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Spodoptera litura (Lepidoptera: Noctuidae) Adult Abundance and Larval Host Plant Use in the Ord River Irrigation Area, Western Australia

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ABSTRACT

Spodoptera litura (Fabricius) is a highly polyphagous lepidopteran pest; however, little is known about its seasonal population dynamics or larval host plant use in northern Australia. Improved understanding of the ecology of *S. litura* is increasingly important given the anticipated expansion of *Bacillus thuringiensis* (Bt) cotton production in the Ord River Irrigation Area (ORIA) in north-eastern Western Australia and the species' status as a pest of cotton. This study investigated the seasonal abundance of adult *S. litura* in the ORIA from May 2023 to November 2024 and used stable isotope analysis of adult tissues to infer larval host plant use, distinguishing between C3 and C4 plant sources. Despite expectations that dry-season cultivation of Bt cotton would not coincide with peak adult *S. litura* activity, substantial numbers of moths were collected within the ORIA during this period. Laboratory-reared insects that had developed exclusively on maize (C4), cotton (C3 non-legume) or pigeon pea (C3 legume) were used to establish baseline $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each plant species. Analysis of $\delta^{13}\text{C}$ values in the wing tissue of pheromone trap-collected *S. litura* adults enabled inference of larval host plant use. Stable isotope analysis revealed that most moths likely originated from C3 plants across the season with smaller numbers developing on C4 plants despite the widespread cultivation of C4 crops, such as maize and sorghum, in the region. These findings suggest that uncultivated C3 plants, including weedy species, may play an important role in sustaining *S. litura* populations within the ORIA. This study provides the first assessment of seasonal *S. litura* host plant use in northern Australia and highlights the importance of understanding landscape-level ecology when developing sustainable pest management strategies.

1 | Introduction

Spodoptera litura Fabricius (Lepidoptera: Noctuidae) is a highly polyphagous herbivore (EFSA Panel on Plant Health et al. 2019; EPPO 2015) that is a pest of a wide range of broad-acre and horticultural crops (EFSA Panel on Plant Health et al. 2019; EPPO 2015; Hamzah and Norsyazwina 2021; Madhu et al. 2023). Although preferences and performance on different host plants have been investigated (Abdullah et al. 2019; Maharjan et al. 2023; Shekhawat et al. 2018; Tuan et al. 2015;

Xue et al. 2010; Zhang et al. 2023), studies often focus on crops other than those that are grown in tropical northern Australia. Despite its wide host range, little is known about host plant use in northern Australia and its influence on seasonal population dynamics.

In the Ord River Irrigation Area (ORIA) in Western Australia, studies from the 1990s suggest that populations of lepidopteran moths typically peak during the summer wet season and decline over the winter dry season (Strickland et al. 2003). To

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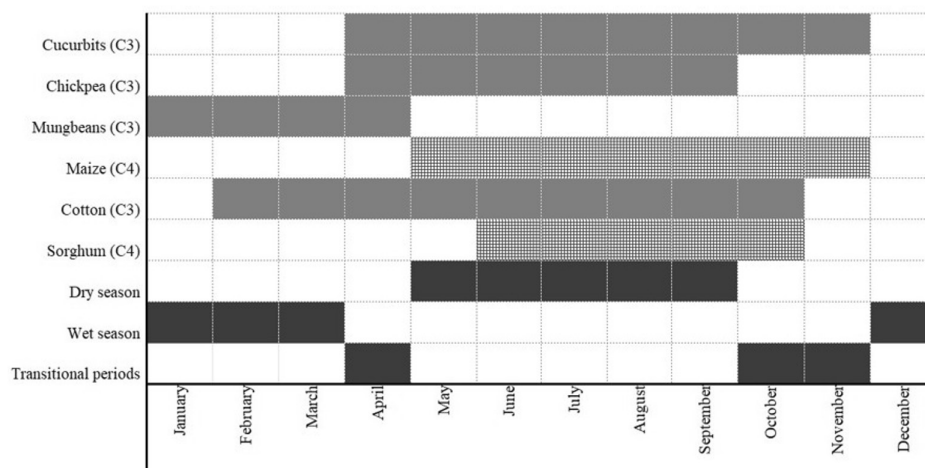


FIGURE 1 | Overview of climatic seasons and predominant cultivated annual crops in the Ord River Irrigation Area (ORIA), Western Australia. Black bars represent the approximate duration of each climatic season (wet and dry season and transitional periods between the seasons), and grey bars show the typical cropping periods for major annual crops; solid grey bars represent C3 crops, whereas white bars with black cross-hatching represent C4 crops.

reduce the risk of cotton crops being exposed to peak pest populations, changing cotton production in the region from the wet season to the dry season was explored, and this is now implemented as standard practice as part of the Bollgard 3 Resistance Management Plan (RMP) for this region (Bayer Crop Science 2024; Davies et al. 2007; Yeates et al. 2013). However, local moth trapping recorded high populations of *S. litura* moths during the dry season (Pers. comm. K. Saligari 2022), suggesting that year-round recruitment occurs. Understanding seasonal host plant use and the relative contributions of the region's diverse cropping system (Figure 1) and its non-crop vegetation to recruitment and population persistence is fundamental to predicting the risk that *S. litura* poses to cotton crops at different times of the year in the ORIA. This is particularly relevant given that Bollgard 3 (BG3) cotton, which expresses Cry1Ac, Cry2Ab and Vip3A proteins, provides a high level of control against *S. litura* (Holman et al. 2025). However, *S. litura* has a naturally low susceptibility to Cry1Ac and Cry2Ab (Tay et al. 2022), with control in BG3 appearing largely reliant on Vip3A (Holman et al. 2025; Tay et al. 2022). Consequently, there may be less redundant killing of *S. litura* by the Cry proteins expressed in BG3 than for other species such as *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) (Holman et al. 2025; Tay et al. 2022), increasing the importance of understanding other factors that could contribute to resistance evolution.

Several methods are available to investigate lepidopteran populations and host plant use, including pheromone trapping, field sampling and controlled host preference studies (Howse et al. 2013; Jost and Pitre 2002; Prasad and Mathyam Prabhakar 2012; Raimondo et al. 2004; Rajapakse and Walter 2007; Scott et al. 2005; Wade et al. 2006). Although these approaches provide some information on population abundance and host plant associations, they can have limitations for determining the origin and movement of insects under field conditions (Carpenter et al. 2013; Raimondo et al. 2004; Serra and Trumper 2006). An alternative approach is stable isotope analysis, which can infer larval host plant use of field-collected insects. Natural differences in carbon and nitrogen isotope signatures retained in

adult tissues can distinguish between those that developed on C4 plants, such as maize, and C3 plants, such as cotton, as larvae (Ambika et al. 2005; Baker et al. 2019; Dittmore et al. 2023; Gould et al. 2002; O'Leary 1988). However, stable isotope analysis can distinguish only between broad plant groups such as C3 and C4 plants, rather than different host plant species, limiting its ability to identify the precise larval host of field-collected moths. Despite this limitation, it provides a useful means of examining the relative contribution of C3 and C4 host plants to moth populations at a landscape-level (Baker et al. 2019; Dourado et al. 2021; Gould et al. 2002; Santos et al. 2023).

