RESEARCH PAPER

Adding non-floral resources increases wild insect abundance but not yield in Australian hybrid carrot crops

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ABSTRACT

Many insect pollinator-dependent crops heavily rely on managed honey bees for pollination, yet flies and other wild taxa can be highly abundant and efficient, thus having potential as complementary pollinators. However, unlike bees, fly pollinator life history requirements and foraging behaviour are often unsupported in agro-ecosystems, or completely unknown. We aimed to determine the effectiveness of different non-floral resources in attracting fly pollinators and supporting their life history requirements, and whether higher fly abundance would result in improved seed yield in hybrid carrot crops. We introduced three decomposing organic resources (carrion, manure, and carrot plant material) to four commercial carrot farms in Northern Tasmania, Australia and sampled fly activity on carrot umbels around each treatment. In total, we recorded 46 adult insect species visiting carrot umbels across all treatments, consisting of 32 Diptera (flies), eight Coleoptera (beetles), four Hymenoptera (bees, wasps and ants), and two Hemiptera (true bugs). We collected 10 fly species and one beetle species as larvae from the resources. Both the carrion and carrot treatments supported five different larval species, while the manure supported two. Only *Lucilia sericata, Australophyra rostrata*, and *Oxysarcodexia varia* were more abundant on carrot umbels around treatments compared to the control, and seed yield around the treatments did not differ to the control. Our results suggest that the inclusion of non-floral resources can fulfil the life history requirements of flies in agroecosystems. However, determining whether the addition of non-floral resources also results in increased yield, requires further investigation.

Introduction

Pollination is a crucial ecosystem service, facilitating the reproduction and dispersal of flowering plants (Kevan & Viana, 2003). Insects play a pivotal role in pollination, serving as primary pollinators for a wide range of plant species (Kearns & Inouye, 1997). This dependency on insect-mediated pollination is of vital importance to horticultural crops, where approximately 80 % of crops are reliant on insect pollination for adequate yields (Aizen et al., 2019). Currently, the European honey bee (*Apis mellifera* Linnaeus 1758) is the primary managed pollinator utilised globally to pollinate most global food crops (Hung et al., 2018). Honey bees are ideal managed pollinators due to their high flower visitation rates and ease of hive management and relocation (Roquer-Beni et al., 2022). However, global reliance on honey bees as primary managed pollinators is concerning, especially as honey bees face several threats such as disease, pathogens, pesticide usage, and *Varroa* mite, all of which adversely affect honey bee health and pollination efficacy (Goulson et al., 2015; Klein et al., 2007; Meixner, 2010; Vanengelsdorp et al., 2008). Other insects are known to visit crop flowers and greater research is needed to determine if their life history requirements can be supported in agricultural landscapes and the extent to which they can be used as supplementary pollinators to support global food security (Osterman et al., 2021).

Wild non-bee insect taxa are understudied crop pollinators that could be incorporated into management practices to supplement managed honey bee pollination on farms (Rader et al., 2020). Among these non-bee taxa, flies are often the most common pollinators observed, and have been documented pollinating crops such as avocado, mango,

blueberry, macadamia, and carrot (Cook et al., 2020b). Research has shown that flies can be efficient crop pollinators (Jauker & Wolters, 2008), more active than honey bees under certain conditions (Rader et al., 2013), and can simultaneously fulfil other beneficial ecological roles such as biocontrol agents (e.g., feeding on pest aphids) (Dunn et al., 2020). Trials have shown flies have been able to produce adequate yields of seed and/or fruit in avocado (Cook et al., 2023), blueberry (Cook et al., 2020a), hybrid celery and fennel (Sánchez et al., 2022a), watermelon (Sánchez et al., 2022b), onion (Currah & Ockendon, 1983), hybrid carrot (Howlett, 2012), mango (Saeed et al., 2016), strawberry (Hodgkiss et al., 2018), and sweet pepper (Jarlan et al., 1997); however, experiments focused on flies as managed pollinators have generally focused on mass releasing laboratory-reared flies within a limited spatial scale (e.g. caged single tree or green house) (Dunn et al., 2020). Additionally, unlike honey bees, most flies require additional resources apart from nectar and pollen to fulfil their life history requirements (Davis et al., 2023a). For example, the common fly pollinator *Eristalis tenax* (Linnaeus, 1758) requires organic matter that is partially submerged in water for larval growth and development (Howlett & Gee, 2019), while several blowfly species require decomposing carrion for reproduction and larval development (Dawson et al., 2021). Therefore, retaining and maintaining fly populations in agroecosystems would be difficult as flies would leave to seek these additional habitat resources. The complex life history of fly pollinators has yet to be fully incorporated into experiments investigating the utility of these insects as supplement pollinators in agroecosystems.

