Host range determination in a novel outbreak pest of sugarcane, *Cacosceles newmannii* (Coleoptera: Cerambycidae, Prioninae), inferred from stable isotopes

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Abstract

1. An outbreak of *Cacosceles newmannii* (Coleoptera: Cerambycidae) was detected for the first time on sugarcane (*Saccharum* spp.) in 2015 in KwaZulu-Natal, South Africa. Although primary host plants of this native species remain unknown, these are central to testing hypotheses concerning the outbreak.
2. We hypothesized that this species has undergone a host plant shift (i.e. a feeding association with a novel host plant).
3. We compared $\delta^{13}C$ and $\delta^{15}N$ ratios of adult beetles retrieved from South African museum collections, collected between 1891 and 2016 (n = 23; 'pre-outbreak'), with samples from infested fields in 2017 (n = 9, 'post-outbreak') and in 2019 (n = 23, 'post-outbreak'), as well as diverse, plausible host plants (n = 42 samples across 10 species) from infested fields and surrounding patches of indigenous and commercial forest vegetation. We used Bayesian isotope mixing models to infer the relative contribution of the different plants to the diet of *C. newmannii*.
4. Pre-outbreak, C_3 plants contributed strongly to the larval diet, whereas post-outbreak, C_4 plants were the largest component of their diet. There was some indication of C_4 plants contributing to their diet pre-outbreak.
5. Our results suggest that the outbreak of this polyphagous beetle was not a dramatic host shift but rather a rapid increase in the proportion of C_4 plants already in their diet.
6. We concluded that plants from the families Fabaceae and Poaceae are the most likely host plants of this species. Nevertheless, the drivers of this rapid outbreak on sugarcane remain poorly determined and should be the focus of future research.

Keywords: Biological invasion, biosecurity, herbivory, niche shift, range expansion, trophic niche.

Introduction

The primary host plants, or host range, of insect pests are not always known but are important for integrated pest management options (Conlong & Rutherford, 2009). This information helps estimate the extent and nature of polyphagy, which in turn provides key information on

biology and host use of insect pests. For example, host range expansions and host shifts are processes through which native species can expand outside of their native habitat (Lefort *et al*., 2014). A host shift can be defined as the continuation of a host range expansion, resulting in a population that forms an association with a novel host plant (Agosta, 2006) that has associated fitness trade-offs with the ancestral host (Diegisser *et al*., 2009). Host range expansions do not result in such fitness trade-offs, allowing the population to simultaneously use its new and ancestral host (Diegisser *et al*., 2009). This can, however, lead to greater damage in the invaded area than in their native range due to the absence of natural enemies and plants with greater susceptibility in the invaded area (Eyre & Haack, 2017). Occasionally, native species can reach the status of a pest within their native range (Lefort *et al*., 2014). Host shifts and range expansions onto commercially grown crops can lead to significant reductions in yields, which in turn can have devastating effects on the economies of countries to which these crops are large contributors. The detection of novel host plant associations is therefore an important contribution to sustainable agricultural production and economic well-being.

A powerful tool to help provide insights into potential dietary sources, and thus host plant associations, is stable isotope analysis (SIA) (Christianen *et al*., 2017; Kamenova *et al*., 2018). Stable isotopes naturally occur in every environment and organism and are safe to handle (Hood-Nowotny & Knols, 2007; IAEA, 2009). Typically, SIA has been used to determine the trophic status of organisms in food webs, track the flow of resources or propagules through ecosystems, track migration patterns of organisms across large spatial scales and identify novel feeding ecotypes within populations of different organisms (reviewed in McCue *et al*., 2020; Quinby *et al*., 2020). For example, SIA has been used to track discrete waves of invasion of Asian longhorn beetles *Anoplophora glabripennis*, which is a European Union quarantine-listed pest (Heinrich & Collins, 2016). It can also be used to distinguish between mass-reared and naturally occurring insects in sterile insect programmes (Hood-Nowotny *et al*., 2016). Stable isotopes do not decay rapidly and allow diverse approaches and insights into invasion biology (McCue *et al*., 2020). Differences in isotopic composition have been found between different tissues and parts of insect bodies. It is therefore important to select the appropriate body parts or tissues for isotope studies (Tsurikov *et al*., 2015). The chitinous exoskeletons of insects has been shown to be chemically stable and are not subject to much change after initial formation (Schimmelmann *et al*., 1986; Van Hardenbroek *et al*., 2012) and are therefore a good option for use in SIAs. Therefore, samples can be included from museum/stored collections (Maguire & Grey, 2006; Murphy *et al*., 2007). This allows access to historic samples that could potentially provide valuable time series information to address key research questions. For example, a study used stable isotope ratios in museum specimens of water beetles to investigate their utility as a proxy for environmental isotope ratios (van Hardenbroek *et al*., 2012).

Stable isotopic ratios of carbon (expressed as $\delta^{13}C$) can provide information regarding the primary sources of energy and thus provide a tool to potentially identify an organism's foraging location or characterize its dietary history (Bodey *et al*., 2011; McCue *et al*., 2020). The $\delta^{13}C$ values of consumers mirror the $\delta^{13}C$ values of primary producers (De Niro & Epstein, 1978). Plants exhibiting different photosynthetic pathways can be distinguished based on their $\delta^{13}C$ values. C_3 plants have mean $\delta^{13}C$ values of −28‰ (ranging from −20‰ to −37‰), whereas C_4 plants have mean $\delta^{13}C$ values of −14‰ (ranging from −12‰ to −16‰) (O'Leary, 1988; Kohn, 2010). Therefore, trees and shrubs (C_3 plants) are isotopically distinct from grasses such as sugarcane (C_4 plants). This allows a distinction to be made between insects feeding on C_3 and C_4 plants. Stable nitrogen isotope ratios (expressed as $\delta^{15}N$) can

provide insights into the trophic level of an animal within a food chain (De Niro & Epstein, 1981). This is because animals at higher trophic levels show higher $\delta^{15}N$ values compared with organisms feeding at lower trophic levels (Bodey *et al*., 2011) due to preferential retention of the heavier isotope (Kamenova *et al*., 2017).

