

Not all isotopes make good geolocators: trophic transfer reveals contrasting geolocation potential in eastern spruce budworm

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Abstract

Stable isotope geolocation of insects assumes that environmental isotope signals are transferred predictably from host plants to consumer tissues, yet this assumption is rarely tested. Using known-origin samples from eastern Canada, we quantified the transfer of $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$, and $^{87}\text{Sr}/^{86}\text{Sr}$ from host Pinaceae foliage to *Choristoneura fumiferana* (eastern spruce budworm, SBW) with linear and mixed-effects models. $\delta^{15}\text{N}$ showed strong, near-proportional transfer ($R^2 = 0.84$) with a large but consistent trophic discrimination factor ($5.32 \pm 0.76\text{‰}$). $\delta^{34}\text{S}$ showed near-proportional transfer ($R^2 = 0.88$), minimal fractionation ($-0.43 \pm 1.15\text{‰}$), and strong regional structure. $^{87}\text{Sr}/^{86}\text{Sr}$ tracked geological variation, but individual moths were close to analytical detection limits; pooled samples recovered near-proportional transfer ($R^2 = 0.85$). $\delta^{13}\text{C}$ showed moderate coupling ($R^2 = 0.48$) with high intra-site variance, and $\delta^2\text{H}$ was weakly related to foliage ($R^2 = 0.08$), with residual scatter (8.96‰) far exceeding analytical uncertainty ($\pm 1.7\text{‰}$). $\delta^{34}\text{S}$ and $^{87}\text{Sr}/^{86}\text{Sr}$, which have well-characterized baselines across eastern Canada, are therefore strong geolocators for SBW and likely for other species in the region. $\delta^{15}\text{N}$ is transmitted with high fidelity, but its geolocation potential depends on foliar baselines that remain to be mapped. $\delta^{13}\text{C}$ and $\delta^2\text{H}$ have limited value at this scale. The weak performance of $\delta^2\text{H}$, a standard geocator, and the strong performance of $\delta^{15}\text{N}$ show that the suitability of an isotope for geolocation is not an intrinsic property of the isotope but emerges from the interaction of environmental spatial structure, trophic transfer, and organismal biology.

Introduction

Multi-isotope geolocation has become a promising tool for studying migratory insect ecology (Bowen and West 2008; Hobson 2008). Insects are notoriously difficult to track using traditional methods because they are small, short-lived, and occur in large populations, which limits our ability to forecast and manage insect-mediated benefits and threats across biodiversity conservation, agriculture, forestry, and public health (Holland et al. 2006; Chapman et al. 2015; Hu et al. 2016). Stable isotopes help overcome these limitations by providing intrinsic markers that insects acquire from their natal environment and enabling the reconstruction of movement and connectivity (Hobson 1999).

Using multiple isotopes simultaneously has advanced insect migration ecology because combining isotopes that have independent patterns of variation allows finer geolocation resolution and more precise ecological inferences on insect mobility at regional to continental scales. For example, Reich et al. used $\delta^2\text{H}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios to reveal moderate migratory connectivity of *Vanessa cardui* (painted lady butterflies) across the Afro-Palearctic, confirming a broad multigenerational parallel migration across the Sahara (Reich et al. 2024). Similarly, Dargent et al. used dual isotope analysis ($\delta^2\text{H}$ and $^{87}\text{Sr}/^{86}\text{Sr}$) to confirm spruce budworm immigration events and distinguish between local and immigrant populations over fine spatial scales (Dargent et al. 2023). However, isotopes are not equally suitable for geolocation: some are strongly structured by stable geographic variables such as precipitation (e.g., $\delta^2\text{H}$) (Bowen et al. 2005) or bedrock geology (e.g., $^{87}\text{Sr}/^{86}\text{Sr}$) (Bataille et al. 2020), whereas others are

more heavily influenced by trophic and metabolic processes that introduce biological noise and weaken spatial signals (e.g., $\delta^{13}\text{C}$) (Martínez del Río et al. 2009) .

Isotopic geolocation relies on the transfer of environmentally structured isotope compositions from substrates to the base of the food chain (e.g., water, minerals to plants) through food webs, and into organism tissues (West et al. 2010). The core principle is that ecosystems exhibit predictable isotope compositions that vary geographically according to climatic, hydrological, and geological conditions at the site of growth, and those compositions are integrated into an organism's tissues, so that it can be used to trace back its origin (West et al. 2010). Ideally, isoscapes for geolocation would be developed using a known-origin dataset (i.e., individuals with verified origin) for the focal species; however, for herbivorous insects this is often impractical and expensive, as it would require rearing individuals across their entire range (West et al. 2010). As a result, isotope baselines are typically generated from environmental substrates (e.g., local water) or primary producers directly linked to those substrates because they can be sampled broadly (Hobson et al. 1999a). For isotopic geolocation to be valid, however, the isotopic composition of these baseline substrates must be reliably related to the isotope composition of the focal organism's tissues (West et al. 2010). Establishing robust baseline-tissue transfer functions is therefore a prerequisite for interpreting geographic origin in insect isotope studies.

In herbivorous insects, spatial isotope patterns are expected to transfer from host plants into insect tissues, allowing direct comparisons between tissue isotopes and environmental isoscapes to infer natal origin (Hobson et al. 1999b, 2019). Tissues with slow turnover, such as chitinous wings, preserve the isotopic composition of the environment in which they formed and are therefore commonly used to determine the natal origin of individuals, particularly after long-distance dispersal (Lindroos et al. 2023). However, during assimilation and incorporation of plants into herbivore tissues, isotopic fractionation produces predictable but non-identical isotope values between consumers and their diets (DeNiro and Epstein 1978, 1981). These offsets, known as trophic discrimination factors (TDFs), arise from trophic metabolic processes such as differential routing of dietary macromolecules or preferential excretion of lighter isotopes (Minagawa and Wada 1984). Additional differences can arise because isotopes are measured in tissues that differ compositionally and biochemically from the baseline substrates (Gannes et al. 1997). For example, insect wings are primarily made of chitin, a long-chain structural polymer, whereas host foliage consists largely of carbohydrates, and these substrates differ in their isotopic composition prior to any metabolic fractionation (Lindroos et al. 2023). As a result, baseline-tissue differences reflect both physiological fractionation and inherent differences in the bulk chemical composition of the tissue being analyzed. TDFs vary among elements: TDFs are generally $\sim +1\text{‰}$ for carbon (DeNiro and Epstein 1978), $\sim +3.4\text{‰}$ for nitrogen (DeNiro and Epstein 1981; Minagawa and Wada 1984; McCutchan et al. 2003), and between -1.5 and 1.5‰ for sulfur (Raoult et al. 2024), although they are often difficult to properly determine due to dietary, metabolic, or ontogenic processes overprinting (McCutchan et al. 2003). While strontium isotopes fractionate during trophic processes, this mass-dependent fractionation is normalized during analysis (Bataille et al. 2020). The transmission of isotope compositions from primary producers to herbivorous insects is rarely validated empirically, introducing a

significant source of uncertainty, particularly for multi-isotope frameworks that rely on accurate and predictable baseline-tissue relationships.

Quantifying baseline-tissue uncertainty is essential for developing robust, interpretable, and biologically calibrated isoscapes (Hobson et al. 2010). The predictive performance of multi-isotope geolocation frameworks depends, in large part, on the predictable trophic fractionation leading to strong, consistent correlations between foliage and tissue isotopes (Wunder et al. 2005; Wunder and Norris 2008). The remaining uncertainty in baseline-tissue transfer is incorporated into the geolocation framework itself with poor correlation strongly decreasing the geolocation potential of the isotope. R packages such as *assignR* (Ma et al. 2020) and *IsoriX* (Courtiol et al. 2019) incorporate transfer-function uncertainty into the isoscape calibration process, propagating both environmental variation and biological error terms into probability-based geographic assignments (Wunder 2012). However, these best practices are rarely followed, and transfer functions are often derived from very limited datasets of unrelated species and applied as fixed corrections, without fully accounting for the true uncertainty in trophic transmission. In a multi-isotope context, failing to propagate this uncertainty can overweight poorly constrained isotopes, inflate apparent spatial precision, and produce erroneous geolocation.

Here, we use *Choristoneura fumiferana*, the eastern spruce budworm (SBW), as a model system to test the assumptions that isotopic compositions are transferred predictably from their host foliage, and evaluate whether trophic fractionation is sufficiently predictable for use in isotope geolocation application. SBW are the most damaging pest in the North American boreal forest and undergo recurrent outbreaks every 30–40 years, resulting in extensive defoliation of their spruce (*Picea spp.*) and balsam fir (*Abies balsamea*) hosts, and leading to major ecological and socio-economic consequences (Chang et al. 2012; MacLean 2016). SBW outbreak expansion is thought to be triggered by massive but sporadic long-distance dispersal events (> 100 km) where gravid females can seed new infestation areas (Johns et al. 2019). Isotopes are a very promising approach for tracking these long-distance dispersal events and to better manage outbreaks (Dargent et al. 2023, 2026).