Additional resources could be added to the crop landscape to support the life history of the targeted fly pollinators. Supporting and promoting fly populations, and insects in general is critical for maintaining ecosystem function, especially as insect populations decline globally due to several factors like deforestation and land-use changes (Wagner et al., 2021). While it has been shown that the addition of floral resources, such as floral strips enhances insect biodiversity and pollination (Carvalheiro et al., 2012; Desaegher et al., 2021; Feltham et al., 2015; Muñoz et al., 2021), the importance of non-floral resources, such as decomposing organic matter for retaining and supporting pollinator diversity has received comparatively less attention (Cook et al., 2020b). Many fly species observed visiting and pollinating crops require decomposing organic matter for larval development (Davis et al., 2023a). Adding non-floral resource could be an effective strategy to support fly populations in agroecosystems and thereby maintain key ecosystem services like pollination (Davis et al., 2023b).

Further research is required to determine the effectiveness of non-floral resources in attracting and retaining fly pollinators in different crop systems. In this study, we investigated how introducing three different non-floral resources to hybrid carrot crops influenced insect pollinator activity and seed yields. Specifically, our study addresses the following three research questions:

1) Does the introduction of non-floral resources support flower visiting insects within carrot seed crops?
2) Does the composition of flower visiting insects vary with different non-floral resources?
3) Do these non-floral resources result in improved seed yields around the non-floral resources?

Materials and methods

Study site

We conducted this study at four commercial hybrid carrot crops (*Daucus carota* L) around the Longford region in Tasmania (TAS), Australia. The four sites were located a minimum distance of 7 kms away from each other and all had managed honey bee hives on site for pollination. The sites ranged in size from 14.8 ha to 26.8 ha and were located in an area dominated by commercial agriculture, surrounded by other crops, livestock, and patches of dry eucalypt woodland. Each site consisted of a single carrot cultivar, with a different cultivar planted at each site (see Appendix A: Table S1 for site details). In hybrid carrot seed production systems, two parent plant lines, the male fertile (pollen-producing) plants and the male sterile (seed bearing) plants are grown in alternating strips within fields. This is to facilitate cross-pollination between the two parent lines, as pollen from the male fertile line must be transferred to the male sterile flowers for successful pollination to occur. Therefore, the plant is dependent on insects for pollination to move the pollen between the plant lines. Currently, managed honey bees are the main pollinator used in hybrid carrot seed crops, however, crop yields are sometimes low, potentially due to a shortfall in pollination (Broussard et al., 2017). To overcome this shortfall, a high hive density is often used, but even this may be insufficient for some varieties (Quarrell et al., 2023).

