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Using hydrogen stable isotope ratios to trace the geographic origin of the population of *Bactrocera dorsalis* (Diptera: Tephritidae) trapped in northern China

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Abstract

The oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), is a generalist feeder that is known to successfully feed and breed on a variety of fruits and vegetables in the tropical and subtropical zones of Asia. Recently, the northward extension of *B. dorsalis* has been reported, and this geographic range increase relates to both climate changes and the rapid development of domestic and international trade and travel. However, it has not been possible to determine the origin of this pest, which is trapped in north China. In this study, traps baited with the sex attractant methyl eugenol were used to collect *B. dorsalis* in Beijing, China. With these traps *B. dorsalis* populations were sampled at the same time in 5 different latitudes of China and used to determine the distribution of $\delta^2\text{H}$ stable isotope values. The relationship between rainwater and the *B. dorsalis* $\delta^2\text{H}$ stable isotope values at these sites was modelled, which then could be used to hypothesize the population origin of *B. dorsalis* trapped in Beijing, China. The results showed that $\delta^2\text{H}$ stable isotope values for *B. dorsalis* from Beijing were not consistent with that of the rainwater in Beijing, but rather were consistent with the results obtained from Fuzhou in southeast China. *Bactrocera dorsalis* trapped in Beijing was not a resident population, and may have come from South China. The fruit and vegetable trade may have vectored the fly northward in China. Our results also showed that $\delta^2\text{H}$ stable isotope technology is a promising strategy for tracing the population origins of invasive fruit flies.

Key Words: fruit fly; biological marker; biosecurity

Resumen

La mosca oriental de la fruta, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), es una plaga generalista que se sabe que se alimenta y se reproduce con éxito sobre una variedad de frutas y verduras en las zonas tropicales y subtropicales de Asia. Recientemente, se ha informado sobre la extensión de *B. dorsalis* hacia el norte, y este aumento geográfico se relaciona tanto con los cambios climáticos como con el rápido desarrollo de comercio y de viajes nacionales e internacionales. Sin embargo, no ha sido posible determinar el origen de esta plaga, que se considera invasiva en el norte de China. En este estudio, se utilizaron trampas cebadas con el atrayente sexual: metil eugenol para recolectar *B. dorsalis* en Beijing, China. Con estas trampas, se tomaron muestras de poblaciones de *B. dorsalis* al mismo tiempo en 5 latitudes diferentes de China y se usaron para determinar la distribución de los valores de isótopos estables $\delta^2\text{H}$. Se modeló la relación entre el agua de lluvia y los valores de isótopos estables $\delta^2\text{H}$ en *B. dorsalis* en estos sitios, que luego se podrían utilizar para establecer la hipótesis del origen de la población de *B. dorsalis* encontrada en Beijing, China. Los resultados mostraron que los valores de isótopos estables $\delta^2\text{H}$ para *B. dorsalis* de Beijing no fueron consistentes con los del agua de lluvia en Beijing, sino que fueron consistentes con los resultados obtenidos de Fuzhou en el sureste de China. Se ha observado *Bactrocera dorsalis* atrapados en Beijing no era una población residente, y puede haber venido del sur de China, desarrollando una población anholocíclica (toda hembras) en Beijing. El comercio de frutas y verduras puede haber traído la mosca hacia el norte en China. Nuestros resultados también mostraron que la tecnología de isótopos estables $\delta^2\text{H}$ es una estrategia prometedora para rastrear los orígenes de la población de organismos invasores.

Palabras Clave: mosca de la fruta; marcador biológico; bioseguridad

The oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), is a generalist feeder that has been known to successfully feed and breed on a variety of fruits and vegetables, including citrus, guava,

litchi, sugar apple, mango, pepper, and papaya (Clarke et al. 2005; Vargas et al. 2012). *Bactrocera dorsalis* is recorded mainly from the tropical and subtropical zones of Asia, but as of 2016 it had spread to

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5 continents, including more than 70 countries and 120 geographical regions (Stephens et al. 2007; Manrakhan et al. 2015). The distribution of *B. dorsalis* is still increasing through invasion of climatically suitable regions throughout the world (De Villiers et al. 2016). Larval feeding of *B. dorsalis* causes the abscission of immature fruits and vegetables, leading to major economic losses to many such crops. Adults of *B. dorsalis* live more than 3 mo in tropical regions, feed primarily on nectar and pollen, and actively fly to seek oviposition sites in fresh fruits or vegetables (Mwatawala et al. 2015).