In this study, we evaluate whether isotopes of hydrogen, carbon, nitrogen, sulfur, and strontium effectively trace SBW origin by measuring the strength and consistency of isotopic transmission from foliage to moth tissues. A strong, predictable correlation would indicate limited metabolic overprinting and support the use of these isotopes as geolocators. Given that SBW are host-specialist folivores that feed almost exclusively on needles of a few species without switching trees, we hypothesize that SBW tissue will reflect local foliar isotopic compositions with consistent offsets due to trophic fractionation. Suitable geolocators must meet three key requirements. First, the environmental baseline must be spatially structured at the scale of the animal range. Second, baseline signal must be transmitted from foliage to consumer tissue with a predictable and near-proportional transfer. Third, the variance introduced within a site (intra-site), by microhabitat heterogeneity, individual physiology, or analytical error, must be small relative to the isotopic contrast between sites. We evaluate each of the five isotopes to propose a hierarchy of isotopes for geolocation in eastern Canada.

Methods

The data and code supporting this study have been deposited in the Purdue University Research Repository (PURR) and are available under DOI: 10.4231/21P1-WX89

Since adult SBW are non-feeding and only live for ~ 10 days, we expect a strong correlation between the isotopic composition of SBW and their host plants. We assessed TDFs between host foliage and SBW by collecting SBW pupae from up to three adjacent trees at 19 sites in Ontario, and 12 sites in Newfoundland (Fig. 1; “known-origin” samples). The sites span a diverse range of environmental conditions which can influence the cycles of our target isotopes systems (Contina et al. 2022). Rather than rearing SBW on isotopically enriched diets in the laboratory, we prioritized field samples to estimate the uncertainty of this relationship under natural conditions. At each known-origin site, we collected needles from either the same trees or within 10 meters of the sampled SBW. In most cases, needles from multiple trees from the same site were pooled for foliar analysis. Known-origin moths in our dataset were collected in the field as pupae, and a few as late-stage L6 larvae, and allowed to eclose in paper bags in the laboratory (~ 22°C). At the collection sites we recorded the geographic coordinates and date of collection. To evaluate the degree of intra-site isotope variations, we intended to analyze three to five moths per site, yet some sites did not have enough pupae whereas others had unsuccessful eclosions or Hymenoptera parasitoidism.

Foliar sample preparation and isotope analysis

General preparation

Analyses of foliar $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$ were performed at the Jan Veizer Stable Isotope Laboratory (University of Ottawa). Dry needles were aggregated by site, rinsed twice with deionized water to remove surface particulates, and dried at 50°C for 48 h. Dried needles were then homogenized to a fine powder and stored in glassine envelopes.

Carbon and nitrogen

Carbon and nitrogen isotope analyses were run separately in August 2023 because of the very large amount of carbon complicating the nitrogen analysis. Ground foliage samples ($\text{C}\% = 48.7 \pm 1.3$; $\text{N}\% = 1.2 \pm 0.3$) were micro-weighed to a target mass of 0.60 ± 0.06 mg into tin capsules (Elemental Microanalysis). Samples were combusted in a vario EL cube elemental analyser (Elementar, Germany) via flash combustion at 1800°C°, equipped with oxidation and reduction reactors packed with copper oxide wires, silver wool, and copper reagents (Elemental Microanalysis, United Kingdom). Combustion was performed using 4.5 O₂ as the combustion gas (Messer, Germany) and 5.0 He (Messer, Germany), as the carrier gas, with gas flows and temperatures set according to manufacturer specifications. Following combustion, water was removed using a Sicapent trap (Merck, Germany) and N₂ was purified by removing CO₂ with an Ascarite trap (Merck, Germany). For $\delta^{13}\text{C}$ analyses, after N₂ was completely

evacuated from the system, the trapped CO₂ was desorbed from the Ascarite trap and analyzed independently. The resulting gases travelled sequentially via a Conflo IV interface (Thermo, Germany) to an isotope ratio mass spectrometer (IRMS, DeltaPlus Advantage, Thermo, Germany), operated using Isodat 3.0 (Thermo, Germany). Corrections for ¹⁷O contributions to CO₂ were applied using the Santrock-Studley-Hayes algorithm. Isotopic deltas were derived relative to a working gas (4.0 CO₂, 4.8 N₂, Messer, Germany) introduced using the Conflo IV and were normalized using LIMS for Light Stable Isotopes (USGS, United States).

Isotopic ratios are reported in δ notation relative to the Vienna Pee Dee Belemnite (V-PDB) scale for carbon and relative to atmospheric N₂ (AIR) for nitrogen. Values were calibrated using a three-point calibration with internal standards: C-51 Nicotiamide (Acros Organics, Belgium; $\delta^{13}\text{C}$: -22.95‰; $\delta^{15}\text{N}$: 0.07‰), C-52 mix of ammonium sulphate and sucrose (Merck, Germany; $\delta^{13}\text{C}$: -11.94‰; $\delta^{15}\text{N}$: 16.58‰), C-54 Caffeine (Alfa Aesar, United Kingdom; $\delta^{13}\text{C}$: -34.46‰; $\delta^{15}\text{N}$: -16.61‰). Those internal standards were previously calibrated against international standards IAEA-CH-6 (-10.45 ± 0.03‰), NBS-22 (-30.02 ± 0.04‰), USGS-40 (-26.39 ± 0.04‰) and USGS-41 (+ 37.63 ± 0.05‰) for $\delta^{13}\text{C}$ and IAEA-N1 (+ 0.43 ± 0.07‰), IAEA-N2 (+ 20.41 ± 0.12‰), USGS-40 (-4.52 ± 0.06‰), and USGS-41 (+ 47.57 ± 0.11‰) for $\delta^{15}\text{N}$. Analytical precision was monitored using a blind internal standard C-55 L-glutamic acid (Merck, Germany; $\delta^{13}\text{C}$: -28.5 ± 0.1‰, n = 3; $\delta^{15}\text{N}$: -3.9 ± 0.1‰, n = 2) and calibrated against the same international standard above. A full set consisting of each of the calibration and blind reference materials was analyzed at the beginning and end of the run, as well as distributed every 30 samples. Calibration and blind reference material were weighted to provide 300 ± 15 µg C and 100 ± 5 µg N. The C-55 L-glutamic acid reference material was analyzed at a range of masses (0.53–2.11 mg) in the middle of each run for linearity. For each run, the analysed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of C-55 glutamic acid fell within the range of accepted calibrated values.

Hydrogen

Bulk ground foliage samples (H% = 6.3 ± 0.3) were micro-weighed to a target mass of 0.25 ± 0.025 mg into silver capsules. Hydrogen isotopic measurements were conducted via high-temperature flash pyrolysis at 1400°C using a temperature conversion elemental analyzer (Elementar Germany) equipped with a zero blank autosampler. He flushing was conducted in the autosampler for 4h prior to any analysis to remove potential water vapor. After pyrolysis, the purified H₂ was introduced to a Thermo Fisher Delta Plus XP isotope ratio mass spectrometer (ThermoFinnigan, Germany) via a Conflo IV interface. Three-point calibration was performed using reference standards: CBS (caribou hoof; -157 ± 0.9‰), KHS (kudu horn; -35.3 ± 1.1‰), and USGS-43 (human hair; -44.4 ± 2.0‰). Analytical precision was monitored using a keratin reference standard, USGS42 (human hair; measured: -71.9 ± 1.1‰, n = 3; standard: -72.9 ± 2.2‰), and two internal standards, *Lymantria dispar* ground wings to act as a chitin matrix match (measured: -62.0 ± 1.57‰, n = 3; long-term average: -64.0 ± 0.8‰) and G-744 (keratin; measured: -58.8 ± 1.2‰, n = 18; long-term average: -60.5‰ ± 0.8‰) distributed throughout analytical runs. $\delta^2\text{H}$ values were determined

by comparative equilibration against the keratin reference materials listed above, which were equilibrated alongside the samples under identical ambient conditions. Reported $\delta^2\text{H}$ values therefore refer to the non-exchangeable hydrogen fraction. One limitation of this analytical approach is that bulk leaf tissue has a different exchangeable-H fraction than keratin but no standard currently exist for plant material. Analytical precision, assessed from repeated measurements of USGS42 and internal chitin standards, was better than $\pm 1.7\text{‰}$. All $\delta^2\text{H}$ values are reported relative to the VSMOW-SLAP international scale.

Sulfur

Sulfur isotope data used in this study were derived from the same foliar and SBW samples reported in Dargent et al. (2026). As the datasets are shared between studies, sample preparation, EA-IRMS procedures, calibration, and quality control were identical. No additional sulfur isotope analyses were performed for this study.