Animal tissues have an isotopic composition that differs from their diet due to isotopic fractionation (McCutchan *et al*., 2003; Caut *et al*., 2009). Therefore, to accurately estimate potential diet sources using stable isotope abundances in animal tissues, knowledge of the isotopic shift between diet and consumer is required (McCutchan *et al*., 2003). This difference between diet and consumer is the trophic discrimination factor (TDF) or trophic enrichment factor, expressed with 'Δ' notation ($\Delta^{13}C$ for carbon and $\Delta^{15}N$ for nitrogen) (Martínez del Rio *et al*., 2009). Studies have shown that TDFs can vary depending on tissue type and turnover rate, diet type, metabolism, environment and developmental state (Vander Zanden & Rasmussen, 2001; McCutchan *et al*., 2003; Caut *et al*., 2009). As TDFs are not always known beforehand or measured for a specific organism used in a study that relies on mixing models, it is common to use previously published TDFs (Martínez del Rio *et al*., 2009).

Beetles in the family Cerambycidae are primarily forest insects and can play an important role in the decomposition of dead trees (Ferreira, 1980). Many species in this family cause significant damage to economically important trees (Craighead, 1950; Haack *et al*., 2010; Eyre & Haack, 2017), and many are economic pests of crops (Wang, 2017). Even though cerambycids are rare pests in sugarcane agro-ecosystems (Long & Hensley, 1972; Carnegie & Conlong, 1994), they can cause extensive crop loss. In Thailand, for example, *Dorysthenes buqueti* is a cerambycid attacking sugarcane and showed a 10-fold population increase within a year after it was first detected in 2003 (Pliansinchai *et al*., 2007). Similarly, in South America, *Migdolus fryanus* has caused extensive damage to the rhizomes of sugarcane (Ferrer, 1994).

Cacosceles newmannii (Thomson 1877) (Coleoptera: Cerambycidae, Prioninae) is a southern African species that has been poorly studied prior to its invasion of South African sugarcane in 2015 (Way *et al*., 2017). Little is published about the biology and ecology of this species (Javal *et al*., 2019a, b; Smit *et al*., 2021). Observations revealed that eggs are laid in the soils and decomposing plant material around the base of mature sugarcane stools. The neonate larvae of *C. newmannii* feed in the roots of these sugarcane stools and eventually enter the growing stalk bases, where they grow and feed, by moving upwards through two to four internodes from the base that they entered. Normally, only one larva is found per infested sugarcane stalk because of their large size. When ready to pupate, the late instar larva will leave the stalk it has been feeding on through its base. Once in the soil under the stool, it will form an earthen cocoon, inside which it pupates. They have a 2-year life cycle in which adults only live about 2–3 months and do not feed (Way *et al*., 2017). A review of the Prioninae (Ferreira, 1980) provides valuable information on the historical distribution of *C. newmannii* and shows that this species has previously been recorded in diverse locations within South Africa, as well as in Mozambique and Eswatini, including areas where sugarcane typically does not occur. Life stages of this beetle are therefore likely to occur on alternative indigenous host plants. *Cacosceles newmannii* can thus be considered a polyphagous insect (Ali & Agrawal, 2012). In addition to sugarcane, the plant family Myrtaceae, specifically *Eucalyptus* species, has been recorded as a host plant of this species (Ferreira, 1980), but as *Eucalyptus* spp. are not native to South Africa, this is most likely not

a primary, endemic host plant of *C. newmannii*. To our knowledge, no further information on host plants of this beetle is readily available.

Larvae of this cerambycid beetle were found for the first time feeding on commercially grown sugarcane in the Entumeni district (28°55′S, 31°19′E) of KwaZulu-Natal, South Africa in 2015 (Way *et al*., 2017). The reason(s) underlying the rapid emergence of this indigenous insect as a crop pest in sugarcane remain unclear (Javal *et al*., 2018). Currently, this species has only been detected as a commercial sugarcane threat in the Entumeni area and nowhere else in the region. Because of the outbreak, large areas of sugarcane were ploughed out (SASRI, 2017) and planted with kikuyu grass *Pennisetum clandestinum* (Hochst. Ex Chiov.) pastures as affected sugarcane farmers switched to more profitable livestock farming. SIAs may provide information regarding possible previous host plants of *C. newmannii* before its detection in sugarcane.

The main objective of this study was to identify the dietary proportions of potential host plants of *C. newmannii* by means of stable light isotope ($\delta^{13}C$ and $\delta^{15}N$) elemental analyses. This was done by applying a Bayesian isotope mixing model (Parnell *et al*., 2010) to the stable isotope data, simultaneously including a sensitivity analysis of TDFs. We hypothesized that *C. newmannii* feeding on sugarcane is a highly novel association. In this study, we set out to answer the following questions: (i) Did *C. newmannii* form an association with a novel host plant (*i.e*. has therefore undergone a host shift)? (ii) Has *C. newmannii* fed on sugarcane previously but never at high enough levels to cause an outbreak? We discuss our results in the context of the pest outbreak on sugarcane in the Entumeni region of KwaZulu-Natal and its potential as a pest insect that spreads into other sugarcane-growing regions with similar host plant complexes.

Materials and methods

Sample collection

To compare specimens collected before and after the outbreak of *C. newmannii* in sugarcane, 24 adult samples were obtained from the insect collections of different South African museums, and 32 adult samples were obtained from infested fields in Entumeni (28°55′S 31°19′E), KwaZulu-Natal, South Africa (Supplementary material, Table S1). The museum specimens were sampled in locations from five provinces in South Africa, and one sample came from a country bordering South Africa, Eswatini (Fig. 1a). Identifications of museum specimens were based on museum labels and were double checked using available literature (Ferreira, 1980). The museum specimens were stored in cupboards and were exposed to chemicals such as Dichloro-diphenyl-trichloroethane, arsenic, Lindane (Benzene hexachloride) and pyrethroids. As we only used chitin from adults, which is a biologically inert material once formed (van Hardenbroek *et al*., 2012) and should not be subject to change with respect to carbon and nitrogen isotopes, we assumed that the preservation method of the museum specimens did not influence the SIA (Schimmelmann *et al*., 1986).