Stable isotope analysis has been applied to investigate host plant use in other noctuid species including *H. armigera* (Baker et al. 2019; Dourado et al. 2021), *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) (Gould et al. 2002; Santos et al. 2023) and *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) (Jia et al. 2021; Nagoshi et al. 2014). For example, in a 3-year study in Texas, USA, <10% of *H. zea* moths were categorised as feeding on C3 plants as larvae early in the cotton season, but this increased to 50% later in the season (Gould et al. 2002). Conversely, in a 4-year study on the Darling Downs, Australia, 80%–95% of *H. armigera* moths were found to have originated from C3 plants early in the cotton season, but this proportion declined to <50% later in the season (Baker et al. 2019). These studies demonstrate the potential of stable isotope analysis to broadly assess host plant use of a species across a landscape.

Understanding the seasonal dynamics and host plant use of *S. litura* is important for identifying factors that contribute to population persistence within the ORIA and for assessing how populations interact with the region's agricultural landscape. This study aimed to (i) examine the seasonal dynamics of *S. litura* populations within and outside the ORIA in Kununurra, Western Australia, over an 18-month period and (ii) use stable isotope analysis to infer host plant use and determine whether patterns of host use reflect the composition of the surrounding cropping landscape.

2 | Material and Methods

2.1 | Study Area

The study monitored *S. litura* moth populations and larval host plant use in the area around Kununurra, Western Australia (−15.65°, 128.70°). The region has a tropical, monsoonal climate with distinct wet and dry seasons. The wet season generally occurs from December to March and the dry season from May to September, with April and October–November representing transitional periods between the two seasons (Figure 1). The region receives an average of 864 mm of annual rainfall, occurring predominantly between November and March (Figure S1). Evaporation is high, exceeding rainfall in all months of the year, resulting in arid conditions during the dry season. The region is characterised by a consistently warm to hot climate, with mean maximum temperatures exceeding 30°C throughout the year. From June to August, the mean minimum temperature falls below 18°C. In July, the mean minimum temperature is 14.8°C, whereas the mean maximum temperature is 30.8°C. In contrast, in the hottest month of November, during the onset phase of the wet season (commonly referred to as the ‘build-up’), the mean minimum and maximum temperatures are 25.1°C and 39.1°C, respectively (Figure S1).

The ORIA constitutes approximately 27,000 ha of mixed production that is irrigated by water from Lake Kununurra (Smith et al. 2005). Currently, the supply channels irrigate approximately 22,000 ha. Within the ORIA, herein referred to as the ‘irrigation area’, a diverse range of crops is produced, including maize, cotton, hybrid seed production, pasture, mungbeans, sandalwood and various horticultural crops (ORDCO 2024). Outside the irrigation area, land is predominantly used for cattle grazing, conservation or unallocated Crown Land, herein referred to as the ‘rangeland’. In the East Kimberley, these rangelands are characterised by tropical savanna vegetation comprising open eucalypt and *Corymbia* woodlands, native grasslands, *Acacia* shrublands, riparian vegetation and disturbed areas supporting both invasive and native plant species, including malvaceous species (Kimberley Rangelands Biosecurity Association, n.d.; Lyons et al. 2014). This diverse vegetation provides a broad range of potential host plants which may be capable of supporting *S. litura* populations outside the irrigated cropping landscape.

2.2 | Moth Abundance Within the Irrigation and Rangeland Areas

Moth populations of *S. litura* were monitored in the irrigation and rangeland area using sex pheromone traps from May 2023 to November 2024. The bucket traps allowed moths to enter from any direction through a gap between the cover and the funnel, which directed them into a bucket containing a dichlorvos (DDVP) cube (Biotrap DDVP cubes, Bio Trap Australia Pty Limited, VIC). An *S. litura* pheromone lure (Entosol Australia Pty Limited, NSW) was placed in the trap. Pheromone lures were replaced monthly and DDVP quarterly as per manufacturers’ guidelines. The bucket traps were hung

on metal poles, with the lure height approximately 1.5 m above the ground.

Six study sites were established within the irrigation area and 13 study sites in rangeland areas surrounding the irrigation area, at distances of 5, 15, 25 and 50 km away from the edge of the irrigation area (Figure S2 and Table S1). Rangeland sites were established along roads extending in different directions from the irrigation area. As sampling was conducted year-round, rangeland sites were restricted to locations that remained accessible during the wet season, limiting the sampling distance along some transects (Table S1). At each site, three pheromone traps were placed approximately 75 m apart along a transect. The traps were emptied and moths counted weekly. Moth samples were collected at specific time points to use in the stable isotope analysis (Table S2). Moth samples were collected and placed in labelled snap-lock plastic bags (18 × 17 cm), before being placed in 25-mL sample jars (LabDirect, Wetherill Park) containing 95% ethanol and stored in the laboratory until preparation for analysis. The use of 95% ethanol for preserving samples does not affect the stable isotope values of insects (Gould et al. 2002).

2.3 | Estimated Proportion of Cultivated C3 and C4 Plants Within the Farming System

Crop area data for cultivated annual crops grown in the irrigation area during the 2023 and 2024 seasons (Table 1) were provided by the Western Australian Department of Primary Industries and Regional Development and Bayer Crop Science (Pers. comm. N. Wilson 2024; Pers. comm. K. Knight and J. Papaioannou 2025). The proportion of C3 and C4 plants within the farming system at different points during the season was estimated using crop planting and harvesting dates obtained in consultation with Ord River District Co-operative Ltd. (ORDCO) (Pers. comm. P. Goldsmith 2024).

2.4 | Isotope Signatures of Moths From Known C3 and C4 Plants

To establish baseline reference carbon and nitrogen isotope values, *S. litura* larvae from a laboratory culture, derived from field-collected populations in Kununurra (refer to Holman et al. (2026) for further details) were reared from hatching to pupation on maize, non-Bt cotton or pigeon pea leaves. Insects were reared individually on leaf material of one plant type in 70-mL food-grade containers. Nine moths reared on each different host plant were prepared for the stable isotope analysis. Maize and cotton are the primary C4 and C3 host plants in the farming system. Pigeon pea is a C3 legume crop and the most commonly grown refuge crop in the region under the Bollgard 3 RMP (Pers. comm. K. Knight 2024), which is designed to prevent *Helicoverpa* spp. developing resistance to the Bt proteins in Bollgard 3 (Bayer Crop Science 2024). Pigeon pea is not a known host plant for *S. litura*, and larvae have not been observed in local pigeon pea refuge crops (Grundy et al. 2020).