Strontium

Foliage samples were cleaned to remove surface dust, lipids, and contaminants by rinsing twice with deionized water followed by an ultrasonic cleaning in a deionized water bath. Samples were then dried at 50°C for 48 h°. Dried foliage samples were digested by microwave digestions using 1 mL of concentrated nitric acid (16 M; Trace Metal Grade distilled for moths; Fisher Chemical, Canada) for 15 min at 250°C using an Anton Paar Multiwave 7000 microwave system (Austria) in Organic High setting. Complete digestion was confirmed by the presence of clear, particle-free solutions. Foliage samples were transferred to 7 ml Savillex PTFE vials. For Sr concentration determination, a 200 μL aliquot of each digest was transferred to a separate Savillex vial, dried, and re-dissolved in 2,000 μL 2% v/v nitric acid with 20 ppb indium as an internal standard. Sr concentrations were measured using an Agilent 8800 triple-quadrupole inductively coupled plasma mass spectrometer (ICP-MS) at the Department of Earth and Environmental Science, University of Ottawa. Calibration standards were prepared from certified single element standards (SCP science; Montreal, Canada). The digest ($\sim 1\text{ mL}$) was dried and re-dissolved in 1 mL 6 M nitric acid for chromatographic Sr preparation. Strontium purification was performed using 100 μL microcolumns packed with Sr-spec Resin[™] (100–150 μm ; Eichrom Technologies, USA). Columns were conditioned with 0.5 mL of 6 M nitric acid prior to sample loading. Based on ICP-MS concentration results, foliage sample volumes were adjusted to achieve a target Sr load of $\sim 100\text{ ng}$. The entire moth digest was used. Matrix elements were removed using 6 M nitric acid, and Sr was subsequently eluted using 0.05 M nitric acid. Purified Sr fractions were dried and re-dissolved in 2% v/v nitric acid for isotopic analysis. For foliage, $^{87}\text{Sr}/^{86}\text{Sr}$ were measured at the MC-ICP-MS Facility, Department of Geological Sciences, University of Cape Town, using a Nu Plasma HR MC-ICP-MS. ^{87}Rb interference was corrected by monitoring ^{85}Rb and applying an established $^{87}\text{Rb}/^{85}\text{Rb}$ ratio. Kr interferences of ^{84}Sr and ^{86}Sr were corrected by subtracting contributions based on the measured On-Peak Zero in the 2% HNO_3 solution used to prepare all standards and samples for analysis. Instrumental

mass fractionation was corrected by normalizing $^{86}\text{Sr}/^{88}\text{Sr}$ to 0.1194 using the exponential law. Strontium isotope compositions are reported as $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Foliage samples were run at concentrations of ~ 200 ppb with all $^{87}\text{Sr}/^{86}\text{Sr}$ data referenced to bracketing analyses of NIST SRM987 (0.710255 ± 0.000023 ; 2 SD; (Waight et al. 2002)). The worst within-run precision was ± 0.000039 (1 SD, $n = 16$). Chitin standard (Alfa Aesar chitin) was also run 0.714016 ± 0.000038 (1 SD; $n = 10$).

Moth sample preparation and isotope analysis

Moth cleaning was done following Dargent et al. (2026). Briefly, moths were washed in beakers containing a 2:1 v/v chloroform:methanol solution to remove lipids, dust, and contaminants. Each individual moth was gently swirled a few times and submerged in the solution for 30 min, followed by subsequent transfers in fresh solution for 3 h, and 15 min. Clean moths were air dried in a class-100 fume hood and stored in glassine envelopes. For hydrogen, carbon, and nitrogen analyses we used wings, which are generally expected to exhibit limited or negligible tissue turnover (Lindroos et al. 2023). Due to extremely low sulfur concentrations in SBW moth wings ($< 0.01\%$; Dargent et al., 2026), the head and thorax were used instead for $\delta^{34}\text{S}$ analyses. Similarly, since strontium concentrations are low in SBW (Dargent et al. 2023) we used full individuals.

Analyses for $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were similar to those for needles but at lower masses ($\delta^2\text{H}$: 0.25 ± 0.025 mg; joint $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$: 0.5 ± 0.05 mg). A matrix-match standard of ground and homogenized spongy moth (*Lymantria dispar*, Linnaeus, 1758) wings was used for blind quality control (measured: $-63.5 \pm 0.8\%$, $n = 5$; long-term average = $-64 \pm 0.8\%$) for $\delta^2\text{H}$ analyses. The C-55 glutamic acid internal standard was used as blind quality control for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with $\delta^{13}\text{C} = -28.5 \pm 0.1\%$ ($n = 3$) and $\delta^{15}\text{N} = -3.9 \pm 0.1\%$ ($n = 2$). Procedures for strontium isotope analysis in moths were similar to foliar analyses. Strontium concentration in SBW tissues is very low (< 1 ppm) and required ultra-clean laboratory procedures to minimize contamination including the use of ultra-clean distilled Trace Metal Grade (Fisher Chemical, Canada) nitric acid for Teflon cleaning, sample digestion and Sr separation. A small, digested aliquot (50 μL) was sampled for Sr concentration analysis, to maximize the remaining amount of Sr for isotope analysis. The Sr concentrations were used to tune the sensitivity of the Nu-Plasma II high-resolution multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS; Nu Instruments) coupled to a desolvating nebulizer (Aridus II[™], CETAC Technologies) at the Pacific Center for Isotopic and Geochemical Research (PCIGR, University of British Columbia). At the PCIGR, standards were run in the range of moth concentrations with reproducibility of $^{87}\text{Sr}/^{86}\text{Sr}$ measurement for 5 ppb NIST SRM987 is 0.71025 ± 0.000023 (1 SD, $n = 138$) and for 1.4 ppb NIST SRM987 0.71019 ± 0.00011 (1 SD, $n = 48$). Chitin standards, 5 ppb Alfa Aesar chitin, yielded a mean $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.714073 ± 0.000128 (1 SD; $n = 10$).

For sulfur isotope preparation and analysis, the SBW samples used in this study were derived from the same individuals and preparation procedures reported in Dargent et. (2026).

Statistical analysis

To quantify trophic transmission relationships between isotope values in host foliage and moth tissue, we used a series of regression-based approaches. For each isotope system, we fitted linear regression models in the form:

$$\text{Moth}_{\text{isotope}} = \beta_0 + \beta_1 \times \text{Foliage}_{\text{isotope}} + \epsilon$$

where β_1 represents the slope (i.e., the transfer function) and β_0 represents the intercept. Because needles were in most cases pooled by site prior to analysis, most sites contribute a single foliar value against which several individual moths are compared. Foliar isotope composition is therefore effectively a site-level predictor. We therefore report simple linear regression (SLR) statistics as descriptive summaries of the foliage-consumer relationship and treat the linear mixed-effects models described below as the inferential models. In the mixed models, foliar isotope composition and the site random intercept are strongly collinear for the same reason, so we interpret the comparison as a test of whether site-level foliage explains differences among site mean moth values, and not as a test of intra-site trophic transmission. Model assumptions were evaluated using residual diagnostics. The strength and reliability of each isotope system was assessed using the coefficient of determination (R^2), regression slope and intercept values, and residual standard error. Isotopes with high R^2 , consistent slopes, and low unexplained variance were considered better geolocators.

Linear mixed-effects models (LMM) were fitted with site included as a random intercept, allowing baseline isotope values to vary among sites while maintaining a common foliage-SBW relationship across all locations. First, we fitted a null mixed-effects model containing only a random intercept for site:

$$\text{Moth}_{\text{isotope}} \sim 1 + (1|\text{site})$$

This model was fit to quantify baseline variance partitioning and assess the degree of spatial clustering in isotope values and was used to estimate intraclass correlation coefficients (ICC), providing a measure of the proportion of total SBW isotope variance attributable to inter-site variation (i.e., site-level clustering). We then incorporated foliage isotope values as a fixed effect while retaining site as a random intercept:

$$\text{Moth}_{\text{isotope}} \sim \text{Foliage}_{\text{isotope}} + (1|\text{Site})$$

This modelling framework allowed foliage isotope values to explain systematic variation in SBW isotope composition while accounting for unmeasured environmental or ecological differences among collection sites. For every isotope system we report the same three models, the simple linear regression (SLR), the null mixed model with site as a random intercept (null LMM), and the mixed model with foliage added as a fixed effect (full LMM), so that model comparisons are consistent across systems. LMMs were fitted using restricted maximum likelihood estimation (REML) to estimate parameters and report them. LMMs were then refitted using maximum likelihood (ML) to allow comparison of models with different fixed-effect structures via Akaike's Information Criterion with a correction for small sample sizes (AICc).

All statistical analyses were performed in R 4.4.1 (R Core Team 2024) using packages tidyverse (Wickham et al. 2019), ggplot2 (Wickham 2016), lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017).