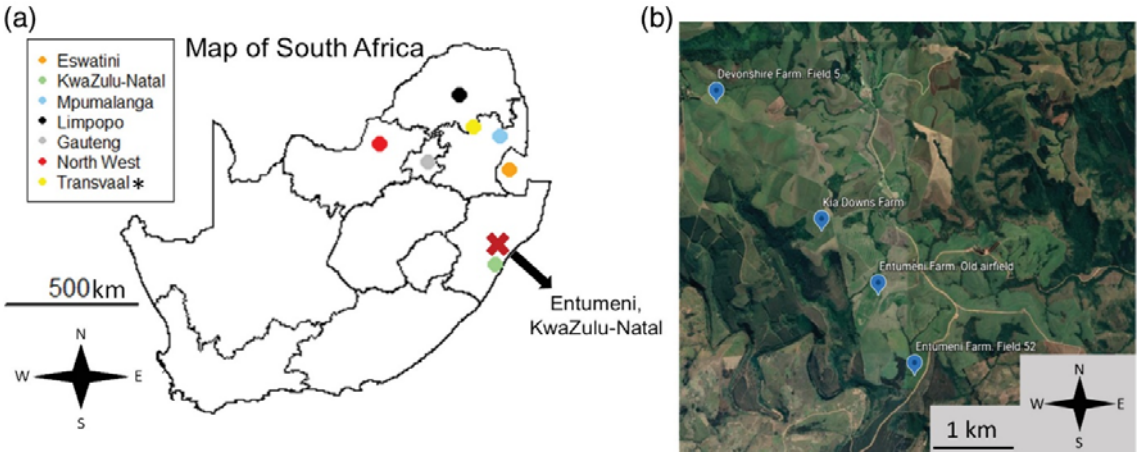

Figure 1. (a) Map of South Africa indicating locations where museum specimens were collected and Entumeni (red cross) in KwaZulu-Natal. * Transvaal (old province of South Africa) currently includes the following provinces: Gauteng, Limpopo, Mpumalanga, North West and KwaZulu-Natal (produced with Rstudio using R version 3.5.3, R core team 2018). (b) Image showing the locations where beetles were collected in 2017 (Entumeni Farm Field 52; Entumeni Farm old airfield) and 2019 (Devonshire Field 5) (produced using Google maps 2020).

Sampling of *C. newmannii* from infested sugarcane fields was done in two separate years, 2017 and 2019, on different farms in the area in each year, which were approximately 5 km from each other. In 2017, the adults that were sampled emerged from recently ploughed out sugarcane fields (Fig. 1b, Entumeni Farm Field 52 (31°19′13″E; 28°56′08″S: 503 m above sea level)) and surroundings (Fig. 1b Entumeni Farm old airfield), and in 2019, the adults sampled emerged from fields that had been replanted with kikuyu grass since 2017 (Fig. 1b Devonshire Farm Field 5 (31°17′20″E; 28°53′58″S: 674 m above sea level)) and surroundings. *Cacosceles newmannii* has a 2-year life cycle in which adults only live about 2–3 months and do not feed (Way *et al*., 2017). Therefore, the isotopic signatures of adults are expected to reflect the isotopic signatures of the food sources they relied upon as larvae in the preceding 2 years, as shown in previous studies (Tsurikov *et al*., 2015). Therefore, only the legs of adults were used for SIA of all *C. newmannii* samples, except for three museum specimens for which a fragment of antenna was used (to preserve the intactness of the specimens). We did, however, use four samples of ecdysed larval cuticle from larvae fed on sugarcane in the SASRI rearing unit for SIA in comparisons with adult sample SIA.

Plant samples were collected in and around the infested sugarcane field agro-ecosystem (Devonshire Farm Field 5 and Entumeni Farm Field 52, Fig. 1b). Leaves, stems and/or roots of plants were placed in bags and labelled accordingly. After transport from the field to the laboratory at ambient conditions, they were stored in the bags at −80 °C prior to processing for SIA, as described below. Plants were identified and classified according to their C_3 or C_4 metabolic pathway (determined based on if the plant was a tree or a grass), family (nine families) and, where possible, species (10 species) (Table 1). If more than one sample per plant was taken (leaves, stems and/or roots), it was labelled accordingly and sent for SIA separately.

Table 1. Mean and standard deviation (SD) of $\delta^{13}C$ and $\delta^{15}N$ values of plants collected at Entumeni, KwaZulu-Natal. n denotes the number of samples

C_3 or C_4	Family	Species	Part sampled	Area sampled	$\delta^{13}C$ (mean ± sd)	$\delta^{15}N$ (mean ± sd)	n
C_4	Poaceae	*Pennisetum clandestinum*	Leaf	Infested fields	−10.7 ± 0.1	3.6 ± 1.1	4
C_4	Poaceae	*Pennisetum clandestinum*	Roots	Infested fields	−13.1 ± 1.3	1.4 ± 0.7	4
C_4	Poaceae	*Saccharum officinarum*	Stem	Infested fields	−13.5 ± 1.2	3.8 ± 1.4	8
C_4	Poaceae	*Panicum maximum*	Leaf	Surrounding vegetation	−12.7 ± 0.2	7.4 ± 0.4	2
C_4	Poaceae	*Panicum maximum*	Roots	Surrounding vegetation	−12.4 ± 0.03	4.7 ± 0.4	2
C_3	Meliaceae	*Trichilia dregeana*	Leaf	Surrounding vegetation	−28.9 ± 0.1	4.9 ± 0.1	2
C_3	Meliaceae	*Trichilia dregeana*	Stem	Surrounding vegetation	−28.5 ± 0.2	4.5 ± 0.2	2
C_3	Fabaceae	*Erythrina lysistemon*	Leaf	Surrounding vegetation	−29.2 ± 0.1	0.49 ± 0.1	2
C_3	Fabaceae	*Erythrina lysistemon*	Stem	Surrounding vegetation	−28.2 ± 0.3	−0.03 ± 0.1	2
C_3	Fabaceae	*Acacia mearnsii*	Leaf	Surrounding vegetation	−29.2 ± 0.04	−0.4 ± 0.1	2
C_3	Moraceae	*Ficus sur*	Leaf	Surrounding vegetation	−26.7 ± 0.3	5.5 ± 0.2	2
C_3	Moraceae	*Ficus sur*	Stem	Surrounding vegetation	−26.7 ± 0.1	2.2 ± 0.2	2
C_3	Myrtaceae	*Syzygium gerrardii*	Leaf	Surrounding vegetation	−30 ± 0.04	4.2 ± 0.2	2
C_3	Myrtaceae	*Syzygium gerrardii*	Stem	Surrounding vegetation	−28.2 ± 0.1	4.6 ± 0.3	2
C_3	Myrtaceae	*Eucalyptus sp*.	Leaf	Surrounding vegetation	−29.3 ± 0.1	2.3 ± 0.1	2
C_3	Apocynaceae	*Tabernaemontana sp*.	Leaf	Surrounding vegetation	−29.1 ± 0.1	10.3 ± 0.2	2

All isotope ratios are presented in delta (δ) notation on the per mille scale (‰).