Bactrocera dorsalis historically had only a narrow distribution in south China (south of 25° N) (Fan 1998). A climate-matching model supported the assumption that *B. dorsalis* could not survive in northern China (Fan 1998; Zhan et al. 2006), and that the area north of the Yangtze River was not suitable for the population survival and overwintering of *B. dorsalis* (Li et al. 2011). Until recently, researchers believed that *B. dorsalis* could not permanently establish in northern China.

In 2008, however, *B. dorsalis* was reported in the Wuxi district of Jiangsu province (31° north latitude), where it caused serious economic damage to citrus fruits due to a high population density (Qi et al. 2008). The domestic trade of fruits and vegetables from south China to north China likely was an important factor enabling the spread of *B. dorsalis* to new areas (Qi et al. 2008). Prior research has shown that the larvae of *B. dorsalis* can be transported in fruits and vegetables to new regions (Goergen et al. 2011; De Villiers et al. 2016). Movement of infested fruits or vegetables in the vectoring process is further supported by the collection of many adults of *B. dorsalis* found in Jun 2012 in apple and peach orchards located close to a large fruit and vegetable wholesale market in Fangshan District of Beijing (Qu & Sun 2013; Wang et al. 2016). In 2014, adult *B. dorsalis* were trapped during Jun in a vineyard (1.2633°E, 39.72°N) in Beijing. In 2015 and 2016, no larval damage or pupae were found in this orchard.

Although the population origin of *B. dorsalis* trapped in northern China has been surmised to be southern China, and based on movement of infested product, this has been surmised only, not experimentally demonstrated. However, the natural variation in a hydrogen stable isotope ratio ($\delta^2\text{H}$) is a useful tool to investigate such questions and determine the temporal-spatial dynamics of ecological pathways (Bortolotti et al. 2013; Voigt et al. 2015). Recently, stable isotope technology has been applied successfully to track the dispersal pathways of birds and insects (Rubenstein & Hobson 2004; Forbes & Gratton 2011). The principle of the technique is that the stable isotope composition found in the tissues of an organism is set by the introduction of H isotopes through its diet, which in turn reflects the signature of the micro-environment in which the organism has grown (Solomon et al. 2009). The stable isotope ratio of $\delta^2\text{H}$ precipitation varies geographically and this ratio is determined by local geophysical and chemical cycles (Wang et al. 2009; Wu et al. 2016). The $\delta^2\text{H}$ stable isotope ratio forms a continuous gradient in China from south to north and from the coast inland (Voigt et al. 2015; Deng et al. 2016). In addition, the $\delta^2\text{H}$ stable isotope has a reliable relationship within ecosystems from low to high trophic levels. The $\delta^2\text{H}$ stable isotope composition of an organism therefore provides a signature of the organism's natal environment through its diet (Holder et al. 2014; Susilawati et al. 2016; Peng et al. 2016).

Stable isotope ($\delta^2\text{H}$) relationships have been used to determine the origin of various insect populations, including *Danaus plexippus* Linnaeus (Lepidoptera: Nymphalidae), *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and *Arhopalus fesus* (Mulsant) (Coleoptera: Cerambycidae), and the results have demonstrated that a stable isotope ratio can be used as a biogeographical location marker (Dawadi & Lugtenburg 2013; Holder et al. 2014, 2015).

We hypothesized that *B. dorsalis* populations were repeated entries in the early season each year, and did not colonize in the area of Beijing because there were no detections in winter. In the present study, $\delta^2\text{H}$ isotope analyses were used to determine the population origin of the first generation of trapped *B. dorsalis* adults collected in Jun 2014 and 2015 in northern China (Beijing).