Trophic Discrimination Factor

Trophic discrimination factors were calculated for each isotopic system as the difference between site-level foliar isotope value and isotope value for each individual SBW collected from the same site. Individual TDFs were calculated as:

$$\text{TDF}_{\text{individual}} = \delta \text{SBW}_{\text{individual}} - \delta \text{foliar}_{\text{site}}$$

where $\delta \text{SBW}_{\text{individual}}$ is the isotope value of an individual moth and $\delta \text{foliar}_{\text{site}}$ is the foliar isotope value for the corresponding site. Individual TDFs were then averaged within each site to obtain site-level TDFs before averaging site-level TDFs across all sampling locations to calculate the mean TDF for each isotopic system.

Results

Carbon

Carbon isotope composition ranged from -32.0‰ to -29.0‰ in foliage ($\delta^{13}\text{C}_{\text{foliar}}$), and -30.5‰ to -25.6‰ in known-origin SBW moths ($\delta^{13}\text{C}_{\text{SBW}}$). The average trophic discrimination factor (TDF) was $2.88 \pm 0.81\text{‰}$. A positive linear relationship was observed between foliar and moth $\delta^{13}\text{C}$ values ($F_{1,36} = 33.17$, $p < 0.001$, $R^2 = 0.48$, $n = 38$ moths from 7 sites). The simple linear regression model (SLR) estimated a slope of 0.96 ± 0.17 ($t = 5.76$, $p < 0.001$) and a non-significant intercept of $1.71 \pm 5.09\text{‰}$ ($t = 0.34$, $p = 0.74$), and a residual standard error of 0.94‰ , consistent with transfer of $\delta^{13}\text{C}$ from foliage to moth tissues. The null linear mixed effect model (LMM) exhibited strong among-site variation in $\delta^{13}\text{C}_{\text{SBW}}$ (ICC = 0.77, conditional $R^2 = 0.77$). Including $\delta^{13}\text{C}_{\text{foliar}}$ reduced among-site variance by 62% (from 1.64 in the null LMM to 0.63 in the full LMM, and SD 1.28‰ and 0.79‰ , respectively) and reduced the ICC to 0.56. Within-site residual variation remained qualitatively unchanged between the LMMs (from 0.491 in the null LMM to 0.492 in the full model) with residual SD of 0.7‰ . The full LMM (AICc = 101.73) outperformed both the null ($\Delta\text{AICc} = 4.73$) and SLR models ($\Delta\text{AICc} = 5.81$). This model showed a positive association between $\delta^{13}\text{C}_{\text{foliar}}$ and $\delta^{13}\text{C}_{\text{SBW}}$ (slope = 1.05 ± 0.35 , $t = 3.01$, $p = 0.03$; Fig. 2A), with $\delta^{13}\text{C}_{\text{foliar}}$ (i.e., the fixed effect) explaining 46% of the variation (marginal $R^2 = 0.46$) in $\delta^{13}\text{C}_{\text{SBW}}$, and the full model explaining 76% of the total variation (conditional $R^2 = 0.76$).

Nitrogen

$\delta^{15}\text{N}_{\text{foliar}}$ values ranged from -6.4‰ to -1.6‰ , while $\delta^{15}\text{N}_{\text{SBW}}$ values ranged from -1.5‰ to 4.1‰ . The average TDF was $5.32 \pm 0.76\text{‰}$. A strong, positive linear relationship was observed between foliar and

moth $\delta^{15}\text{N}$ values ($F_{1,36} = 183.7$, $p < 0.001$, $R^2 = 0.84$, $n = 38$ moths from 7 sites). The SLR estimated a slope of 1.05 ± 0.08 ($t = 13.55$, $p < 0.001$) with an intercept of $5.57 \pm 0.33\text{‰}$ ($t = 16.79$, $p < 0.001$), and a residual standard error of 0.79‰ , indicating proportional transfer of $\delta^{15}\text{N}$ from foliage to moth tissues. The null LMM exhibited substantial among-site variation in $\delta^{15}\text{N}_{\text{SBW}}$ (ICC = 0.97, conditional $R^2 = 0.97$). Inclusion of $\delta^{15}\text{N}_{\text{foliar}}$ as a fixed effect reduced among-site variance by 85% (from 4.26 in the null LMM to 0.65 in the full LMM, and SD 2.06‰ and 0.81‰ , respectively) and reduced ICC to 0.83. As with $\delta^{13}\text{C}$, within-site residual variation remained the same between the LMMs (both 0.129) with a relatively small residual SD of 0.34‰ . The full LMM (AICc = 59.57) outperformed both the null ($\Delta\text{AICc} = 11.71$) and SLR models ($\Delta\text{AICc} = 34.73$). This model showed a strong positive association between $\delta^{15}\text{N}_{\text{foliar}}$ and $\delta^{15}\text{N}_{\text{SBW}}$ (slope = 1.07 ± 0.19 , $t = 5.75$, $p < 0.01$; Fig. 2B), with $\delta^{15}\text{N}_{\text{foliar}}$ explaining 81% of the variation (marginal $R^2 = 0.81$) in $\delta^{15}\text{N}_{\text{SBW}}$, and the full model explaining 97% of the total variation (conditional $R^2 = 0.97$).

Hydrogen

SBW moths ($\delta^2\text{H}_{\text{SBW}}$ range -130.0‰ to -79.0‰) and their corresponding foliage samples ($\delta^2\text{H}_{\text{foliar}}$ range -156.0‰ to -112.5‰) showed a significant but very weak positive linear relationship ($F_{1,124} = 10.16$, $p < 0.01$, $R^2 = 0.076$, $n = 126$ moths from 19 sites). The SLR estimated a slope of 0.21 ± 0.07 ($t = 3.19$, $p < 0.01$) with an intercept of $-70.99 \pm 8.74\text{‰}$ ($t = -8.12$, $p < 0.001$), and a residual standard error of 8.96‰ . The null LMM exhibited some among-site variation in $\delta^2\text{H}_{\text{SBW}}$ (ICC = 0.26, conditional $R^2 = 0.26$). Inclusion of $\delta^2\text{H}_{\text{foliar}}$ as a fixed effect reduced among-site variance by 24% (from 22.22 in the null LMM to 16.83 in the full LMM, and SD 4.71‰ and 4.1‰ , respectively) and reduced ICC to 0.21. Within-site residual variation was similar and relatively high between the LMMs (64.38 and 64.2) and thus had a relatively large residual SD of $\sim 8\text{‰}$. The full LMM (AICc = 907.29) outperformed both the null ($\Delta\text{AICc} = 2.79$) and SLR models ($\Delta\text{AICc} = 6.9$). This model also had a weak positive association between $\delta^2\text{H}_{\text{foliar}}$ and $\delta^2\text{H}_{\text{SBW}}$ (slope = 0.22 ± 0.1 , $t = 2.24$, $p < 0.04$; Fig. 3A), with $\delta^2\text{H}_{\text{foliar}}$ explaining only 0.8% of the variation (marginal $R^2 = 0.08$) in $\delta^2\text{H}_{\text{SBW}}$, and the full model explaining 27% of the total variation (conditional $R^2 = 0.27$). Given the large degree of within-site variation in $\delta^2\text{H}_{\text{SBW}}$ we aggregated SBW individual values to site averages and explored whether it improved the fit of foliar-to-moth $\delta^2\text{H}$ relationship. This approach is not feasible for our LMMs since the number of sites would equal the number of observations. Aggregating the data improved model fit ($F_{1,18} = 5.62$, $p = 0.03$, $R^2 = 0.24$, $p = 0.03$, 20 moth averages from 20 sites) but explained less variation in $\delta^2\text{H}$ than the full LMM. Despite this improvement in the SLR fit, the slope remained the same and far from 1 indicating strong attenuation of $\delta^2\text{H}$ transmission.

Sulfur

As in Dargent et al. (2026) a strong positive linear relationship was observed between $\delta^{34}\text{S}_{\text{foliar}}$ and $\delta^{34}\text{S}_{\text{SBW}}$ ($F_{1,72} = 517$, $p < 0.001$, $R^2 = 0.88$, $n = 74$ moths from 23 sites), with SLR regression yielding a slope of 0.94 ± 0.04 ($t = 22.74$, $p < 0.001$), an intercept not significantly different from 0 ($-0.07 \pm 0.25\text{‰}$, $t = -0.27$, $p = 0.79$), and a residual standard error of 1.21‰ , consistent with proportional isotopic transfer. The average TDF was $-0.43 \pm 1.15\text{‰}$. The null LMM showed strong among-site variation in $\delta^{34}\text{S}_{\text{SBW}}$ (ICC = 0.96, conditional $R^2 = 0.96$). The inclusion of $\delta^{34}\text{S}_{\text{foliar}}$ as a fixed effect reduced model variance by 92% (from 13.5 in the null LMM to 1.11 in the full LMM, and SD 3.67‰ and 1.05‰ , respectively) and reduced ICC to 0.68. Within-site residual variation remained the same in both LMMs (both 0.51) with residual SD of 0.71‰ . The full LMM (AICc = 213.55) outperformed both the SLR ($\Delta\text{AICc} = 28.5$) and null models ($\Delta\text{AICc} = 53.2$). This model shows qualitatively the same foliar to SBW relationship as the SLR, that is, a strong, positive and near proportional association between $\delta^{34}\text{S}_{\text{foliar}}$ and $\delta^{34}\text{S}_{\text{SBW}}$ (slope = 0.93 ± 0.06 , $t = 14.48$, $p = 0.03$; intercept not significantly different from 0; Fig. 3B), with $\delta^{34}\text{S}_{\text{foliar}}$ explaining 86% of the variation (marginal $R^2 = 0.86$) in $\delta^{34}\text{S}_{\text{SBW}}$, and the full model explaining 96% of the total variation (conditional $R^2 = 0.96$).