Stable isotope analysis

Small fragments of beetle (larval cuticle, adult legs or antennae) and plant samples (leaves, stems and/or roots) were placed in labelled 2-mL micro-centrifuge tubes and then dried overnight at 60 °C in an oven. The samples in the tubes were then placed in airtight bags and couriered to the Stable Isotope Laboratory at the Mammal Research Institute (MRI), University of Pretoria. At the MRI, beetle samples were placed inside 1-mL Eppendorf tubes filled with ultra-pure water and washed using an ultrasonic bath (Eins Sci Professional Ultrasonic cleaner, United Scientific South Africa) for 15 min to remove any chemicals used in museum collections. As chitin is a biologically inert material (van Hardenbroek *et al*., 2012) and is unlikely to change with respect to carbon and nitrogen isotopes, a water wash to remove any potential surface contaminants was thought to be adequate. The samples

were then placed in new 1-mL Eppendorf tubes and dried overnight in an oven at 60 °C. Between 0.5 and 0.6 mg of the clean and dried insect sample was weighed out to three decimal points (using a Mettler Toledo Mk5 microbalance) into pre-cleaned (with toluene) 6 × 4-mm tin capsules (Elemental Microanalysis, United Kingdom), folded into a circular shape using sterilized forceps and placed into a 96-well microplate. After each sample was placed in the microplate, the forceps were sterilized using 70% ethanol before being used again to avoid contamination.

The plant samples were dried overnight at 60 °C and then homogenized using a mechanical homogenizer (Bead Bug (TM) microtube homogenizer, LASEC South Africa) with 2 × 5 mm-diameter sterilized glass beads (supplied by LASEC) for 2 min at 3500 rpm. After the plant samples were homogenized, aliquots of between 1.1 and 1.2 mg were weighed out into pre-cleaned 6 × 4-mm tin capsules, folded and added to the microplate. Each sample (plant and insect) was given a unique ID to keep track of where samples were placed in the microplate.

The samples were combusted at 1020 °C using an elemental analyser (Flash EA 1112 Series) coupled to a Delta V Plus stable light isotope ratio mass spectrometer *via* a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany). Two laboratory running standards (Merck Gel: $\delta^{13}C = -20.26‰$, $\delta^{15}N = 7.89‰$, C% = 41.28, N% = 15.29 & DL-Valine: $\delta^{13}C = -10.57‰$, $\delta^{15}N = -6.15‰$, C% = 55.50, N% = 11.86) and a blank sample were run after every 11 unknown samples to ensure no contamination across samples and robust precise readings. The output was a ratio of the heavy to light isotopes of a particular element (in this case carbon or nitrogen), which was then converted into a delta (δ) value (Hood-Nowotny & Knols, 2007; Tsurikov *et al*., 2015).

The $\delta^{13}C$ and $\delta^{15}N$ values obtained for the in-house Merck Gel and DL-Valine standards were used to calibrate the sample results and provide the instrumental precision for the run, which was <0.06‰ for carbon and nitrogen. All stable carbon isotope values are presented in delta (δ) notation on a per mille (‰) scale, relative to the Vienna PeeDee belemnite international standard for carbon and air for nitrogen, using the standard equation:

$$\delta X(‰) = (R_{sample}/R_{standard}) - 1$$

where X = ^{13}C and R represents $^{13}C/^{12}C$ (Coplen, 2011).

Statistical analysis

Preliminary analyses and comparisons of beetle body parts for $\delta^{13}C$ and $\delta^{15}N$ showed little variation between legs and antennae and, similarly, little variation between larvae and adults, in the few samples that were obtained (Supplementary material). To determine any difference in the isotopic ratios of leaves, stems and/or roots from the same plant, a main-effects generalized linear model was used to analyse the $\delta^{13}C$ data and the $\delta^{15}N$ data. A time series graph of $\delta^{13}C$ and $\delta^{15}N$ data of *C. newmannii* from 1891 to 2019 using *C. newmannii* specimens with a known sampling year (n = 51 samples, Supplementary material, Table S1) was generated to determine if there were any patterns in stable isotope data over this period. The $\delta^{13}C$ and $\delta^{15}N$ values of *C. newmannii* from infested sugarcane fields (after the outbreak) from two different years, 2017 and 2019, were compared using a t-test for independent samples. Key assumptions for statistical tests were checked and not violated in results

presented. Statistica 13 (TIBCO Software, United States) software was used to perform the statistical tests, and differences were accepted as significant at a 5% probability level.

Mixing models and sensitivity analyses

To determine the relative contribution of plant samples to the diet of *C. newmannii*, a Bayesian stable isotope mixing model (SIAR) was used (SIAs in R; Parnell *et al*., 2010). The mixing models were run with R studio (R version 3.5.2, R core team, 2018). Given the limited variation among parts of plants or insects, isotopic values of plant parts (leaves, stem and/or roots) were pooled, and *C. newmannii* parts (legs and antennae) were also pooled for the mixing model analysis. To run SIAR, the isotopic compositions of sources (plants), consumers (*C. newmannii* adults) and isotopic fractionation values (TDFs) between diet and consumer were assigned.