2.5 | Seasonal Patterns of Host Plant Use Shown by Isotopes

Samples of moths were collected from pheromone traps at six key time points representing major changes in the farming system, defined by the presence or absence of maize, cotton and sorghum, and the phenological stage of cotton when present (Table 2). The six stages of the farming system were defined as follows: (i) cotton flowering with no maize or sorghum present; (ii) cotton flowering, maize planted, but no sorghum planted; (iii) cotton reaching cut-out with maize and sorghum planted; (iv) cotton defoliated or harvested; (v) most crops harvested; and (vi) absence of cotton, maize and sorghum in the farming system. At each point, a subsample of up to 10 moths collected from the pheromone traps at each site in the irrigation area was used for stable isotope analysis. There were no moths or fewer than 10 moths available at some sites at certain collection times (Table S2). Moths were collected for stable isotope analysis from April 2023, prior to the establishment of the complete moth trap network (in May 2023), to June 2024, a period covering nine collections within the farming system (Table 2).

2.6 | Sample Preparation and Stable Isotope Analysis

Each moth sample was rinsed with 95% ethanol and allowed to air dry. Samples were placed individually into labelled DL-sized envelope (PPS, Officeworks) and oven-dried at 70°C for 48 h. A 0.7 (± 0.1) mg wing tissue sample was dissected from each moth and placed in pressed tin capsules. Stable isotope analyses of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in wing tissue were carried out using an Elementar PreciSION Isotope Ratio Mass Spectrometry (IRMS), coupled with an Elementar Vario Isotope Cube at the Stable Isotope Geochemistry Laboratory in the School of the Environment at the University of Queensland, Brisbane. Calibration was performed by a three-point normalisation using standards USGS40, USGS41a and USGS4. Precision for USGS40 (L-glutamic acid) was $\pm 0.1\%$ (1σ) for $\delta^{13}\text{C}$ and $\pm 0.2\%$ (1σ) for $\delta^{15}\text{N}$. Carbon isotope values ($\delta^{13}\text{C}$) values are reported relative to VPDB (Vienna Pee Dee Belemnite) (Friedman et al. 1982) and nitrogen isotope values ($\delta^{15}\text{N}$) relative to atmospheric nitrogen (AIR), with all isotope values expressed in per mil (‰).

TABLE 1 | The area (hectare/ha) of different cultivated annual crops planted within the Ord River Irrigation Area (ORIA), Western Australia, in the 2023 and 2024 cropping seasons.

Crop category	Crop	Area planted (ha)		Photosynthetic group
		2023	2024	
Broadacre	Maize	4800	5290	C4
	Bollgard 3 cotton	2190	1807	C3
	Unsprayed non-Bt cotton refuge	8	23	C3
	Unsprayed pigeon pea refuge	63	71	C3
	Mungbean	145	150	C3
	Chickpea	800	800	C3
	Sorghum	1190	1200	C4
	Legumes	170	170	C3
	Other beans and field crops	1035	450	C3
Horticulture	Cucurbits	1250	1250	C3

TABLE 2 | Six stages of the Ord River Irrigation Area farming system were defined according to the phenological stage of cotton and/or absence of cotton, maize and/or sorghum. Moths were collected from nine time points from April 2023 to June 2024.

Farming system stages	Season 1	Season 2
Cotton flowering with no maize or sorghum present	Mid-April 2023	Mid-April 2024
Cotton flowering, maize planted, but no sorghum planted	Mid-May 2023	Late May 2024
Cotton reaching cut-out with maize and sorghum planted	Late June 2023	Late June 2024
Cotton defoliated and/or harvested	Mid-September 2023	No samples collected
Most annual crops within the farming system harvested	Late November 2023	No samples collected
Absence of cotton, maize and sorghum in the farming system	Early February 2024	No samples collected

2.7 | Statistical Analysis

All statistical analyses were conducted in Genstat Version 24 (VSN International 2024). To account for differences in sampling effort, total monthly moth counts for each trap were divided by the number of weekly collections completed within that month, thereby standardising counts where traps were not deployed for the entire month or where fewer collections were completed. A generalised linear mixed model (GLMM) was used to test the effects of location and the season on the adjusted weekly moth collections, with collection location (irrigation or rangeland area), sampling month-year and their interaction as the fixed effects. The model included random effects for location and trap nested within location (traps within location) to account for potential variation between sites and among traps within sites.

A one-way ANOVA was conducted to test for differences between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in laboratory moths reared on maize, pigeon pea and cotton. Estimates of the proportion of C3 and C4 plants within the farming system at key seasonal time points were derived from the planted areas of cultivated annual crops, along with their typical planting and harvest dates, to assess the composition within the cropping system at each point in time. Larval diet (C3 plants, C4 plants or a mixture of each) was inferred from the $\delta^{13}\text{C}$ of the wings of trap-collected moths, following the approach adapted from Gould et al. (2002). The proportion of individuals within each dietary category was quantified at each sampling time point. A principal components cluster analysis using a covariance matrix was used to investigate the multivariate structure in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data. Because only two variables were included, the analysis was restricted to two principal components, which together accounted for all variation in the dataset. Clustering was subsequently applied to the data in the reduced two-dimensional PC space to evaluate potential grouping patterns.

3 | Results

3.1 | Moth Abundance Within the Irrigation and Rangeland Area

Moths were caught year-round within the irrigation area and in the surrounding rangeland; however moth abundance differed temporally (Figure 2). There was trend for higher moth abundance within the irrigation area compared to the rangeland, but this was not statistically significant ($F=3.7$; $\text{df}=1, 16.3$; $p=0.072$). However, there was a significant interaction between collection location and sampling month-year ($F=14.2$; $\text{df}=18, 994.2$; $p<0.001$), indicating that seasonal patterns in moth abundance differed between the irrigation area and rangeland. In the rangeland, moth abundance declined progressively throughout the winter dry season from June each year and into the early wet season. In contrast, although a decline was also observed in the irrigation area, the pattern was more variable, with a marked reduction in abundance occurring from October 2023 to February 2024 and from October 2024.