Strontium

$^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ ranged from 0.710630 to 0.729022, whereas $^{87}\text{Sr}/^{86}\text{Sr}_{\text{SBW}}$ values ranged from 0.708252 to 0.723825. We first analysed individual moth $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Although we expected to see no fractionation between foliage and moths (see Discussion), the average TDF offset between foliar, and moth was at -0.00033 ± 0.00231 . SLR regression showed a positive relationship between foliar and SBW moth $^{87}\text{Sr}/^{86}\text{Sr}$ ratios ($F_{1,56} = 82.64$, $p < 0.001$, $R^2 = 0.60$, $n = 58$ moths from 18 sites). The simple linear regression model (SLR) estimated a slope of 0.60 ± 0.07 ($t = 9.09$, $p < 0.001$), an intercept of 0.28 ± 0.05 ($t = 5.99$, $p < 0.001$), and a residual standard error of 0.002, consistent with partial isotopic transfer from foliage to SBW tissues (Fig. 3C). The null linear mixed effect model (LMM) exhibited strong among-site variation in $^{87}\text{Sr}/^{86}\text{Sr}_{\text{SBW}}$ (ICC = 0.62, conditional $R^2 = 0.62$). Including $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ (marginal $R^2 = 0.58$) reduced among-site variance by 92% (from 7.16×10^{-6} in the null LMM to 6.04×10^{-7} in the full LMM, and SD 2.68×10^{-3} and 7.77×10^{-4} , respectively), reduced the ICC to 0.12, but explained a similar amount of variance (conditional $R^2 = 0.63$). Within-site residual variation remained qualitatively unchanged between the LMMs (from 4.35×10^{-6} in the null LMM to 4.33×10^{-6} in the full model) with residual SD of 2.08×10^{-3} . Nonetheless, the SLR (AICc = -540.33) outperformed both the full ($\Delta\text{AICc} = 1.88$) and null LMM models ($\Delta\text{AICc} = 26.97$). To evaluate whether low sample mass or analytical precision influenced these results, pooled SBW samples were prepared by amalgamating multiple known-origin moths per site into a single homogenous sample. Pooled $^{87}\text{Sr}/^{86}\text{Sr}$ ratios ranged from 0.709985 to 0.718336, while foliage ratios for the same set of sites ranged from 0.709819 to 0.717943. Pooled samples exhibited a stronger, near proportional relationship between $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ and $^{87}\text{Sr}/^{86}\text{Sr}_{\text{SBW}}$ ($F_{1,10} = 58.24$, $p < 0.001$, $R^2 = 0.85$, $n = 12$ pooled-moth samples from 12 sites). This SLR estimated a slope of 1.25 ± 0.16 ($t = 7.63$, $p < 0.001$), an intercept not significantly different from 0 (-0.18

± 0.12 , $t = -1.52$, $p = 0.16$), and a residual standard error of 0.001 (Fig. 3C). An ANCOVA showed that approach for strontium extraction (single vs. pooled moths) significantly influenced the detected relationship between foliar and SBW $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (interaction term: $t(66) = -2.44$, $p = 0.017$). The pooled-moth samples had a significantly steeper slope than the individual SBW data, which is better aligned with $^{87}\text{Sr}/^{86}\text{Sr}$ trophic transfer expectations. Visual inspection of residuals from both SLR models against a proxy of available strontium in a sample (i.e., ^{88}Sr intensity) indicates that individual SBW samples contain lower concentrations of strontium, and that lower ^{88}Sr intensity was associated with higher error in the SLR models.

Discussion

Carbon

Across heterogeneous environmental conditions, $\delta^{13}\text{C}_{\text{SBW}}$ exhibited a positive but moderate relationship with $\delta^{13}\text{C}_{\text{foliar}}$, indicating variable trophic transmission of carbon isotope signals from host foliage to SBW tissues. Although the relationship was statistically significant, approximately a quarter of the variance in $\delta^{13}\text{C}_{\text{SBW}}$ remained unexplained. Site accounted for a significant component of variation, and inclusion of $\delta^{13}\text{C}_{\text{foliar}}$ as a fixed effect significantly improved model performance. Nevertheless, substantial intra-site and inter-site variability remained, limiting the utility of $\delta^{13}\text{C}$ for provenance applications at the spatial scale examined here.

This variability is likely driven in part by the inherent sensitivity of $\delta^{13}\text{C}_{\text{foliar}}$ to fine-scale environmental heterogeneity. Foliar $\delta^{13}\text{C}$ integrates photosynthetic discrimination processes governed by the ratio of intercellular to atmospheric CO_2 , which responds to stomatal conductance and carboxylation capacity (Farquhar et al. 1989; Diefendorf et al. 2010). Consequently, water availability, vapour pressure deficit, precipitation seasonality, irradiance, canopy microclimate, and edaphic conditions can all influence foliar $\delta^{13}\text{C}$ values (Farquhar et al. 1989; Diefendorf et al. 2010). Many of these processes operate at microhabitat scales and can generate approximately 1‰ variation within stands and even among branches of the same tree. Additional heterogeneity may arise from within-canopy gradients in atmospheric CO_2 concentration and isotopic composition generated by canopy photosynthesis and inputs of isotopically light CO_2 from soil respiration (Farquhar et al. 1989). These processes can produce substantial local variation in $\delta^{13}\text{C}_{\text{foliar}}$, weakening the assumption of site-level baseline homogeneity required for isotope-based geographic assignment.

At the consumer level, SBW development is expected to introduce additional variance through ontogenetic shifts in carbon allocation and fractionation. $\delta^{13}\text{C}$ in insect tissues can shift during growth, starvation, and metamorphosis due to preferential catabolism of ^{13}C -depleted lipid reserves and changes in assimilation and respiration (Doi et al. 2007). These processes can generate within-population $\delta^{13}\text{C}$ differences of $\sim 1\text{‰}$ (Doi et al. 2007), which are consistent with the intra-site variability

we observed in SBW and the high TDF (mean TDF of $2.88 \pm 0.81\%$) relative to other Lepidoptera raised in the laboratory (Quinby et al. 2020). Larval feeding conditions in the field are likely heterogeneous, and thus $\delta^{13}\text{C}_{\text{SBW}}$ may reflect variation in both foliar baseline signals and individual physiological state, reducing geographic specificity. This variability is consistent with evidence that $\delta^{13}\text{C}$ trophic discrimination is less predictable than for other isotope systems and is strongly influenced by metabolic routing and macronutrient composition rather than trophic position alone (McCutchan et al. 2003).

Although foliar $\delta^{13}\text{C}$ exhibits broad climatic gradients and site explained a significant component of variation in our LMM, substantial intra-site variability, variable trophic transmission, and additional physiological fractionation at the consumer level reduced the geographic specificity of the signal. Consequently, despite the presence of broad spatial structure, $\delta^{13}\text{C}$ does not provide sufficient resolution for reliable standalone geolocation of individual SBW across the spatial extent examined here.

Nitrogen

$\delta^{15}\text{N}_{\text{foliar}}$ explained most of the variation observed in $\delta^{15}\text{N}_{\text{SBW}}$ and reduced unexplained inter-site variance by 85% showing a near proportional relationship. These findings indicate that spatial variation in $\delta^{15}\text{N}$ was primarily driven by differences among sites and that these differences were largely captured by $\delta^{15}\text{N}_{\text{foliar}}$. The strong performance of both the LMM model, and even the SLR, demonstrates that geographic variation in host-tree isotopic baselines is transferred predictably to SBW tissues. This result is consistent with the literature showing that plant $\delta^{15}\text{N}$ reflects ecosystem-level nitrogen cycling processes that vary across climatic, edaphic, and biogeochemical gradients (Amundson et al. 2003). Consequently, where geographically structured foliar $\delta^{15}\text{N}$ baselines occur, as reported across many terrestrial ecosystems and supported by our limited $\delta^{15}\text{N}_{\text{foliar}}$, these patterns should be expressed predictably in $\delta^{15}\text{N}_{\text{SBW}}$, fulfilling the requirements for isotope geolocation. The strong agreement between foliar and moth $\delta^{15}\text{N}$ values suggests that uncertainty associated with $\delta^{15}\text{N}$ geolocation will arise primarily from modeling the foliar isotope landscape rather than from the trophic transfer itself.