The relative contribution of sampled plants to the diet of *C. newmannii* was successively tested by grouping plants in the following way: i) C_3 and C_4 plants, ii) plant families and iii) plant species (Table 1). The relative contribution of the following plant families was tested: Meliaceae, Fabaceae, Moraceae, Myrtaceae, Apocynaceae and Poaceae. These families contain species known to be host plants of cerambycid species of the subfamily Prioninae, which includes *C. newmannii* (Ferreira, 1980). The relative contribution of the following plant species was tested: *Trichilia dregeana*, *Erythrina lysistemon*, *Acacia mearnsii*, *Ficus sur*, *Syzygium gerrardii*, *Saccharum officinarum*, *Panicum maximum*, *Pennisetum clandestinum Tabernaemontana* sp. and *Eucalyptus* sp. (the last two species could not be identified to the specific species). A requirement for mixing models is that the consumer isotopic values must fall within the range of corrected food source isotopic values for each element (in this case C and N) (Phillips *et al*., 2014). There was one plant sample that did not meet this requirement, *Tabernaemontana* sp., and was therefore excluded to prevent unrealistic model solutions. As *Tabernaemontana* sp. was the only plant sampled in the family Apocynaceae, this plant family was also excluded from further analyses.

For each plant assemblage (C_3 vs. C_4; families; species), museum specimens were assigned as group 1, samples collected on Entumeni Farm in 2017 group 2 and samples collected on Devonshire Farm in 2019 group 3.

Initially, each mixing model was run assuming TDFs for carbon and nitrogen from the literature (McCutchan *et al*., 2003). The same mixing models were then re-run using TDFs estimated specifically for *C. newmannii* using the following formula (Kurle *et al*., 2013):

$$\Delta^{13}C_{tissue-diet} = \delta^{13}C_{tissue} - \delta^{13}C_{diet} \text{ (and similarly for N).}$$

For published TDFs, we assumed trophic discrimination factors for carbon and nitrogen to be 0.5 ± 0.19‰ for $\delta^{13}C$ and 2.3 ± 0.24‰ for $\delta^{15}N$, as recommended by McCutchan *et al*. (2003) for terrestrial animals. For estimated TDFs, we used $\delta^{13}C$ and $\delta^{15}N$ ratios of eight plant samples of sugarcane and nine *C. newmannii* samples from infested sugarcane fields at Entumeni Farm in 2017. TDFs for *C. newmannii* samples were calculated by subtracting mean stable isotopic values of sugarcane samples from those of the beetles (Supplementary material, Table S2).

In all SIAR models, we set the following model parameters: concentration dependents = 0, iterations = 200 000 and 'burn-in' (number of initial iterations discarded) = 50 000.

Results

Stable isotope analysis

The carbon and nitrogen isotopic compositions of 46 plant samples (Table 1) and 56 *C. newmannii* samples (Supplementary material, Table S1) were determined. Although there was significant variation in $\delta^{13}C$ and $\delta^{15}N$ between plant species ($P < 0.001$), there was no significant difference in $\delta^{13}C$ between the different parts of the plants sampled ($P = 0.613$). There was, however, significant variation in $\delta^{15}N$ between different parts of the plants ($P < 0.001$).

When $\delta^{13}C$ and $\delta^{15}N$ values of *C. newmannii* museum specimens were plotted, the data showed that $\delta^{13}C$ values remained relatively stable over the years 1891–2016. The $\delta^{15}N$ data are more variable, and this is most likely due to the variety of locations and soil types where the specimens were sampled. The museum specimens collected in the most recent years before the outbreak in sugarcane show more negative $\delta^{13}C$ and $\delta^{15}N$ values compared with the specimens collected at infested fields in 2017 and 2019 (Fig. 2).

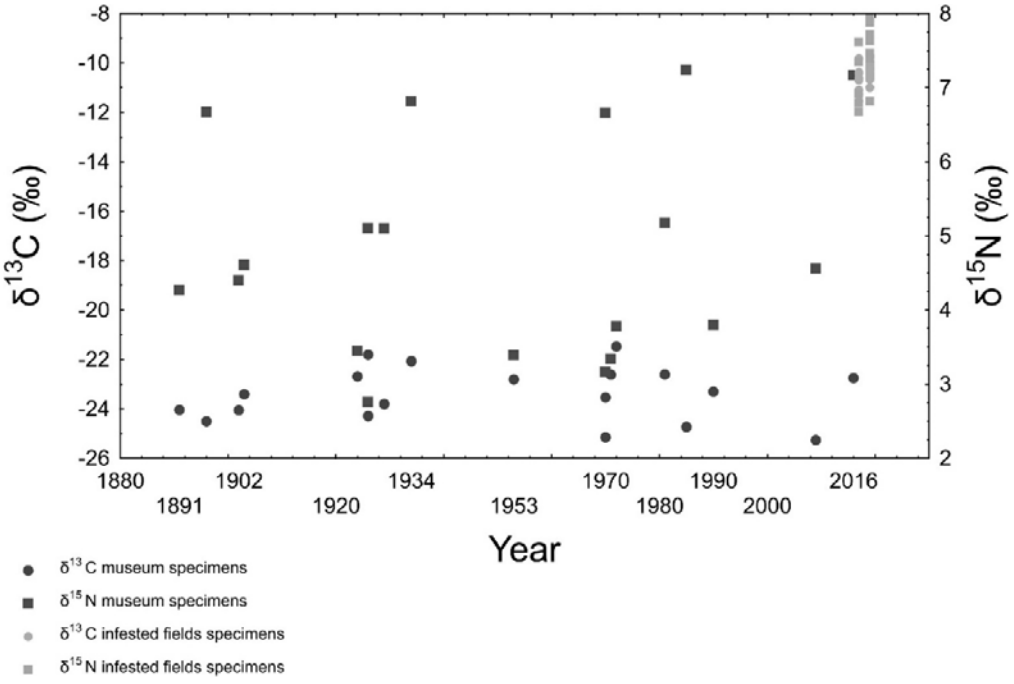

Figure 2. Scatterplot of $\delta^{13}C$ (left *y*-axis) and $\delta^{15}N$ (right *y*-axis) for museum ($\delta^{13}C$ in blue and $\delta^{15}N$ in red) and infested field ($\delta^{13}C$ in green and $\delta^{15}N$ in orange) samples of *C. newmannii* plotted according to year collected. $\delta^{13}C$ is illustrated by dots, and $\delta^{15}N$ is illustrated by squares.