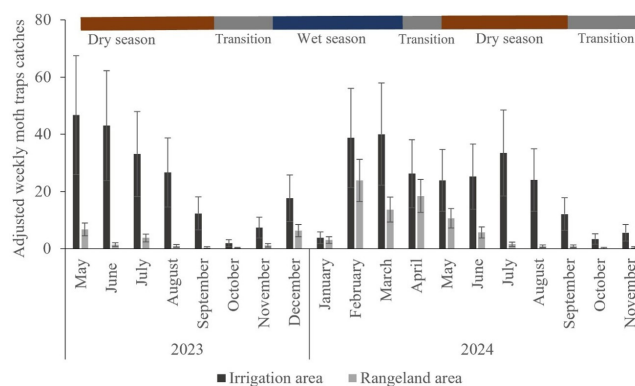


FIGURE 2 | Adjusted* mean weekly moth catches per trap in the irrigation and rangeland areas of Kununurra from May 2023 to November 2024. The climatic and transitional seasons are indicated by the bars positioned above the graph. *To account for variation in sampling effort, weekly moth catches were pooled within each month for each trap and divided by the number of weekly collections completed during that month. This standardisation accounted for missed weekly collections and months in which traps were not sampled for the entire month.

3.2 | Estimated Proportion of Cultivated C3 and C4 Plants Within the Farming System

A variety of cultivated C3 and C4 crops is grown within the irrigation area, with the largest areas planted to maize, cotton, cucurbits and sorghum (Table 1). The composition of cultivated C3 and C4 plants within the irrigation area varied throughout the cropping season (Supp. Figure 3). In mid-April 2023, early February and mid-April 2024, all actively growing crops were C3 plant species, including cotton and mungbeans. The proportion of C4 crops increased during the dry season, with maize contributing the most to this increase (Table 1). C4 plants accounted for between 52% and 71% of the overall crop area during the dry season (between May and November 2023 and May and June 2024).

3.3 | Isotope Signatures of Moths From Known C3 and C4 Plants

The host plant that larvae were reared on in the laboratory affected $\delta^{13}\text{C}$ values ($F_{2,26}=2581.5$; $p<0.001$; Figure 3A), and moths reared on C4 maize ($\delta^{13}\text{C}=-13.8$) were distinct from those reared on C3 cotton ($\delta^{13}\text{C}=-30.1$) and pigeon pea ($\delta^{13}\text{C}=-30.6$). The host plant that larvae were reared on in the laboratory also affected $\delta^{15}\text{N}$ values ($F_{2,26}=257.6$; $p<0.001$; Figure 3B), and moths reared on the three plants had different values from each other (maize, $\delta^{15}\text{N}=-0.3$; cotton, $\delta^{15}\text{N}=14.2$; pigeon pea, $\delta^{15}\text{N}=6.3$).

3.4 | Seasonal Patterns of Host Uses Shown by Isotopes

3.4.1 | Carbon

A total of 430 *S. litura* moths collected from moth traps in the irrigation area between April 2023 and June 2024 were subject to stable isotope analysis (Table S2). The frequency distribution of

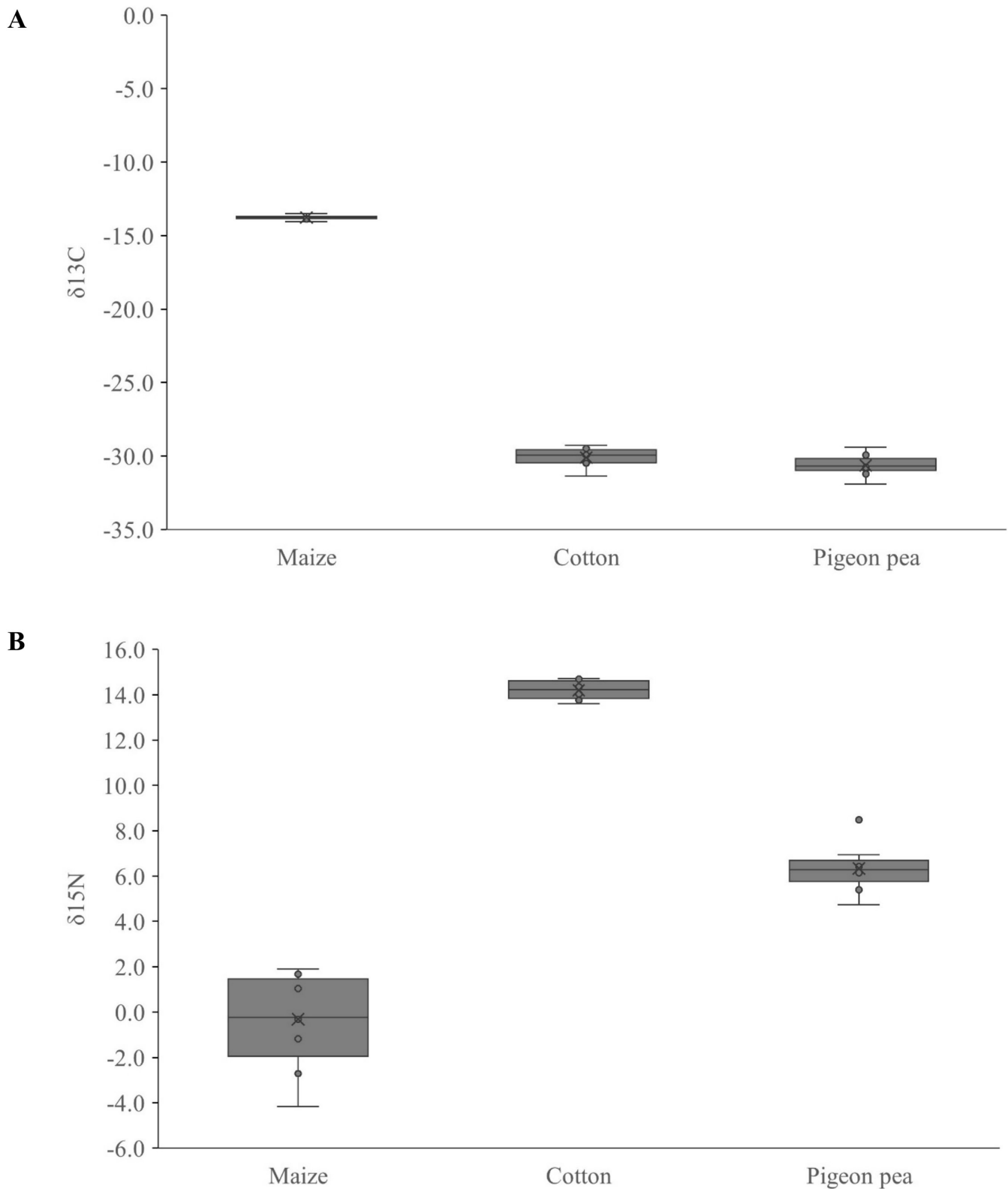


FIGURE 3 | Box and whisker plots showing $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) isotope values of moths reared on maize, cotton and pigeon pea. Boxes represent the interquartile range (IQR), with the median indicated by a horizontal line. Whiskers show the range excluding outliers, which are plotted as individual points.

$\delta^{13}\text{C}$ values fell into two distinct groups (Figure 4). The predominant group, representing 92% of the samples, exhibited values between -25 and -32 , whereas a second group, comprising 5% of the samples, showed values between -14 and -16 (Figure 4).