This is surprising considering that the TDF observed between moths and their host trees exceeds commonly reported values of approximately 2–4‰ for herbivorous vertebrates (Minagawa and Wada 1984) and insects (Quinby et al. 2020). Although elevated, this value falls within the upper range reported for Lepidoptera reared in laboratory studies using artificial diets (Tibbets et al. 2008). The consistency of this TDF across environmentally distinct sites suggests that it is largely governed by stable physiological processes rather than local differences in diet quality or environmental conditions affecting nutritional stress and elevating consumer $\delta^{15}\text{N}$ through increased protein catabolism (McCutchan et al. 2003).

Several non-exclusive mechanisms may contribute to the elevated TDF observed in SBW. First, metamorphosis in holometabolous insects involves extensive tissue reconstruction and nitrogenous waste production, processes that can substantially alter isotopic composition (Tibbets et al. 2008). During pupation, isotopically depleted exuviae and meconium are expelled, enriching remaining tissues

in ^{15}N and producing isotopic shifts of up to 5‰ enrichment in other insects (Doi et al. 2007; Tibbets et al. 2008). Second, temperature may indirectly affect fractionation through its influence on metabolic rate, protein turnover, and nitrogen excretion pathways. Increased growth and protein cycling enhance deamination and preferential loss of ^{14}N , thereby enriching tissues in ^{15}N (DeNiro and Epstein 1981). Consistent with this mechanism, *Spodoptera littoralis* exhibits a $\delta^{15}\text{N}$ increase of approximately 1.5‰ under rapid growth conditions (Charberet et al. 2023). Whatever its cause, this large TDF did not weaken the strong foliar-moth relationship observed in our study, indicating that it is both predictable and readily accommodated within a transfer-function for isotope geolocation.

Although the transfer function identified here is highly promising, several steps are required before $\delta^{15}\text{N}$ can become an effective geolocator. First, broader geographic sampling is needed across the SBW range to characterize the magnitude and spatial structure of $\delta^{15}\text{N}_{\text{foliar}}$ (Amundson et al. 2003; Craine et al. 2009; Souza-Silva et al. 2026). Second, the effects of physiology and nutritional stress on $\delta^{15}\text{N}_{\text{SBW}}$ should be quantified more explicitly. Adult SBW are short lived and do not feed following emergence, relying entirely on nutrient reserves acquired during larval development. Consequently, catabolism during adulthood or migration could theoretically modify isotopic composition (Doi et al. 2007). Experimental studies examining nutritional stress could potentially identify isotopic changes associated with catabolism which are likely to occur in migratory individuals. Third, the temporal stability of foliar and moth $\delta^{15}\text{N}$ values should be evaluated across years and outbreak phases. Although nitrogen cycling in boreal forests is generally conservative and tightly regulated (Amundson et al. 2003), climatic variability and severe SBW outbreaks may alter local nitrogen dynamics through enhanced nutrient recycling from frass and insect carcasses (Christenson et al. 2002). Quantifying the magnitude of these temporal effects will be necessary to determine how much year-to-year variation contributes to geolocation uncertainty.

Nitrogen isotopes are rarely considered as geolocators because mixed diets and physiological variability frequently obscure geographically structured isotopic signals. In host-specialist insects such as SBW, however, the combination of a highly restricted larval diet, limited intra-site isotopic variability, and the absence of adult feeding following emergence creates unusually favorable conditions for $\delta^{15}\text{N}$ geolocation. Our results demonstrate that $\delta^{15}\text{N}$ signals are transferred predictably from host foliage to adult moths, retain strong site-level structure, and exhibit a highly consistent trophic discrimination factor across diverse environmental conditions. These findings suggest that $\delta^{15}\text{N}$ may represent an underappreciated candidate for reconstructing the mobility of SBW across eastern Canada. The remaining task is to characterize the spatial and temporal structure of foliar $\delta^{15}\text{N}$ baselines across the full geographic range of the species.

Hydrogen

$\delta^2\text{H}_{\text{SBW}}$ was only weakly related to $\delta^2\text{H}_{\text{foliar}}$ ($R^2 = 0.08$), and within-site residual variation ($\text{SD} \sim 8\text{‰}$) greatly exceeded analytical uncertainty. Three factors likely contribute. First, the range of $\delta^2\text{H}_{\text{foliar}}$ among

our sites ($\sim 44\%$) is small relative to continental precipitation gradients, so within-site variance is large compared with the spatial signal at this scale. Second, hydrogen in consumer tissues derives from both dietary organic hydrogen and body water, and their relative contributions vary with physiology and development (Hobson et al. 1999b; Magozzi et al. 2019). Third, bulk foliage and chitin differ in their proportion of exchangeable hydrogen, which normalization of bulk foliage against keratin standards may not fully correct (Soto et al. 2017; Magozzi et al. 2021). Replacing foliar $\delta^2\text{H}_{\text{foliar}}$ with precipitation $\delta^2\text{H}$ did not improve the relationship. $\delta^2\text{H}$ remains effective at continental scales, where isotopic gradients exceed within-site variance, but at the regional scale examined here its signal-to-noise ratio is too low for reliable geolocation of individual SBW.

Sulfur

$\delta^{34}\text{S}_{\text{SBW}}$ values closely tracked the isotopic composition of host foliage (Dargent et al. 2026). A near-zero mean TDF ($-0.43 \pm 1.15\%$), is consistent with the established theory that sulfur exhibits negligible trophic fractionation due to the conservation of essential dietary amino acids (i.e., cysteine and methionine; Trust & Fry, 1992). When disentangling the trophic transfer effects from the underlying spatial structure, we found strong site-level structuring, indicating that $\delta^{34}\text{S}_{\text{SBW}}$ variability is attributable to inter-site rather than intra-site variation. Inclusion of $\delta^{34}\text{S}_{\text{foliar}}$ reduced unexplained inter-site variance by 92%, and the full model explained 96% of the total variance, as expected if $\delta^{34}\text{S}_{\text{SBW}}$ variation was predominantly structured by baseline foliar $\delta^{34}\text{S}_{\text{foliar}}$ composition. The large variation in $\delta^{34}\text{S}_{\text{foliar}}$ across the study region (1.5% to 15.6%) is consistent with environmentally driven sulfur cycling controlled primarily by atmospheric deposition and marine aerosols as observed on the larger scale $\delta^{34}\text{S}_{\text{foliar}}$ isoscape (Dargent et al. 2026). The low trophic discrimination, high-transfer fidelity, and strong regional structure make sulfur isotopes a strong geolocator for tracking SBW movements (e.g., Dargent et al., 2026).

Strontium

$^{87}\text{Sr}/^{86}\text{Sr}_{\text{SBW}}$ covaried positively with $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ ratios, indicating that SBW retain geographically derived strontium isotopes from their larval host foliage. This result is consistent with the theoretical basis of strontium isotope geolocation, with bioavailable Sr being directly transferred from bedrock to soils, plants, and primary consumers (Capo et al. 1998; Bataille and Bowen 2012). The wide range of $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ across sites further reflects the strong geological heterogeneity of eastern Canada, where ancient Precambrian rocks occur alongside younger sedimentary and Appalachian formations that generate distinct bioavailable $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ (Le Corre et al. 2025).

Despite this clear baseline relationship, the regression suggests attenuation from $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ to $^{87}\text{Sr}/^{86}\text{Sr}_{\text{SBW}}$. No fractionation of Sr isotopes is expected because mass-dependent fractionation is corrected for during analysis. Therefore, the difference between $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ and $^{87}\text{Sr}/^{86}\text{Sr}_{\text{SBW}}$ usually

occur with mixing of two isotopically distinct sources or analytical limitations. Based on the stronger but scattered model residuals at lower ^{88}Sr signal intensity (Fig. 4), we hypothesize that this attenuation was best explained by analytical limitations. Sr concentrations in individual SBW approached the lowermost limits of the best MC-ICP-MS sensitivity, often close to 1 ng of Sr. Under such low Sr concentrations, the signals for ^{87}Sr and ^{86}Sr are extremely low and error rapidly propagates in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. Contamination and blanks are unlikely to be the cause as the residuals are scattered without clear bias supporting our findings that procedural blank contribution was less than 5% for most samples.

To overcome these analytical limitations, we pooled individuals by site to increase the amount of Sr available during purification and analysis. This approach produced a stronger foliage-moth correlation ($R^2 = 0.85$) and a slope whose 95% confidence interval fell within the expected 1:1 relationship (Fig. 3C). Pooled samples exhibited higher signal intensity and reduced dispersion relative to individual ones (Fig. 4), and residual magnitude declined with increasing ^{88}Sr signal intensity, consistent with an analytical rather than a biological source of error when operating at the edge of the instrument's detection limit. Additionally, pooling samples might also average out biological micro-variation among individuals feeding at the same site.