Materials and Methods

SAMPLE COLLECTION IN NORTHERN CHINA

Bactrocera dorsalis collections were carried out in the Fangshan district of Beijing, North China, and the surrounding rural areas in Jun 2014 and 2015. Adults were attracted to traps with an attractant (methyl eugenol) in an organic Bolongbao grape vineyard (1.2663°E, 39.72°N) using the random 5-point collection method of Zhao et al. (2015) with at least 10 m between sampling points. *Bactrocera dorsalis* was trapped in early Jun to collect the first generation of *B. dorsalis* populations in that year (the first arrivals into the district). Every 2 wk, the traps were examined, and the sex attractant renewed. All collected *B. dorsalis* individuals were transferred to small vials with 100% alcohol. Although adults were collected in traps, field surveys of fruit did not result in any collections of eggs or larvae in fruit from the field investigation.

Also, we collected samples of *B. dorsalis* adults from a nearby fruit and vegetable market of Xinfadi (1.2723°E, 39.82°N) using the same trapping method. All collected adult samples were taken back to the laboratory and stored at -20°C for further $\delta^2\text{H}$ determination (see below for method).

COLLECTION AND HANDLING OF SAMPLES FOR $\Delta^2\text{H}$ STABLE ISOTOPE ANALYSIS

Bactrocera dorsalis adults, collected in a variety of locations in southern and central China, were placed individually into Eppendorf (EP) tubes and transferred to a drying closet at 60°C for 48 h. After flies had been allowed to dry for 48 h, samples were transferred to a mortar for grinding. To guarantee the fineness of samples, each individual *B. dorsalis* was ground for more than 10 min. The entire body of the *B. dorsalis* adult was ground, which represents the isotope ratios derived from the fruit consumed by its larva, marking its geographic origin, through its match with hydrogen isotopes in rainwater (Bortolotti et al. 2013). Ground samples were weighed using a microbalance (0.0001 g) and wrapped in silver paper for further $\delta^2\text{H}$ analysis. Samples were stored at room temperature for 2 to 3 d to equilibrate before examination by spectrometer. Finally, an isotope ratio mass spectrometer (Thermo Scientific MAT 253, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) was used to examine the $\delta^2\text{H}$ stable isotope of *B. dorsalis* samples through the differences of neutron number in hydrogen.

SAMPLE COLLECTION IN SOUTHERN AND CENTRAL CHINA

We collected *B. dorsalis* in southern and central China, in Haikou, Guangzhou, Fuzhou, Wuhan, and Yixing, using the same trapping methods as described above for northern China. These location samples represent an anticipated latitudinal gradient of $\delta^2\text{H}$ stable isotope values. All sampling sites were located with a Geographical Position System (GPS) and their elevation recorded at the same time.

We obtained the precipitation $\delta^2\text{H}$ stable isotope values for each sample location from Waterisotopes.org of the Online Isotopes in Pre-

precipitation Calculator (OIPC), which is provided by the International Atomic Energy Association (IAEA) and the World Meteorological Organization (WMO). This database includes the global net precipitation station and provides the water $\delta^2\text{H}$ values of stable isotopes (Yann et al. 2013).

Using the laboratory method described above, we determined the $\delta^2\text{H}$ stable isotope values of the flies collected from each location in southern and central China. Those data were then used to construct a standard curve equation of $\delta^2\text{H}$ stable isotopes relating values in *B. dorsalis* to those of geographical locations.

STATISTICAL ANALYSIS

The relative abundance of the $\delta^2\text{H}$ stable isotope (heavy vs light H) in each *B. dorsalis* fly relative to the international calibration standard, Vienna Standard Mean Ocean Water (VSMOW) (Tanaka & Nakamura 2013), was determined using the equation below:

$$\delta^2\text{H} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where $\delta^2\text{H}$ is the ratio of heavy hydrogen element isotopic (deuterium) to the light hydrogen element stable isotope (protium), which is an international standard of isotope measurement. R_{sample} is the ratio of heavier to lighter hydrogen element isotopes in the *B. dorsalis* sample, and R_{standard} is the hydrogen element isotopic ratio of VSMOW.