Experimental design

Three resource treatments and a control were used in this study, decomposing kangaroo mince ('carrion'), horse manure ('manure'), carrot plant material ('carrot'), and a control consisting of no resource ('control'). These resources were chosen as they were most likely to attract fly species that were previously observed on carrot umbels in field surveys in TAS (Gaffney et al., 2011). The kangaroo mince was purchased from Greens Quality Meats, Dynnyrne TAS, horse manure from Margate, TAS and the carrot plant material was collected on site at each trial location. We placed 1 kg of each resource in a 5-litre bucket with 500 ml of tap water and 500 g of soil collected from each trial location, while an empty bucket was used for the control treatment. On-site soil was incorporated into each resource to introduce local microbes to facilitate decomposition and release of volatile organic compounds (Crippen et al., 2015). Only 1 kg of resource was used per replicate as we were interested in determining which species were attracted to the resources and used the resources as a breeding substrate, not whether the resources could support a large quantity of larvae. A lid was secured to the top of the bucket to prevent access by scavengers and three 2–3 cm^2 holes were cut along the top edge of the bucket to allow access by insects. The buckets were hung approximately 1 m off the ground on a wooden stake and placed along the outside of the crop field (no >30 cm away from the crop edge) so they were not in the way of machinery and workers. Three replicates of each treatment were placed at each site, totalling 12 replicates per site, and 48 in total across the four sites. Treatments were placed around the whole perimeter of a crop field at a minimum distance of 50 m apart. This distance was chosen due to the size of sites, and it is the minimum distance required to maintain independence between replicates when attracting saprophagous insects (Perez et al., 2015). Treatments were placed in the field on the 16–17th December 2022 just as the primary umbels began to open and resources were left to decompose naturally for 12–13 days before sampling commenced. This time frame was chosen to allow all resources to at least begin decomposing before sampling occurred as different necromass decays at different rates and we wanted to observe what species are attracted to each resource during decomposition (Butterworth et al., 2022).

Sampling

Sampling was conducted between 09:00–15:00 on sunny days with no rain, and sampling occurred at least five times per site. Sampling occurred between 29th December 2022 – 7th January 2023, lasting a total of 12 days. To count pollinator abundance and diversity, a 5 m transect was observed for 5 min at each replicate. Each transect was conducted along the male sterile row, beginning at the edge of the field adjacent to a bucket, and extending toward the centre of the crop field. Only insects that were directly on the carrot umbels were recorded. Species that could not be visually identified to species-level on site were

identified to morphotypes and representatives of each species were collected for identification. Within every transect, the number of blooming umbels were recorded and separated into four categories: 0–49, 50–99, 100–149 & 150+ umbels. Ambient temperature was also recorded at each transect using Kestrel Drop 2 data loggers (Nielsen-Kellerman Company). At the conclusion of the sampling period, on 8th January 2023, all replicate resources were searched for eggs, larvae, and pupae, and representatives of each type present were collected for identification. All collected specimens were stored in 70 % ethanol. Adult specimens were identified to species level where possible, or to the lowest possible taxonomic level (e.g., genus or family) using several taxonomic keys (Colless, 1982; Crosskey, 1973; Johnston & Wallman, 2021; Li & Yeates, 2018; McAlpine, 2012; Pont, 1973; Wallman, 2001) and the online resource "What bug is that?" provided by CSIRO, Australia. *Calliphora stygia* (Fabricius, 1781), and *Calliphora hilli* Patton 1925 were grouped together for analysis as they could not be reliably distinguished in the field. When morphological techniques were not possible, some adult specimens and all larvae were identified using analysis of the cytochrome oxidase 1 barcoding region. DNA extraction, amplification and sequencing were completed commercially using the Australian Genome Research Facility (AGRF; Melbourne, Australia) using the primer pair LCO1490-L & HCO2198-L, previously shown to be successful across a range of Diptera (Gibson et al., 2011; Johnston et al., 2021; Meiklejohn et al., 2013). Sequences were edited using Ugene (Okonechnikov et al., 2012) and compared against sequences deposited in the GenBank database via nucleotide BLAST facilitating species identification.

Carrot seed yield

Yield samples of the number of seeds produced from carrot umbels was also collected. At crop maturity, on the 6th March 2023, 20 primary umbels were collected from a 10 m radius around each replicate and transported to the seedPurity laboratory for yield determination. Primary umbels were only collected from the male sterile row. Harvested samples were packed in net bags and held in a laboratory air drying chamber (30 C° and 25 % RH) for 1 week. After drying, the samples were threshed and debearded using a laboratory thresher (Wintersteiger, Salt Lake City, USA). Seed cleaning was performed using a laboratory sized clipper-cleaner (Blount Agri-Industrial, Indiana, USA) and a South Dakota seed blower (Seedburo, Chicago, USA). Seed yields were determined on a dry weight basis by the low constant temperature method (ISTA, 1993). We calculated the average total seed weight per umbel around each replicate (g/umbel).