One moth exhibited a $\delta^{13}\text{C}$ value of -8 , indicating that its carbon was derived from a C4-based diet with a more enriched isotopic signature than that observed in the laboratory-reared insects fed on maize (Figure 3A). A small number of samples were

positioned between these peaks (Figure 4), possibly indicating that larvae developed on a mixed diet comprising both C3 and C4 plant hosts.

There was no overlap of $\delta^{13}\text{C}$ values of insects reared on C3 plants (cotton = -30.1 ; pigeon pea = -30.6) and those reared on a C4 plant (maize = -13.8). Based on these known values (Figure 3A) and the distinct peaks observed in the field-collected moths (Figure 4), individuals with $\delta^{13}\text{C}$ values between -25 and -32 were inferred to represent predominantly C3-derived diets, whereas those with values between -8 and -16 were inferred to represent predominantly C4-derived diets. Individuals with $\delta^{13}\text{C}$ values between these ranges were considered indicative of potentially mixed feeding on both C3 and C4 host plants.

The proportion of moths that predominantly fed on C3 or C4 host plants as larvae, or those that likely fed on mixed C3 and C4 host plants varied across the season (Figure 5). In April and May 2023, most moths (97% and 93%, respectively) had values indicating larval feeding on C3 plants. In June 2023, most moths (90%) continued to reflect a predominantly C3 plant larval diet, whereas 10% of moths had values suggesting a mixed larval diet of C3 and C4 host plants. By September and November 2023, as the cropping season ended, the proportion of moths feeding predominantly on C3 plants as larvae increased to 92% and 100%, respectively. During the wet season in February 2024, outside the primary cropping period, the highest proportion of moths showing predominantly C4 host plant use as larvae (25%) was recorded (Figure 5). At this time 14% of moths had values indicating a larval diet of mixed C4 and C3 host plants, but most moths (61%) had $\delta^{13}\text{C}$ signatures

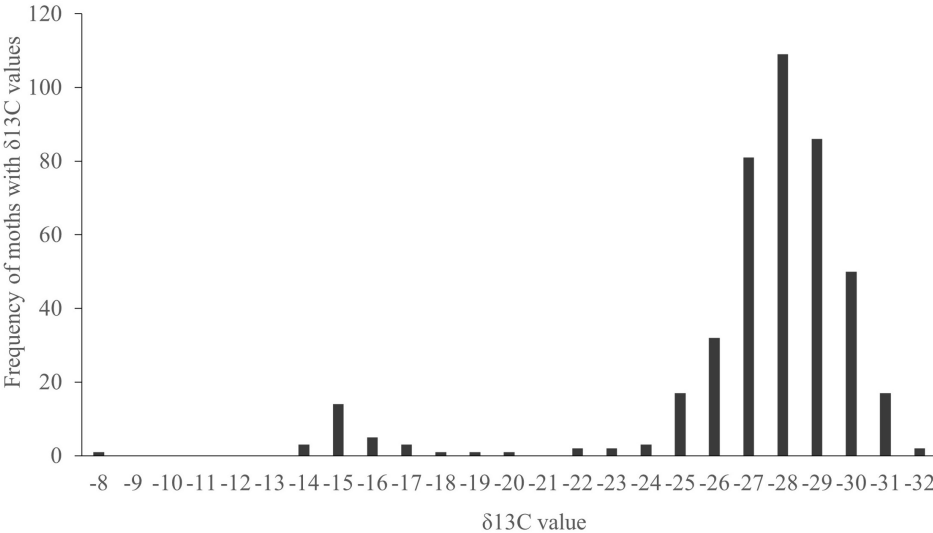


FIGURE 4 | The frequency of $\delta^{13}\text{C}$ values from the wing samples of 430 moths collected from traps within the Ord River Irrigation Area between April 2023 and June 2024. The x-axis indicates the possible range of $\delta^{13}\text{C}$ values, whereas the y-axis indicates the number of moths that had these $\delta^{13}\text{C}$ values.

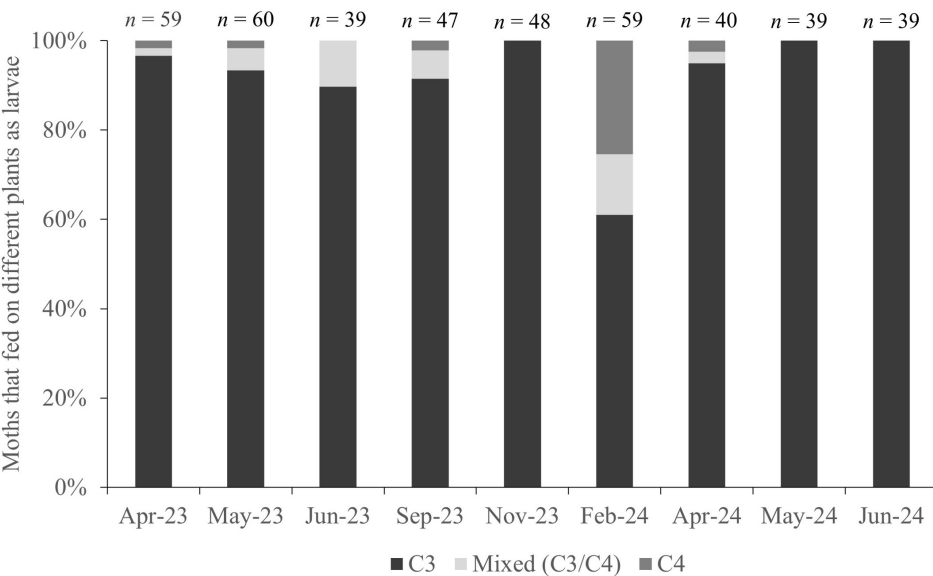


FIGURE 5 | Percentage of insects that likely developed on diets consisting of predominantly C3, C4 or mixed (C3 and C4) host plants at key time points within the Ord River Irrigation Area from April 2023 to June 2024.

that indicated that they had developed predominantly on C3 host plants as larvae (Figure 5). In April 2024, 95% of moths had values indicating larval feeding on predominantly C3 host plants, increasing to 100% in May and June 2024 (Figure 5).

Comparison of cultivated C4 crop area with inferred larval host use showed contrasting seasonal patterns (Figure 6). Although C4 crops accounted for 52%–71% of the cultivated crop area between May and November 2023, relatively few moths had $\delta^{13}\text{C}$ values indicative of predominantly C4 or mixed C3 and C4 larval host use during this period. Conversely, the highest proportion of $\delta^{13}\text{C}$ values which suggested a larval diet of predominantly C4 host plants (25%) or mixed C3 and C4 host plants (14%) occurred in February 2024, when no cultivated C4 crops were growing in the irrigation area (Figure 6 and Figure S3). In May and June 2024, C4 crops accounted for 63%

and 59% of cultivated crop area, respectively, although all analysed moths had $\delta^{13}\text{C}$ values indicative of a predominantly C3 larval diet (Figure 6).