Samples from the infested sites on Entumeni Farm collected in 2017 had an average $\delta^{13}C$ value of -10.72 ± 0.52‰, which was significantly lower ($P < 0.05$) compared with the average $\delta^{13}C$ (-10.29 ± 0.32‰) of those collected from Devonshire Farm in 2019. *Cacosceles newmannii* samples from the infested sites collected in 2017 had an average $\delta^{15}N$ value of

7.78 ± 0.94‰, which was significantly lower ($P < 0.05$) compared with the average $\delta^{15}N$ (8.55 ± 0.95‰) of those collected in 2019 (Fig. 3).

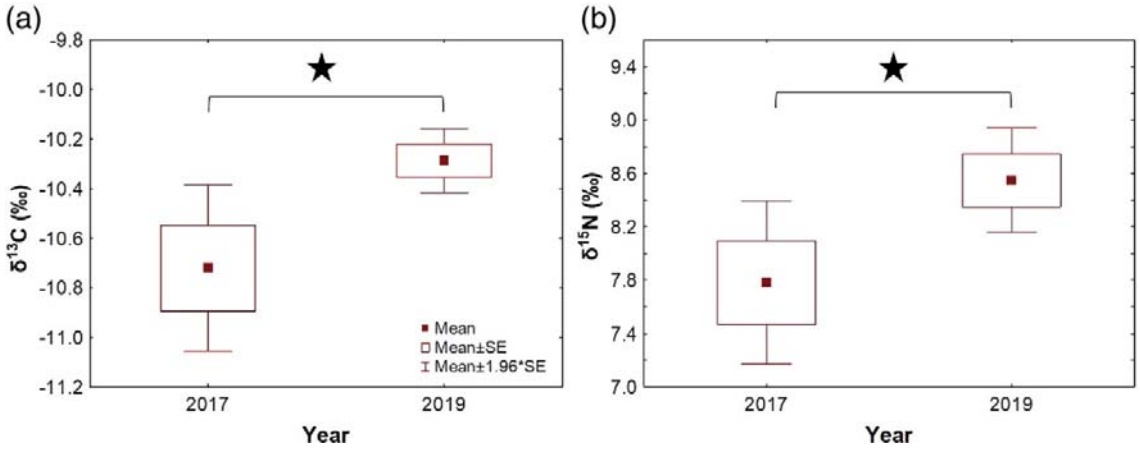

Figure 3. Boxplots showing the (a) $\delta^{13}C$ and (b) $\delta^{15}N$ values of *C. newmannii* adults collected in 2017 (n = 9) and 2019 (n = 23). Stars indicate statistically significant difference ($P < 0.05$) in average $\delta^{13}C$ and $\delta^{15}N$ between 2017 and 2019, determined using t-tests for independent samples.

Mixing models and sensitivity analyses

The results for each plant assemblage (C_3 vs. C_4; families; genera) is given using published TDFs (McCutchan *et al*., 2003) and TDFs estimated specifically for *C. newmannii* (see Supplementary material, Table S2). The carbon TDF ($\Delta^{13}C$) we estimated for *C. newmannii* was 2.8 ± 0.5‰, and for nitrogen, we estimated a value of 4.0 ± 0.9‰ (Supplementary material, Table S2).

C_3 versus C_4 plants

Running the model using published TDFs (Fig. 4a), the results showed that C_3 plants comprised the largest component in the diet of museum specimens with an average contribution of 70%. There was a small C_4 contribution of 30% to the diet of museum specimens. The average C_4 contribution to the diet of *C. newmannii* collected from the from infested fields of Entumeni Farm in 2017 was 99%, whereas the average contribution of C_3 plants was 1%. The same pattern of C_4 and C_3 contribution was seen for 2019 samples collected at Devonshire Farm.

