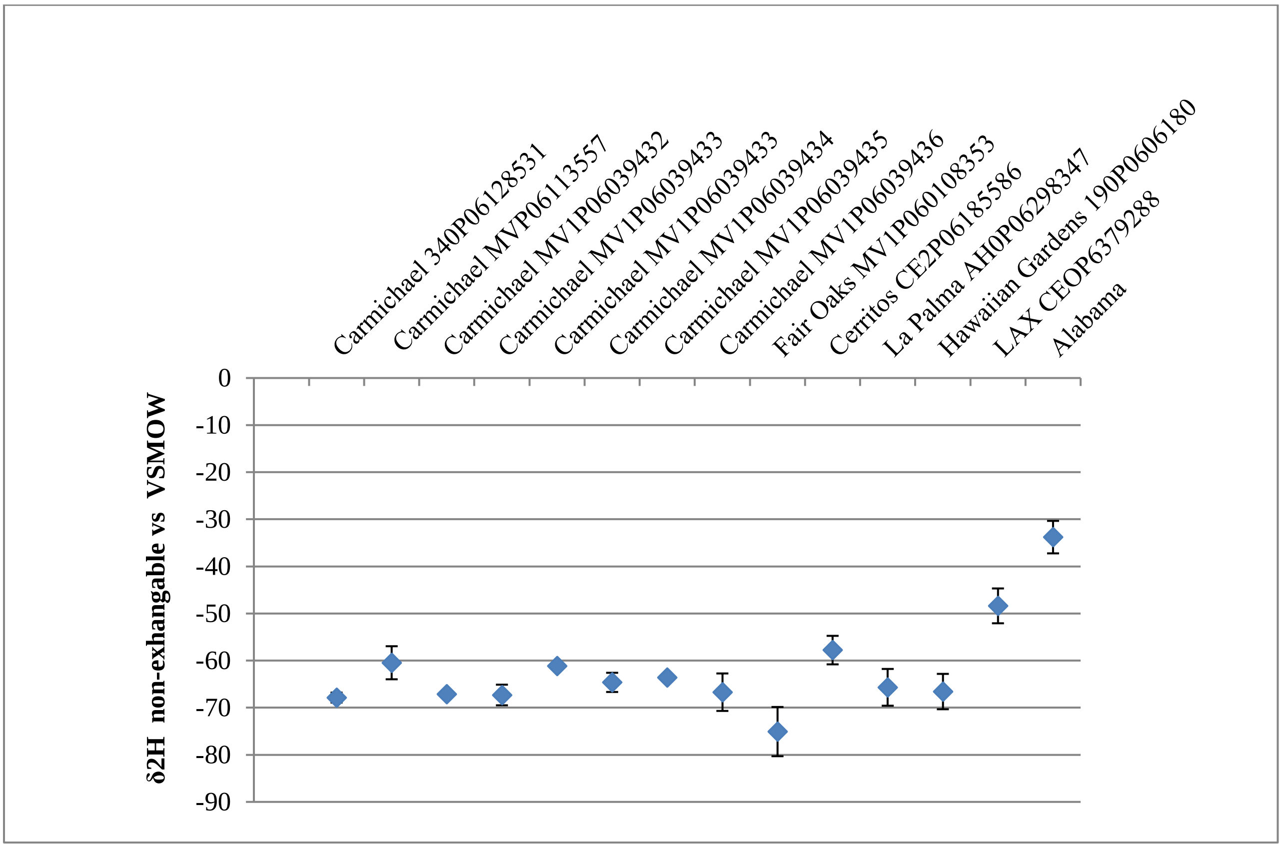


Figure 2. Histogram of all samples supplied measured to date. X-axis is non-exchangeable hydrogen isotope values.

Figure 3. Average non-exchangeable hydrogen isotope values against beetle description error bars are one standard deviation.



References

HUNGATE, B. A., KEARNS, D. N., OGLE, K., CARON, M., MARKS, J. C. & ROGG, H. W. 2016. Hydrogen Isotopes as a Sentinel of Biological Invasion by the Japanese Beetle, Popillia japonica (Newman). PLOS ONE, 11, e0149599.