3.4.2 | Nitrogen

The frequency distribution of $\delta^{15}\text{N}$ values had one distinct but broadly distributed group (Figure 7). This group, comprising 94% of the samples, exhibited values between 3 and 11, encompassing the mean $\delta^{15}\text{N}$ value of laboratory-reared insects fed pigeon pea (6.3) (Figure 3B). Overall, field-collected moths exhibited $\delta^{15}\text{N}$ values ranging from 0.2 to 21.6, a range that extended from just above the laboratory-reared maize mean (−0.3) to above the laboratory-reared cotton mean (14.2) (Figure 3B).

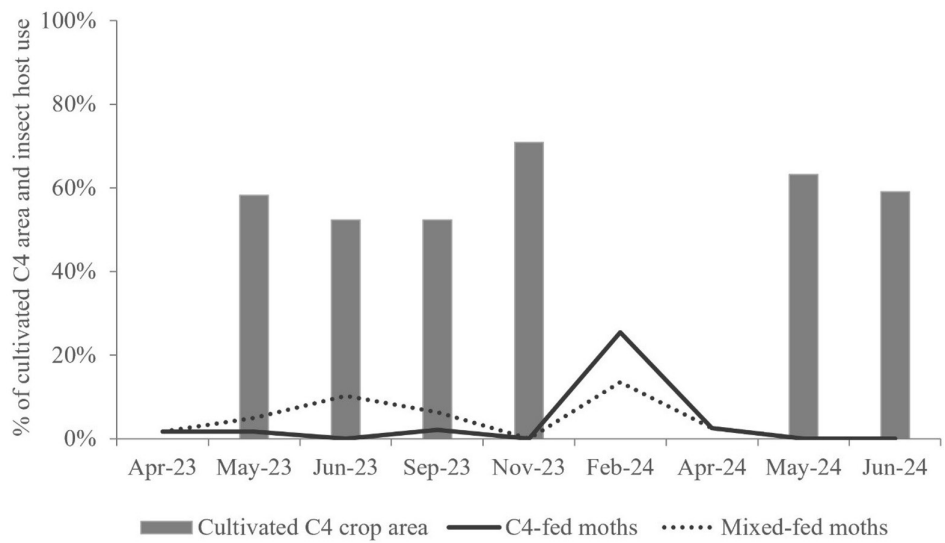


FIGURE 6 | Estimated percentage of cultivated crop area comprising C4 plants and the percentage of *Spodoptera litura* moths with $\delta^{13}\text{C}$ values indicative of predominantly C4 or mixed C4/C3 larval host use within the Ord River Irrigation Area from April 2023 to June 2024.

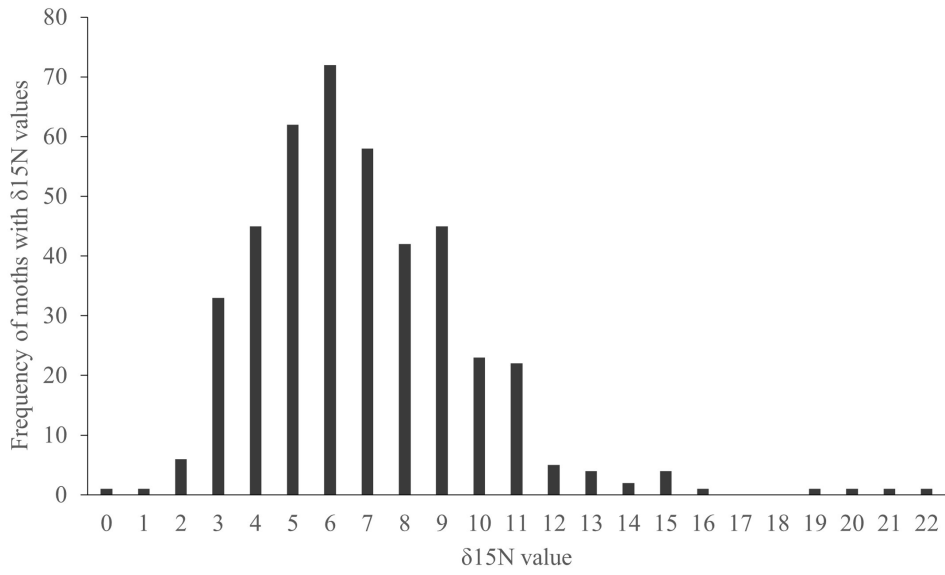


FIGURE 7 | The frequency of $\delta^{15}\text{N}$ values from the wing samples of 430 moths collected from traps within the Ord River Irrigation Area. The x-axis indicates the possible range of $\delta^{15}\text{N}$ values, whereas the y-axis indicates the number of moths that had these $\delta^{15}\text{N}$ values.

3.4.3 | Groupings of Samples

Principal component analysis (PCA) was undertaken to explore whether samples formed natural groupings based on their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The first two principal components explained 100% of the total variation in the dataset (PC1: 62.6%, PC2: 37.4%). PC1 was primarily associated with carbon, whereas PC2 reflected variation associated with both carbon and nitrogen. Density-based clustering of the PCA scores identified a single dominant cluster across varying density thresholds, suggesting that variation with the samples occurs along a continuous gradient rather than forming distinct sample groupings (Figure S4). Similarly, visualisation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed substantial overlap among farms (Figure S5A) and collection dates (Figure S5B). Although February 2024 contained a greater proportion of moths with $\delta^{13}\text{C}$ values consistent with larval development on C4 host plants, these individuals remained part of the overall continuous isotope distribution rather than forming a distinct multivariate group.

4 | Discussion

The timing of cotton production in the ORIA was changed to the dry season, in part to minimise the exposure of lepidopteran populations to Bt proteins (Yeates et al. 2013). However, findings from this study suggest that the abundance of *S. litura* moths remains relatively high during the dry season when cotton is actively grown (Figure 2), resulting in continued exposure to Bt proteins. Moth abundance sharply declined from October 2023 and remained low throughout the wet season until February 2024 (Figure 2), a period that coincides with absence of actively growing Bt cotton in the landscape. Therefore, the risk of *S. litura* populations being exposed to Bt proteins appears greatest during the dry season, when moths are highly abundant within the irrigation area and BG3 cotton is actively growing in the field.

Although patterns in moth abundance in the rangelands were broadly similar to those observed in the irrigation area, the timing of population declines occurred earlier. Moth abundance in the rangelands was low in May 2023, peaked in February 2024 and gradually declined through to July 2024, with moths captured in traps remaining very low thereafter (Figure 2), likely reflecting changes in the availability of seasonal plants. A resource bottleneck is likely to occur when native vegetation dries off (James et al. 1999) or is destroyed through prescribed burns for land management (Environmental Protection Authority 2006), reducing the availability of host plants in the rangelands during the dry season.