Stable isotope values of $\delta^2\text{H}$ stable isotopes (i.e., precipitation and *B. dorsalis*) were examined using a Gaussian distribution. ANOVA was used to compare the differences of the $\delta^2\text{H}$ stable isotope for *B. dorsalis* among different geographical locations (Duncan's method). Then, the relationship between the $\delta^2\text{H}$ stable isotopes from trapped *B. dorsalis* in the 5 aforementioned geographical locations and the local water was then established using a simple linear regression model, which was a standard curve with a linear equation for calculating the theoretical $\delta^2\text{H}$ stable isotopes. The ANOVA also was used to examine the differences of the $\delta^2\text{H}$ stable isotope values for *B. dorsalis* between Xinfadi market and Bolongbao grape vineyard (Duncan's multiple range test). The theoretical values of the *B. dorsalis* $\delta^2\text{H}$ stable isotope then could be obtained through the equation of standard curve at a given water $\delta^2\text{H}$ stable isotope value. We also calculated the diet $\delta^2\text{H}$ stable isotope values of *B. dorsalis* trapped in Beijing by using the equation. All statistical analysis was performed using R 3.4.1. (R Development Core Team 2016).

Results

The $\delta^2\text{H}$ stable isotope values of *B. dorsalis* samples decreased from south to north, which was consistent with the relationship between water and the $\delta^2\text{H}$ stable isotope values. The $\delta^2\text{H}$ stable isotope value of *B. dorsalis* was the highest (-80.3 ± 3.78) in Haikou and lowest (-93.5 ± 4.34) in Yixing (Table 1). Additionally, the $\delta^2\text{H}$ stable isotope values of *B. dorsalis* among 5 geographical locations had significant differences (Table 1).

The $\delta^2\text{H}$ value of water in Guangzhou for 2015 to 2016 was -38 , based on the monthly average precipitation), which corresponded to a $\delta^2\text{H}$ value for flies of -82.3 ± 3.86 , empirically derived for *B. dorsalis* trapped in that location. Based on the 5 geographical locations analyzed, the relationship between the $\delta^2\text{H}$ stable isotope values for *B. dorsalis* and water can be described well by a simple linear regression ($y = 2.8268x + 23.745$; $r = 0.8169$; $P_{1,24} < 0.001$; Fig. 1). This indicates a strong relationship between $\delta^2\text{H}$ in the precipitation and in the local fruit flies, with the flies being less enriched with the heavy isotope of deuterium.

Based on this standard curve and given precipitation in this northern area, the theoretical values of $\delta^2\text{H}$ stable isotope in the *B. dorsalis* from the Fangshan district of Beijing, north China, should be -130.85 . However, the $\delta^2\text{H}$ stable isotope of *B. dorsalis* in the Xinfadi market in Beijing actually ranged from -81.6 to -93.9 , which is significantly higher, more enriched for the heavy isotope of deuterium, than that of the theoretical fly values ($F_{1,9} = 8.98$; $P < 0.001$; Table 2). Similarly, the $\delta^2\text{H}$ stable isotope values for *B. dorsalis* in Bolongbao grape vineyard in Beijing ranged from -81.6 to -90.3 , which also were significantly higher than the theoretical values of -130.85 for that latitude ($F_{1,9} = 9.74$; $P < 0.001$; Table 2). Furthermore, there were no differences of the $\delta^2\text{H}$ stable isotope values for *B. dorsalis* between Xinfadi market and Bolongbao grape vineyard ($F_{1,9} = 0.23$; $P = 0.64$; Table 2).

Conversely, the $\delta^2\text{H}$ stable isotope value of *B. dorsalis* from the Xinfadi market in Beijing (-88.14 ± 4.71) was not statistically different from the water $\delta^2\text{H}$ stable isotope of Fuzhou (-87.8 ± 4.85), in southern China ($F_{1,9} = 0.46$; $P = 0.51$; Table 1). In the Xinfadi market (the origin of flies from imported fruit), the $\delta^2\text{H}$ stable isotope value of *B. dorsalis* was -87.17 ± 3.66 , which also was consistent with some locations (Fuzhou of Fujian province) in southern China, according to the standard curve equation ($F_{1,9} = 0.34$; $P = 0.57$; Table 2; Fig. 1). This suggests that the *B. dorsalis* trapped in the Xinfadi market and Bolongbao grape vineyard in Beijing likely originated from further south, within the known geographic range for this species.