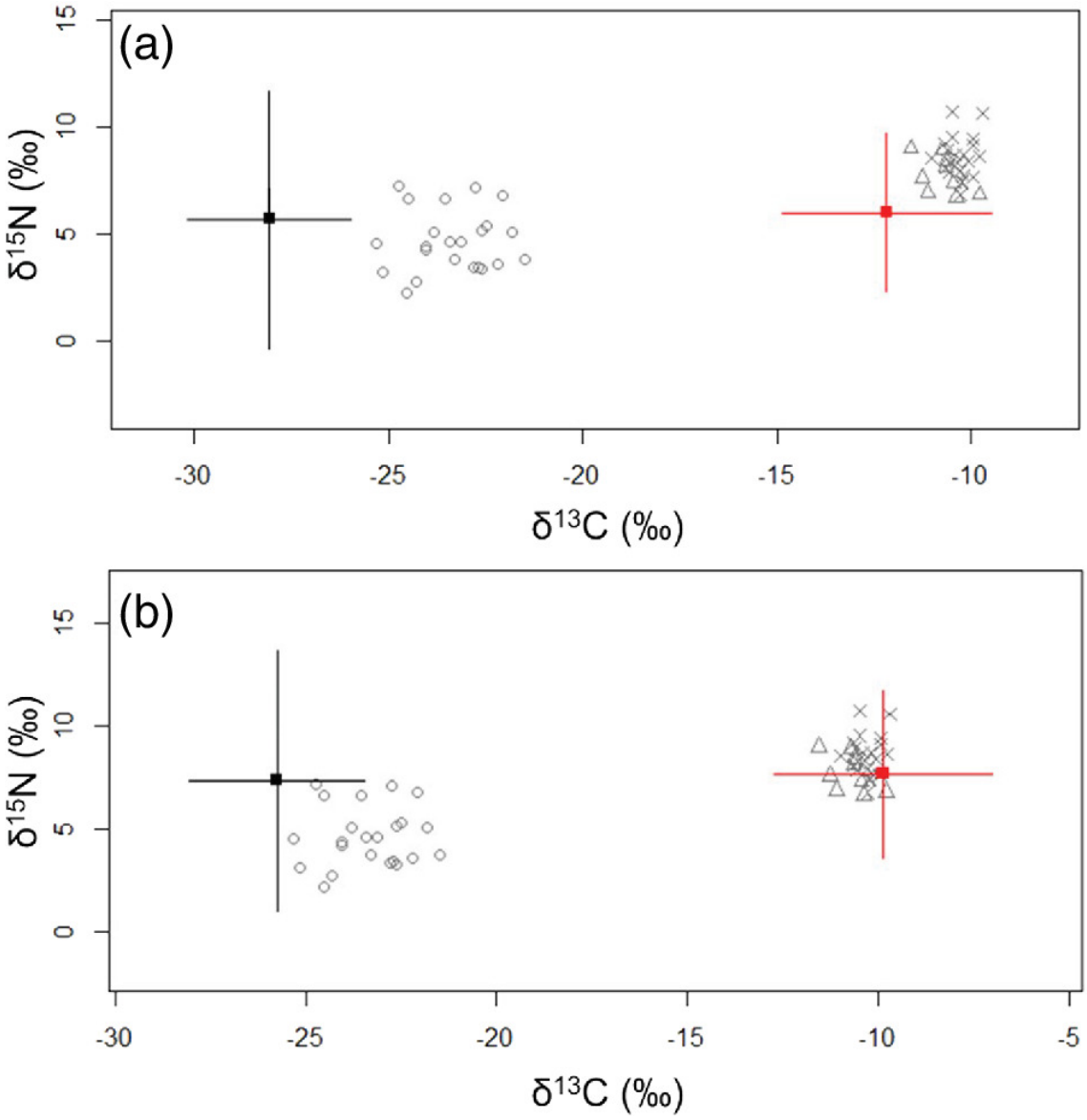

Figure 4. Biplot showing $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (mean ± SD) of C_3 (black cross) and C_4 (red cross) plants and isotopic signatures of museum (group 1, circles), Entumeni Farm in 2017 (group 2, triangles) and Devonshire Farm in 2019 (group 3, small crosses) adult *C. newmannii* samples using (a) published trophic discrimination factors and (b) estimated trophic discrimination factors.

The model using TDFs estimated specifically for *C. newmannii* (Fig. 4b) yielded results showing that C_3 plants were the largest component in the diet of the museum specimens; the average contribution was 84%, and the average C_4 contribution was 15%. The average C_4 contribution to the diet of *C. newmannii* collected in 2017 was 93%, whereas the average contribution of C_3 plants was 1%. The average C_4 contribution to the diet of *C. newmannii* collected in 2019 was 96%, whereas the average contribution of C_3 plants was 4%.

Plant families

When we ran the model using published TDFs, the results showed that the plant family which showed the highest average contribution to the diet of museum specimens was Fabaceae

(34%). For 2017 and 2019 samples, Poaceae had the highest average contribution to their diet (84% and 97%, respectively). When we ran the model using estimated TDFs, similar results were found. Fabaceae (69%) was observed to be the highest for museum specimens and Poaceae the highest for 2017 (90%) and 2019 (94%) (Table 2).

Table 2. Average contributions (%) of plants grouped according to family and species levels using published trophic discrimination factor (TDFs), estimated TDFs, according to the diets of museum specimens and 2017 and 2019 samples

	Published TDFs			Estimated TDFs		
Plant family and species	**Average contribution to diet (museum) (%)**	**Average contribution to diet (Entumeni 2017) (%)**	**Average contribution to diet (Devonshire 2019) (%)**	**Average contribution to diet (museum) (%)**	**Average contribution to diet (Entumeni 2017) (%)**	**Average contribution to diet (Devonshire 2019) (%)**
Fabaceae	34	2	1	69	2	1
Erythrina lysistemon	15	2	1	25	1	1
Acacia mearnsii	16	2	1	36	1	1
Poaceae	29	84	97	16	90	94
Pennisetum clandestinum	12	13	5	8	38	23
Saccharum officinarum	10	16	5	5	23	18
Panicum maximum	8	50	85	4	30	55
Moraceae	15	5	1	6	3	2
Ficus sur	10	4	1	5	2	1
Myrtaceae	13	4	1	5	2	1
Syzygium gerrardii	9	4	1	4	1	1
Eucalyptus sp.	12	3	1	9	1	1
Meliaceae	10	5	1	4	2	2
Trichilia dregeana	9	5	1	4	1	1

Plant species

When plants were grouped according to species level, and we ran the model using published TDFs, the results showed that *Acacia mearnsii* contributed the most (average 16%) to the diet of museum specimens. For 2017 and 2019 samples, the results showed that *Panicum maximum* contributed the most (average 50% and 85%, respectively) to their diets. When we ran the model using estimated TDFs, *Acacia mearnsii* also had the highest average contribution (36%) to the diet of museum specimens. For 2017 samples, the results showed that *Pennisetum clandestinum* contributed the most to their diet, with an average proportion of 38%. For 2019 samples, *Panicum maximum* had the highest average contribution (55%) (Table 2).

Discussion

Our study brings together multiple independent lines of evidence to yield novel insights into diet and host plant use in a southern African Cerambycidae beetle that has become a rapid biosecurity risk on sugarcane grown in the region. We provide the first determination of the host plants, other than sugarcane, for *C. newmannii* based on SIA, as well as a novel temporal (longitudinal) analysis of museum specimens. In the process, we test and reject our original hypothesis that this species feeding on sugarcane is a highly novel association for reasons outlined below. Although TDFs are required for studies on the SIAs of animal diets, such data are only available for a small number of species. Therefore, researchers often use the best approximation values of similar species from the literature (Hervías *et al*., 2014; Catry *et al*., 2016), although this may be criticized (Kelly *et al*., 2012; Quinby *et al*., 2020). This emphasizes the importance of deriving species-specific TDFs, or assessing the validity of TDFs employed, when undertaking SIA studies to determine food sources. We are confident our TDFs used in the mixing models are relatively robust as using different TDFs gave only slightly different quantitative results, hardly affecting the major qualitative outcomes. The only mixing models that showed different qualitative outcomes between different TDFs were for 2017 and 2019 samples when plants were grouped according to species. The model using previously published TDFs resulted in *Panicum maximum* having the highest average contribution to both 2017 and 2019 samples, whereas using our own estimated TDFs resulted in *Pennisetum clandestinum* being the highest average contributor to 2017 samples and *Panicum maximum* to 2019 samples (Table 2). This suggests that the results of isotope (diet) mixing models are more sensitive to the change in TDFs when sources are not pooled into groups of higher taxonomic classification.