It would be expected that the abundance of *S. litura* moths in the rangelands follows this seasonal pattern; however, the timing and persistence of populations into the dry season may vary annually depending on the quantity of rainfall and its distribution. Whether, and to what extent, this resource bottleneck in the rangelands influences local migration and the populations within the irrigation area is unclear. Determining the extent of movement between the rangelands and irrigation area would improve understanding of the contribution of moths developing outside the cropping system to populations within the ORIA.

Similar to other lepidopteran species (Baker and Tann 2013; Dittmore et al. 2023; Gould et al. 2002; Santos et al. 2023), stable isotopes could be used to differentiate whether *S. litura* larvae developed on C3 or C4 host plants (Figure 3A). Experimental insects reared on maize (C4), cotton (C3 non-legume) and pigeon pea (C3 legume) showed distinct $\delta^{13}\text{C}$ values enabling separation between C4 and C3 plants (Figure 3A). Further discrimination between legume and non-legume C3 plants was achieved through $\delta^{15}\text{N}$ values (Figure 3B). Legumes typically exhibit lower $\delta^{15}\text{N}$ values than non-legumes due to nitrogen fixation (Wanek and Arndt 2002), a pattern reflected in larvae, as demonstrated by Ambika et al. (2005) and this study (Figure 4B). These findings contrast with Baker and Tann (2013), who reported no differences in $\delta^{15}\text{N}$ values in *H. armigera* moths reared on cotton or pigeon pea. They attributed this to either poor nitrogen fixation in pigeon pea or the common practice of injecting anhydrous ammonia into cotton production soils, as a nitrogen fertiliser (CRDC 2018), masking isotopic differences between host plants (Baker and Tann 2013). In contrast, the use of more conservative nitrogen inputs, primarily urea, in Kununurra, likely in response to the greater uncertainty in the availability of soil nitrogen following the wet season due to variable rainfall and mineralisation rates (Davies et al. 2007), may explain the clearer $\delta^{15}\text{N}$ separation observed in this study. Continued establishment of baseline $\delta^{15}\text{N}$ values is critical for assessing whether C3 legumes and non-legume plants, such as pigeon pea and cotton, remain distinguishable in adult moths, particularly if nitrogen management practices change within the farming system.

Despite C4 plants comprising up to 71% of the total cultivated cropping area during the season, this was not reflected in the carbon isotope values of moths collected from traps within the irrigation area (Figure 6). From April to November 2023, only a small proportion of individuals exhibited $\delta^{13}\text{C}$ values resembling the maize (C4) control, ranging from 0% to 2.5% at key points across the cropping season (Figure 6). Although maize was the largest annual crop sown by area (Table 1) and is a known host plant of *S. litura* (Ahmad et al. 2013; Maharjan et al. 2023), larval development on this crop is likely reduced due to insecticide applications targeting *S. frugiperda*, which established in northern Australia in 2020 and has a strong preference for maize (Volp et al. 2022).

The highest proportion of moths with values suggesting larvae predominantly fed on C4 plants (25%) was observed in February 2024 (Figure 6), when no cultivated C4 crops were present. A plausible explanation is that these moths may have developed as larvae on uncultivated and/or tropical grasses, which become widespread across the irrigation and rangeland landscape during the wet season and subsequently dry off during the dry season. However, C4 photosynthesis is not restricted to grasses, and C4 dicotyledonous plants, including some plant species in the families Chenopodiaceae, Aizoaceae and Portulacaceae, also occur (Gregg et al. 2019). Chenopods are common in the broader Kimberley region (Cresswell et al. 2011) and have previously been proposed as a potential source of C4 isotope signatures in *Helicoverpa punctigera* Wallengren (Lepidoptera: Noctuidae) where development on grasses is unlikely (Bawa et al. 2021).

PCA and visualisation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed substantial overlap among farms and collection dates (Figure S5A,B),

despite the bimodal distribution of $\delta^{13}\text{C}$ values. Rather than forming discrete multivariate groups, isotope signatures formed a continuous distribution. This suggests that larval host plant use occurs across a continuum of resources within the landscape rather than representing distinct ecological populations associated with particular farms or collection periods, consistent with the highly polyphagous nature of *S. litura*. Carbon isotope values of moths collected from pheromone traps indicated that larvae predominantly fed on C3 plants, suggesting that one or more host plants using the C3 photosynthetic pathway are likely to contribute substantially to *S. litura* populations within the ORIA (Figures 5 and 6). Furthermore, the predominant grouping of nitrogen isotope values ($\delta^{15}\text{N}$ range between 3 and 11; Figure 7) most closely aligned with values from experimental insects reared on pigeon pea, a C3 legume crop ($\delta^{15}\text{N}=6.3$; Figure 3B), rather than those reared on cotton or maize (Figure 3B). However, as pigeon pea is not considered a host plant (Purwar et al. 2007) and larvae have not been observed in local refuge crops (Grundy et al. 2020), this similarity may indicate feeding on other leguminous plants. The broad range of nitrogen isotopic values further suggests that multiple plant species contribute to the larval diet of *S. litura* rather than a single dominant host plant.

In Kununurra, the primary cultivated C3 crop is BG3 cotton (Table 1). Although BG3 cotton has demonstrated high efficacy (>99% mortality) against *S. litura* larvae in laboratory bioassays (Holman et al. 2025), small numbers of individuals did survive and complete development under experimental conditions. Some trap-collected individuals exhibited carbon and nitrogen isotopic values similar to those of experimental insects reared on non-Bt cotton. Structured refuges are integral to resistance management strategies of Bt cotton production in Australia and are designed to delay resistance in *H. armigera* (Knight, Grundy, et al. 2021; Knight, Head, and Rogers 2021). However, in 2023 and 2024, only 8 and 23 ha of unsprayed non-Bt cotton refuge, respectively, were planted on a single farm in the region, with the remainder of structured refuges planted to pigeon pea (Pers. comm. J. Papaioannou 2024). Consequently, non-Bt cotton represented only a minor component of the landscape and was therefore unlikely to have been a major source of the *S. litura* population. However, the extent to which BG3 cotton contributed to the local *S. litura* population remains uncertain. To investigate whether larvae can complete development on BG3 cotton, future studies could analyse moth samples for gossypol (Head et al. 2010; Orth et al. 2007). However, as larvae feeding on native hibiscus species could also result in gossypol-positive individuals, investigations are needed to assess the specificity of this method for use in regions, such as Kununurra (Craven et al. 2003; Wilson 1974), where native hibiscus species are prevalent. In addition to cotton, various horticultural crops represent key C3 plantings in Kununurra (Table 1). However, when considering the intensive insecticide programmes typically associated with horticultural production (Navarro et al. 2021), it seems unlikely that a substantial proportion of the moths originated from these crops.