Discussion

Our research showed that $\delta^2\text{H}$ stable isotope technology could be used to determine the likely population origin of *B. dorsalis*. Previous research also has found that some migratory insects could be traced to their population origins using stable isotope technology (Brattstrom et al. 2010). In plant quarantine especially, population tracing of the emergent species and invasive species could be achieved by determining the $\delta^2\text{H}$ stable isotope composition during commodity trading (Holder et al. 2015).

The stable isotopes were used to determine the population origins of *B. dorsalis*, and such information could be valuable for detecting alternative hosts of invasive species or invasive pathways on populations outside their original environment (Voigt et al. 2015; Adams et al. 2016). The survival of larval *B. dorsalis* on hosts such as mango, sugar apple, and lychee, may lead to individuals being transferred to

Table 1. The $\delta^2\text{H}$ stable isotope values of *Bactrocera dorsalis* populations for five geographical locations in China.

Places (Regions)	Longitude (East)	Latitude (North)	$\delta^2\text{H}$ of <i>B. dorsalis</i> (measured values)	$\delta^2\text{H}$ of water (theoretical values)
Hainan province (Haikou, $n = 5$)	110.20	20.04	$-80.3 \pm 3.78a$	-37
Guangdong province (Guangzhou, $n = 5$)	113.27	23.13	$-82.3 \pm 3.86b$	-38
Fujian province (Fuzhou, $n = 5$)	119.30	26.07	$-87.8 \pm 4.85c$	-39
Hubei province (Wuhan, $n = 5$)	114.30	30.59	$-91.3 \pm 0.42d$	-40
Jiangsu province (Yixing, $n = 5$)	119.82	31.34	$-93.5 \pm 4.34e$	-42

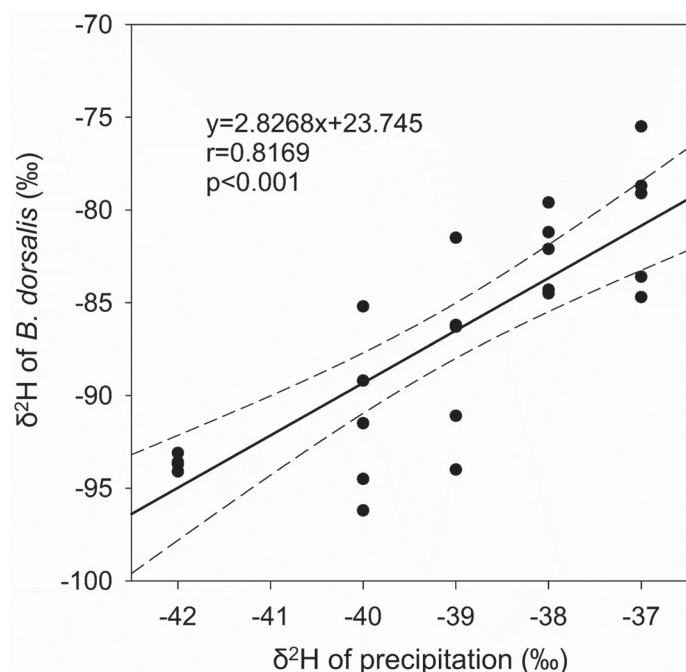


Fig. 1. Implied relationship standard curve equation between *Bactrocera dorsalis* and precipitation based on a $\delta^2\text{H}$ stable isotope (solid line indicates the linear regression and dash lines indicate the 95% confident intervals).

new environments. The stable isotopes or elemental markers could be used to determine the host species of *B. dorsalis* when the emergent generation of *B. dorsalis* was trapped in the field (Rubenstein & Hobson 2004; Holder et al. 2014).