Many stable isotope studies of trophic relationships assume a small increase (usually between +0.8‰ and +1.0‰) in $\Delta^{13}C$ from one trophic level to the next (McCutchan *et al*., 2003). This study shows that this is not necessarily the case for a terrestrial invertebrate feeding on C4 plants as the $\Delta^{13}C$ calculated from field-collected specimens was 2.8 ± 0.5‰. The shift in $\Delta^{15}N$ between diet and consumer is usually assumed to be between +2.6‰ and +3.4‰ (De Niro & Epstein, 1981). However, the $\Delta^{15}N$ calculated for *C. newmannii* was 4 ± 0.9‰.

It is important to note that the estimates of TDFs could have a degree of uncertainty. The results of the mixing models showed that the *C. newmannii* samples we initially assumed to be only feeding on sugarcane (as they emerged from sugarcane fields), and subsequently used to estimate the TDFs (2017 samples), fed on more than one food source. Ideally, when calculating species-specific TDFs, animals should be fed on a single food source. However, this should not have major effects on our results as these beetles fed mainly on plants in the same family, Poaceae, which all have similar isotopic values (Table 1). Preliminary analyses of larvae and adults feeding on sugarcane (Supplementary material) suggested little among-stage $\delta^{13}C$ and $\delta^{15}N$ variation and thus add confidence in any comparisons across ontogeny or pooling stages.

The results for the mixing models, of either published estimates or our own estimated TDFs, showed that *C. newmannii* historically fed primarily on C_3 plants, although there was a small percentage of C_4 plants contributing to the diet of the museum specimens. Our results suggest that *C. newmannii* fed on some C_4 plants previously, never at high levels – this was unlikely a major dietary nutrient source. Their preference for C_4 plants in the family Poaceae, however, increased drastically in 2017 and even more in 2019. Our results suggest plants in the Fabaceae family, specifically *Acacia mearnsii* and *Erythrina lysistemon*, were the most likely

host plants of this insect prior to their occurrence in sugarcane. *Acacia mearnsii* is not native to southern Africa and can therefore not be a primary endemic host plant of *C. newmannii*. *Erythrina lysistemon* is, however, indigenous, and *C. newmannii* could have fed on this plant species before moving onto *Acacia mearnsii*. Further work is required to disentangle the specific sequence of events and any host-related preference between these tree species.

Furthermore, our results showed that *C. newmannii* fed on a mixture of C_4 plants post-outbreak (2017 and 2019). There was a statistically significant increase in both $\delta^{13}C$ and $\delta^{15}N$ ratios between 2017 and 2019 in *C. newmannii* adults collected at Entumeni. These data showed that the isotopic signatures of *C. newmannii* changed significantly within a period of 2 years. This change could be explained by the difference in abundance of the crops found at the two locations at the time of sampling. Other site parameters may also have contributed to this variation, such as various soil parameters and altitude (Supplementary material, Table S3). This rapid change in diet observed over such a short period of time in *C. newmannii* showed that this species is, however, opportunistic and feeds on what is available, with outbreaks perhaps driven to some extent by a decline in an alternative resource that was previously abundant.

It has been hypothesized that phytophagous insects tend to avoid C_4 plants due to these plants being a poorer-quality food source compared with C_3 plants (Caswell *et al*., 1973). Even though another study showed no significant difference between the utilization of C_3 and C_4 plants, a consistent trend towards heavier utilization of C_3 species was found (Boutton *et al*., 1978). Whether this is indeed the case here is, however, unclear, although this could be one potential explanation for the small contribution of C_4 plants in the diet of *C. newmannii* pre-outbreak. However, insects can adapt to feeding on C_4 vegetation as seen in grasshoppers that are able to digest the nutrient-rich bundle sheath cells of C_4 grasses (Barbehenn, 2005). Further research is needed regarding the ability of *C. newmannii* to digest C_4 plant foods to determine if this is a viable explanation for the outbreak of this insect in sugarcane.

Several potential sources of variation can influence the outcomes of SIA if they are not considered (McCue *et al*., 2020; Quinby *et al*., 2020). For example, the importance of accounting for lipids in stable isotope analysis has been emphasized in a number of studies as these may introduce variation in whole-body isotopic signatures (De Niro & Epstein, 1978; Post *et al*., 2007). Before determining the stable isotope ratios in our samples, we did not use a solvent wash to remove any potential lipids. The reason for this was to prevent any loss of scarce material of precious museum specimens. In addition, lipids are mainly an issue when investigating hydrogen isotopes and, to a much lesser extent, influence carbon isotopes (Heinrich & Collins, 2016). Life stage (ontogeny) or body part variation can also introduce a potential bias, although here, we focused on data of adults' chitinous structures only in our main analyses. Nevertheless, our preliminary investigations suggested nothing particularly unusual (e.g. unusually high isotope enrichment or depletion) between stages or body parts or between museum specimens compared with field-collected individuals in either $\delta^{13}C$ or $\delta^{15}N$. Had this been the case, we would be less confident in the interpretation of our results and hence any conclusions drawn here.

As we found plant families Fabaceae and Poaceae to contain the most likely host plants in all mixing model outputs, management efforts seeking to control *C. newmannii* should be focussed on these plant families. Furthermore, *C. newmannii* likely did not form a particularly novel association with sugarcane as a host plant to experience an abrupt host shift but rather rapidly increased the dietary proportion of a prior group of host plants, probably

driven by declines in alternative, previously relied-upon resources or other anthropogenic pressures (e.g. informal logging) combined with favourable environmental conditions for population growth. Regardless of the precise underlying drivers, this study provides an example of a native insect that has reached pest status within its native range, causing extensive damage to a crop that is a large contributor to the overall economy of several tropical and subtropical countries (Goebel & Sallam, 2011). The drivers of this rapid outbreak should be the focus of future research.

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Authors' contributions

CS, MJ and JST conceived the project and designed the study; DEC and JST secured the funding; DEC coordinated field collection of insects and plants; CS and MJ collected the museum samples; GH and CS performed stable isotope analyses in samples; CS analysed the data; CS, MJ, GH, DEC and JST contributed to data interpretation; CS and JST drafted and revised the manuscript; and MJ, GH and DEC contributed to the interpretation of and writing the manuscript. All authors reviewed and approved the final manuscript.

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