The biological characteristics of SBW further support the use of $^{87}\text{Sr}/^{86}\text{Sr}$ as a geolocator for this particular species. Adult moths do not feed after emergence and have relatively short lifespans, meaning that their tissue Sr is derived almost entirely from larval feeding sites (Dargent et al. 2023). The contribution of sites in the LMM, along with the well-characterized spatial structure of $^{87}\text{Sr}/^{86}\text{Sr}$ across eastern Canada (Le Corre et al. 2025) further support the use of $^{87}\text{Sr}/^{86}\text{Sr}$ as a geolocator. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios provide direct information on natal habitat that is largely independent of climatic influences that affect other isotope systems, such as hydrogen, carbon, and nitrogen (Reich et al. 2021). Because geological variation often occurs over relatively fine spatial scales, strontium isotopes can provide geographic resolution that complements climate-driven tracers (Reich et al. 2021; Bataille et al. 2021). Although similar lithologies may generate isotopically indistinguishable regions at continental scales, integrating $^{87}\text{Sr}/^{86}\text{Sr}$ with complementary isotope systems has repeatedly been shown to improve geolocation accuracy and precision (Crowley et al. 2021; Reich et al. 2021; Bataille et al. 2021).

Our results demonstrate that $^{87}\text{Sr}/^{86}\text{Sr}$ ratios exhibit conservative trophic transmission from host foliage to SBW and retain information derived from underlying geological substrates. The principal limitation for insect geolocation identified in this study was analytical rather than ecological, arising from the low Sr concentrations present in individual moths. Consequently, future applications should focus on increasing analytical precision through larger sample masses, pooled analyses, or methodological developments that improve measurement sensitivity at low Sr concentrations. Once these constraints are addressed, $^{87}\text{Sr}/^{86}\text{Sr}$ has considerable potential as a geolocation tracer for SBW, particularly when incorporated into multi-isotope geolocation frameworks.

Limitations

First, sample sizes differ substantially among isotope systems and are small for carbon and nitrogen (7 sites) relative to sulfur (23 sites), hydrogen (19 sites) and strontium (18 sites), so the strength of evidence is not uniform across the systems we compare; the nitrogen result, which we identify as among the most promising, rests on the smallest dataset in the study. Second, different tissues were analysed for different elements, wings for $\delta^2\text{H}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, head and thorax for $\delta^{34}\text{S}$, and whole individuals for $^{87}\text{Sr}/^{86}\text{Sr}$, because of differences in elemental concentration; the resulting transfer functions are therefore not strictly comparable across systems, and each incorporates a tissue-specific offset in addition to any physiological fractionation. Third, foliage was pooled by site prior to analysis, so intra-site foliar heterogeneity was not quantified and foliar composition acts as a site-level predictor. This matters most for $\delta^{13}\text{C}$, where we attribute intra-site variance in moth values to within-canopy heterogeneity and ontogenetic effects, but where unmeasured foliar heterogeneity is an equally plausible and untested explanation. Fourth, sampling was restricted to Ontario and Newfoundland and does not span the full SBW range, so the transfer functions reported here should be treated as regionally calibrated.

Conclusion

Isotope geolocation in insects is limited by the effective propagation of isotopes from the environment to the animal, yet this step is rarely investigated. By quantifying it directly in a host-specialist insect herbivore, we show that the five isotope systems most commonly used show different levels of suitability for geolocation. $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ display near-proportional transfer with a consistent trophic discrimination factor and low intra-site variance and are the strongest geolocators in this system. However, unlike $\delta^{34}\text{S}$ which has a well-established isoscape across eastern Canada, the $\delta^{15}\text{N}$ patterns still need to be characterized and confirmed before $\delta^{15}\text{N}$ could be used as a geocator. Sr isotopes are transmitted conservatively, as expected, but their limitation for tiny-insect ecology is analytical rather than biological. $\delta^{13}\text{C}$ and $\delta^2\text{H}$ are poorly propagated due to microhabitat variation in photosynthetic discrimination and by mixed dietary and environmental hydrogen sources respectively, and neither has strong geocator potential at the study scale presented here. Although $\delta^2\text{H}$ is among the most widely used isotopes for continental-scale geolocation, the high intra-site variance observed here substantially reduced its discriminatory power at the spatial scale examined, illustrating that isotope performance depends strongly on the geographic scale of application. The $\delta^{15}\text{N}$ result is the least expected. Nitrogen isotopes are seldom used to infer geographic origin because mixed and shifting diets usually decouple consumer values from any single baseline. In SBW that decoupling does not occur because larvae feed on the needles of one or a few host trees, adults do not feed at all, and the tissues we measure are formed before dispersal begins. The life history that makes this insect so difficult to track by conventional means is precisely what makes its $\delta^{15}\text{N}$ applicable for geolocation. The same reasoning should extend to other host-specialist herbivores with non-feeding adults, a life history shared by many of the lepidopteran pests whose movements are hardest to observe. More generally, our results caution against applying published baseline isoscapes to a new species without first measuring the transfer function and considering the study scale. A large but highly consistent trophic offset, as we observe for

nitrogen, is no obstacle to geolocation, whereas a small but variable one, as we observe for carbon, is challenging to overcome.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

This study involved insects and conifer foliage only and did not require ethics approval.

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Author contribution

statement BH, CPB and FD designed the study. BH and FD collected the samples. BH and PLR performed the laboratory and analytical work. BH, FD and CPB performed the statistical analyses. JNC and MSR contributed methods and data. BH wrote the first draft. All authors contributed to revisions and approved the final manuscript. CPB and FD supervised BH and gathered funding for this project.

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Data availability

The data and code supporting this study have been deposited in the Purdue University Research Repository (PURR) and are publicly available under DOI: 10.4231/21P1-WX89

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Figures



Figure 1

Known-origin sample sites for host conifer foliage (*Abies balsamea* and *Picea* spp.) and spruce budworm (*Choristoneura fumiferana*) across eastern Canada. Points indicate site location and lines point to the stable isotope analyzed for foliage and budworm tissues, including hydrogen ($\delta^2\text{H}$), carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), sulfur ($\delta^{34}\text{S}$), and strontium ($^{87}\text{Sr}/^{86}\text{Sr}$).

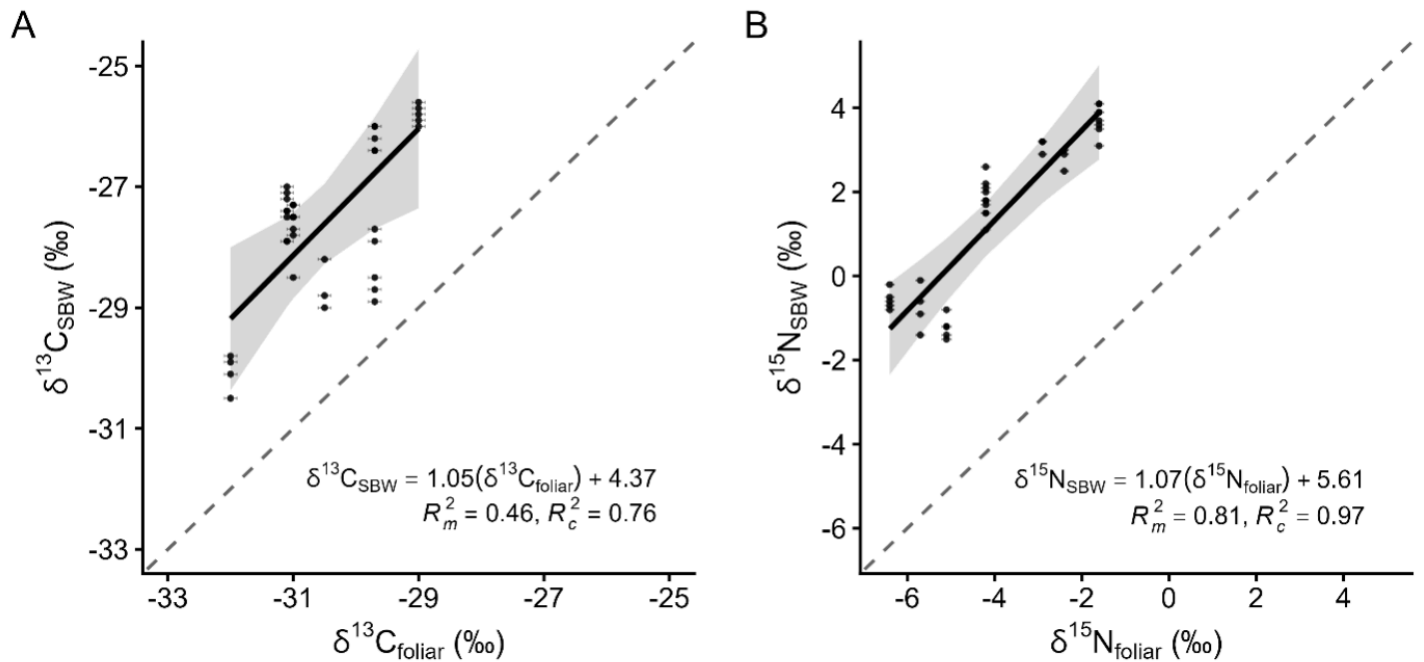


Figure 2

Linear relationships describing trophic transmission of (A) $\delta^{13}\text{C}$ and (B) $\delta^{15}\text{N}$ from host foliage (Pinaceae) to known-origin SBW tissues (*Choristoneura fumiferana*) across sites in eastern Canada ($\delta^{13}\text{C}$: 7 sites, 38 moths; $\delta^{15}\text{N}$: 7 sites, 38 moths). Each point represents a foliage sample, and its corresponding moth samples from the same site. Solid lines indicate population-level fixed effects from linear mixed-effects models (with site of collection treated as a random intercept) and marginal 95% confidence intervals (shaded ribbon), while the dashed lines denote the 1:1 relationship for reference. Error bars represent ± 1 standard deviation of the internal reference material (C-55: glutamic acid) within the analytical run, reflecting analytical uncertainty. Marginal (R_m^2) and conditional (R_c^2) R^2 values reported.