Three hypotheses warrant further investigation regarding potential C3 host plants for *S. litura* in the ORIA. First, legume crops such as chickpea and mungbeans may contribute to the

recruitment of *S. litura*. However, these crops are cultivated over limited areas and during a restricted period of the year (e.g., mungbeans are typically planted in January–February and harvested in April). Additionally, *Helicoverpa* species are also a key pest of these crops and managed by synthetic insecticides (Bird et al. 2018; Sharma et al. 2010). Second, uncultivated C3 plants, such as weeds, particularly leguminous weeds, may contribute to the recruitment of *S. litura*. As the season progresses, the reduction in irrigation, defoliation/desiccation and harvest of crops likely reduces weed availability, potentially contributing to the decline in *S. litura* abundance within the ORIA (Figure 2). Other *Spodoptera* species can survive on weeds, particularly in the absence of their preferred host plants or during the non-production periods ('off-season') (Adnan et al. 2024; Fortuna et al. 2024; Moraes et al. 2020). Third, tree crops such as mango and sandalwood, which are widely planted in the region and have been reported as hosts for *S. litura* overseas (Choi et al. 2013; Gupta et al. 2015; Padma et al. 2023), may serve as hosts in Australia, although this is untested. Future research could examine whether these crops serve as host plants by assessing the presence of larvae. Surveys should also target weeds, which are commonly found in agricultural fields (Khan et al. 2022; Manalil et al. 2017; Walker et al. 2005), alongside field edges and irrigation channels.

Factors beyond host plant availability may also contribute to the observed decline in the abundance of lepidopteran moths as determined through moth traps (Figure 2). These include increased mortality due to natural enemies present in the landscape (Langellotto and Denno 2004; Letourneau et al. 2009; Tscharnkte et al. 2007), impact of weather conditions, such as temperature, on development (Fand et al. 2015; Fu et al. 2025) and the influence of wind direction on adult dispersal and movement (Wakamura et al. 1990).

Together, the abundance and isotope results have potentially contrasting implications for resistance management (Figures 2 and 6). The high abundance of *S. litura* during the cotton production period increases the opportunity for exposure to Bt proteins and subsequent selection (Griffitts and Aroian 2005). Conversely, if a substantial proportion of the population develops on uncultivated or other non-Bt host plants, these hosts could function as an unstructured refuge, potentially contributing susceptible alleles to the population and diluting resistance alleles (Jin et al. 2015; Li et al. 2017). Moths migrating from the surrounding rangelands may provide an additional source of individuals that have remained unexposed to Bt proteins for several generations. Recruitment from non-Bt hosts within and surrounding the irrigation area may therefore partially counter the increased opportunity for Bt selection associated with the high abundance of *S. litura* during the cotton production period. However, the extent of this effect is unknown and will depend on the relative contribution of different host plants to the population and the movement and mating of moths across the landscape. Further research is therefore needed to determine the importance of these interacting factors for resistance management.

The experimental insects provide a reference for what trap-collected moths might look like isotopically if they had fed on the same host plants. The C3, C4 and mixed-diet categories assigned

to field-collected moths were inferred from the isotope values obtained from the experimental host plants in conjunction with the distribution of $\delta^{13}\text{C}$ values in field-collected moths. These categories should therefore be considered indicative rather than definitive classifications of larval host plant use. The number of control replicates was limited to nine moths for each plant species assessed, and the experimental data are only representative of the specific plants tested rather than all C4 or C3 species, or all legumes and non-legumes. Consequently, characterising the carbon and nitrogen isotope ranges of other plants cultivated within the region, such as chickpeas and mungbeans, as well as prominent uncultivated plants, would assist in assessing the broader dietary classifications used in this study and improve the interpretation of potential larval host use. Although the findings are preliminary, they nonetheless represent the first assessment of *S. litura* population dynamics and possible types of plants used as larval hosts in Kununurra, WA, using stable isotope analysis. The study therefore provides a baseline for future ecological research on *S. litura* in northern Australia and highlights key directions for further investigation, particularly in relation to potential host plants, seasonal resource bottlenecks and landscape-level movement in sub-arid tropical climates.

In conclusion, the abundance of *S. litura* moths within the ORIA, in north-eastern Western Australia, remains relatively high during periods when cotton is actively grown, resulting in populations having continued exposure to Bt proteins. The isotope analysis of moths suggests that the larval diet is not predominantly C4 plants as would be expected based on the crop composition across the irrigation area. Despite C4 plants, such as maize and sorghum, comprising a large proportion of the total cultivated plants, few moths had carbon isotope values that indicated larval development on these plant species. Instead, C3 plants appear to be contributing substantially to the development of *S. litura* populations within the irrigation area, particularly relative to their representation among cultivated crops. Further research is needed to identify which C3 plants support these populations, with targeted surveys on plants potentially helping to identify the important sources of recruitment.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The mean monthly maximum and minimum temperature (°C), recorded from 1986 to 2024, and mean monthly rainfall (mm), recorded from 1971 to 2024, for Kununurra, Western Australia. Climate data from Australian Government Bureau of Meteorology (Kununurra Aero; site number: 002056) [http://www.bom.gov.au]. **Figure S2:** Location of moth trap sites in the rangeland area in relation to the Ord River Irrigation Area (ORIA), Western Australia. The white outline indicates the ORIA boundary. Sites 1–6 were located within the irrigation; GPS locations for these sites are not provided to protect the privacy of individual farms. Sites 7–19 were located in the surrounding rangeland. Site numbers, distances and directions from the ORIA are provided in Table S1. **Figure S3:** Estimated percentage of cultivated C3 and C4 plants within the Ord River Irrigation Area (ORIA) from April 2023 and June 2024. **Figure S4:** PCA of carbon and nitrogen values of the 430 trap collected moth samples. Samples are plotted in two-dimensional PCA space based on the first two components. PC1 is primarily associated with carbon, while PC2 represents a contrast between carbon and nitrogen. The continuous spread of points indicates a gradient of variation with no distinct groupings, consistent with the density-based clustering results. **Figure S5:** The visualisation of carbon and nitrogen values of 430 moths collected from six farm sites (A) and over nine points in time (B). **Table S1:** Study site

number, location, distance from the Ord River Irrigation Area (ORIA) boundary, aspect and GPS coordinates of moth trapping sites within the ORIA and surrounding rangeland, Western Australia. GPS coordinates for farm sites are withheld to protect landholder privacy. **Table S2:** The number of moth subsamples collected for stable isotope analysis from study (farm) sites within the Ord River Irrigation Area (ORIA), Western Australia, collected at nine different time points from April 2023 to June 2024.