Geologically driven $\delta^2\text{H}$ stable isotopes reflect the geochemical cycle characteristics of the origin point and type of climate (precipitation) as intrinsic markers (Holder et al. 2014). Because $\delta^2\text{H}$ stable isotopes could be applied to determine the population origin of emergent species and invasive species, stable isotope technology has great potential for use in plant quarantine (Holder et al. 2015).

In this study, the $\delta^2\text{H}$ stable isotope ratio of sampled *B. dorsalis* trapped in Beijing was higher than the theoretical values. This inconsistency revealed that the *B. dorsalis* population came from south China or another country at a similar latitude to southern China where this species is widespread. The movement of infected fruits and vegetables may be the most important avenue for causing the population spread of *B. dorsalis* in China (Wang et al. 2015).

The $\delta^2\text{H}$ stable isotope also is transmitted within the food web from low trophic level species to high trophic level species at a constant fractional distillation (Dawadi & Lugtenburg 2013; Górká et al. 2017). However, the mechanism of fractionation of the $\delta^2\text{H}$ stable isotope in

the precipitation-host-fruit fly relationship is not clear (Bortolotti et al. 2013), and the transmitting mechanism of the $\delta^2\text{H}$ stable isotope in the food web of the ecosystem is an unexplored field for future work (Weber et al. 2017). Other stable isotopes (S, P, and N) should also be evaluated as potential markers for population tracing because the availability of several additional markers would enhance the accuracy and validity of stable isotope technology (Murray et al. 2016).

In a terrestrial ecosystem, the $\delta^2\text{H}$ stable isotopic is one of the most important elements for tracing diets and origins due to the stable fractionation associated with plant photosynthetic pathways. Some experts have suggested that flight wings may be more suitable markers because they are largely metabolically inert after adult emergence (Holder et al. 2014). One of the major advantages of using such a technique for invasive species such as *B. dorsalis* is that this pest only feeds on one fruit throughout the larval stage on plant hosts, while the adult stage of *B. dorsalis* is relatively non-feeding, which mean that the adult signatures of H isotopes only would be derived from larval feeding, and would not be altered or masked due to adult feeding (Wang et al. 2009). Thus, the application of $\delta^2\text{H}$ stable isotopes may be a reliable technology to track population origins or original hosts if conspicuous differences exist among population isotopic signatures. Such $\delta^2\text{H}$ stable isotope technology also could be used to determine the population origins of invasive species.

Based on the research reported herein, we conclude that the first generation of *B. dorsalis* in 2014 and 2015 in Beijing is not a resident population, and may come from southern China. The fruit and vegetable trade may have vectored the fly northward in China. Further research is needed to evaluate whether combining $\delta^2\text{H}$ element stable isotope data with other element isotopic and trace element concentration profiles would be useful for determining accurate insect provenance (Nagoshi et al. 2007; Ziegler et al. 2016). Stable isotopes, including $\delta^2\text{H}$ and many other isotope elements, may be an important technology for precise population tracing in the field of biosecurity (Hobson et al. 1999; Simard et al. 2008; Hood-Nowotny et al. 2011).

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Table 2. The measured values and theoretical values of *Bactrocera dorsalis* $\delta^2\text{H}$ stable isotope from the Xinfadi market and the Bolongbao grape vineyard (the capital letters indicated the differences of *Bactrocera dorsalis* $\delta^2\text{H}$ stable isotope between measured values and theoretical values, the lower case letters indicated the differences of *Bactrocera dorsalis* $\delta^2\text{H}$ stable isotope between Xinfadi market and Bolongbao grape vineyard).

Sampling sites	$\delta^2\text{H}$ of <i>B. dorsalis</i> (Measured value)	$\delta^2\text{H}$ of <i>B. dorsalis</i> (Theoretical value)
Xinfadi market ($n = 5$)	-88.14 ± 4.71 Ba	-130.85 ± 5.18 Aa
Bolongbao grape vineyard ($n = 5$)	-87.17 ± 3.66 Ba	-130.85 ± 4.24 Aa

The theoretical values were derived from the standard curve equation.

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