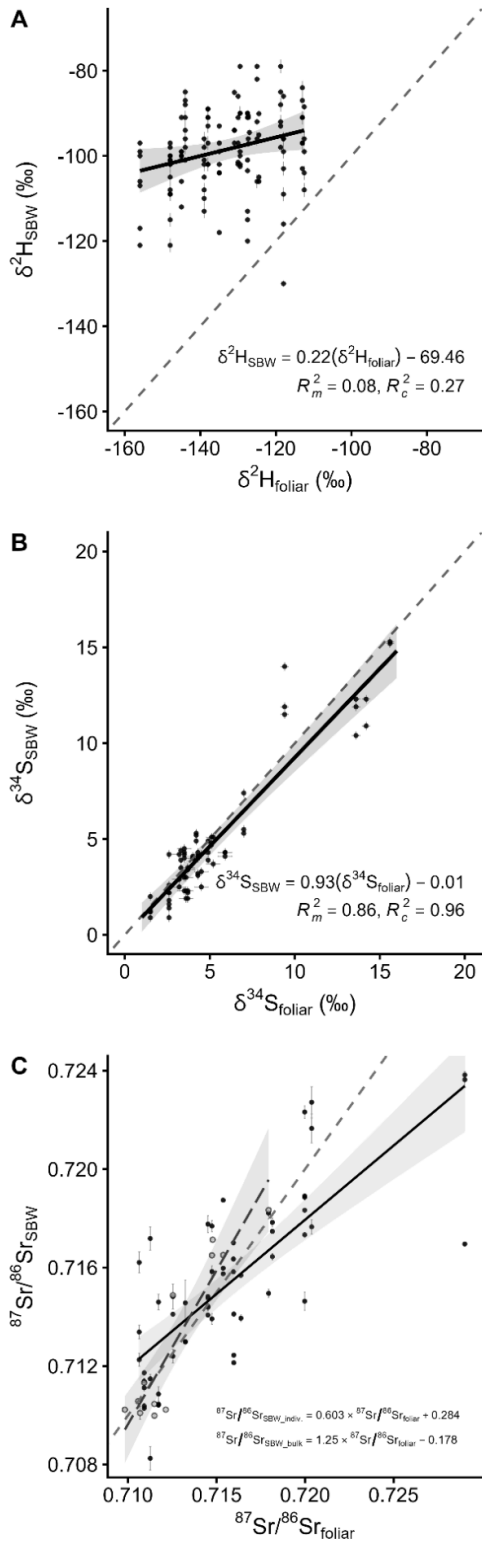


Figure 3

Linear relationships describing trophic transmission of (A) $\delta^2\text{H}$, (B) $\delta^{34}\text{S}$, and (C) $^{87}\text{Sr}/^{86}\text{Sr}$ from host foliage (Pinaceae) to known-origin SBW tissues (*Choristoneura fumiferana*) across sites in eastern Canada ($\delta^2\text{H}$: 19 sites, 126 moths; $\delta^{34}\text{S}$: 23 sites, 74 moths; $^{87}\text{Sr}/^{86}\text{Sr}_{\text{indiv.}}$: 18 sites, 58 moths; $^{87}\text{Sr}/^{86}\text{Sr}_{\text{pool.}}$: 12 sites). (A, B) Each point represents a foliage sample, and its corresponding moth

samples from the same site. Solid lines indicate population-level fixed effects from linear mixed-effects models (with site of collection treated as a random intercept) and marginal 95% confidence intervals (shaded ribbon). Marginal (R^2_m) and conditional (R^2_c) R^2 values reported. (C) Includes both individual moth samples (filled circles) and site-level pooled samples (open circles) excluded from the individual sample regression. Solid (individual moths) and broken (pooled moths) lines represent ordinary least squares regressions with 95% confidence intervals (shaded). Dashed lines indicate the 1:1 relationship for reference. Error bars represent analytical uncertainty based on internal reference materials within each analytical run, reflecting analytical uncertainty.

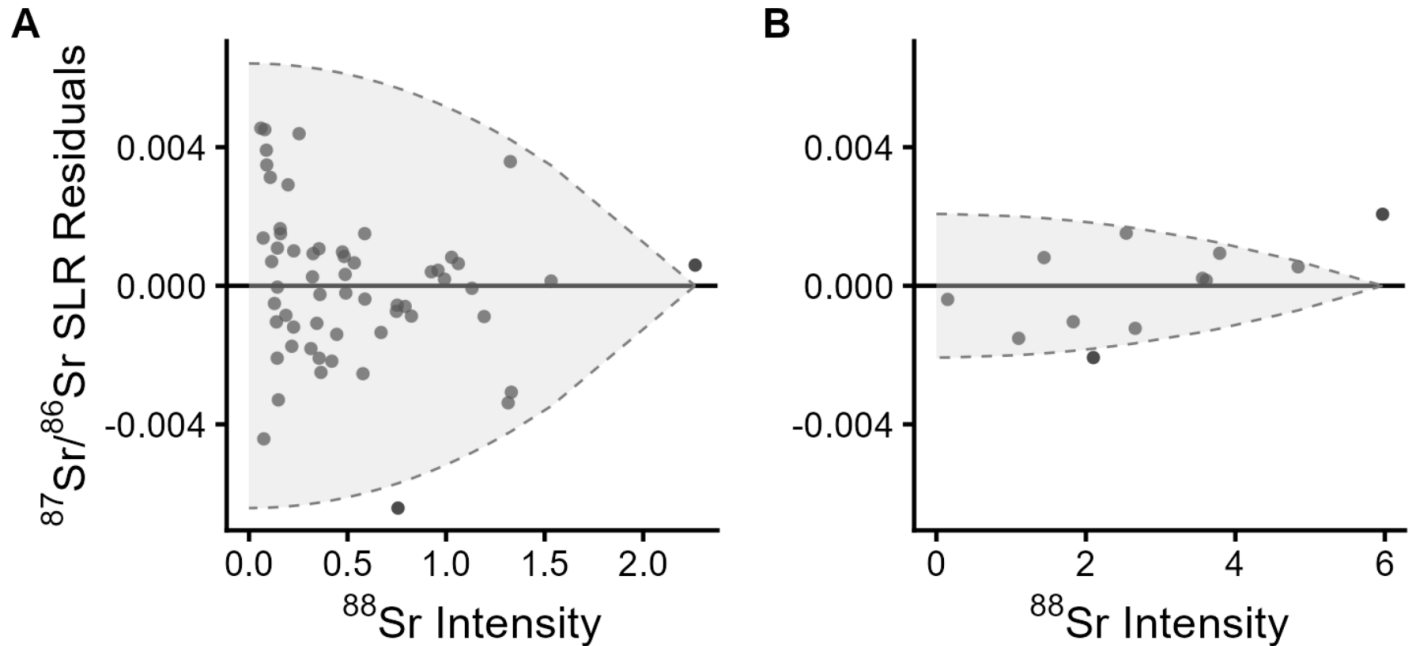


Figure 4

Residual patterns of $^{87}\text{Sr}/^{86}\text{Sr}$ trophic transfer as a function of ^{88}Sr signal intensity. (A) Residuals from the ordinary least-squares regression of $^{87}\text{Sr}/^{86}\text{Sr}_{\text{SBW}}$ versus $^{87}\text{Sr}/^{86}\text{Sr}_{\text{foliar}}$ for individual SBW samples plotted against ^{88}Sr signal intensity (a proxy for overall available Sr). (B) Equivalent residual plot for site-level pooled moth samples. Note that panels share a common y-axis but differ in x-axis range. Shaded envelopes represent the dispersion of residuals across the gradient of ^{88}Sr intensity, and bounds the maximum residual variance across the observed intensity.