Data analysis

To determine the effectiveness of the treatments in attracting pollinators we performed a series of analyses on different metrics of pollination. First, we conducted a generalised linear mixed model (GLMM) to determine how the treatments influenced insect abundance on umbels. We used treatment (categorical variable with 4 levels) as a fixed effect and included ambient temperature as a covariate in the model. We also included a quadratic term for temperature into the model to account for the likely non-linear relationship between insect abundance and temperature due to physiological reasons (Rojano et al., 2021). We included site as a random effect to account for differences between cultivars and we nested replicate ID in site to account for repeated measures. We also included the number of umbels as a random effect in the model to account for an uneven number of umbels between transects. We used a negative binomial distribution and the R package "glmmTMB" (Brooks et al., 2017) to fit the GLMM. The R package "performance" (Lüdecke et al., 2021) was used to assess the model performance. We also conducted a pairwise comparison of the GLMM predicted variables using the R package "emmeans" (Lenth, 2023).

Second, we conducted a permutational multivariate analysis of variance test (PERMANOVA) to determine how the treatments influenced the insect community composition on umbels. We used a Bray-Curtis dissimilarity matrix to calculate the distance matrices, and used treatment as a fixed effect. We also included site and number of umbels as random effects. Rare species, defined as those that had an overall abundance less than or equal to 5 across all samples were removed prior to analysis. A complete species list can be seen in Appendix A: Table S2. We used the R package "vegan" (Oksanen et al., 2022) to conduct the PERMANOVA. We visualised the insect community by plotting the ordination as a non-metric multidimensional scaling (nMDS) plot.

Third, we conducted a Bayesian ordination and regression analysis to determine how individual insect species responded to the treatments. We used the same data (with rare species removed) and variables as the PERMANOVA. We used a negative binomial distribution and two latent variables. We plotted the X-coefficient estimates as effect sizes and interpreted the effects as significant if their 95 % credible intervals did not cross the zero-effect posterior median line. We used the R package "boral" (Hui, 2016) to conduct the Bayesian analysis.

Finally, we conducted another GLMM to determine how the treatments influenced carrot seed yield. We used average dry seed yield (g/umbel) around each replicate as a response variable, treatment as a fixed effect and site as a random effect. We used a gaussian distribution and the same R packages as the previous GLMM. All analyses were performed in R version 4.2.3 (R Core Team, 2023) and plots were created using the R package "ggplot2" (Wickham, 2016).

Results

In total we conducted 205 transect counts and found 46 insect species on hybrid carrot umbels across all treatments, consisting of 32 Diptera, eight Coleoptera, four Hymenoptera and two Hemiptera (Appendix A: Table S2). The sepsid fly *Parapalaeosepsis plebeia* (Meijere, 1906) was found to be the most abundant species on umbels, followed by the blowfly *Calliphora accepta* Malloch, 1927 (Appendix A: Table S2). Honey bees (A. *mellifera*) were the fifth most abundant species observed. Six fly species (*C. stygia, Lucilia sericata* (Meigen, 1826), *Oxysarcodexia varia* (Walker, 1836), *Australophyra rostrata* (Robineau-Desvoidy 1830), *Muscina stabulans* (Fallén, 1817) and *P. plebeia*) were observed visiting carrot umbels and also found as larvae on one or more of the resources provided (Table 1).

Larval species diversity differed between the treatments. In total, we collected larval representatives from 11 insect species. Most species (10)

Table 1
Number of replicate resources where insect larval species collected directly from the decomposing carrion, manure, and carrot material treatments. Total replicates for each resource = 12 and * indicates that the species was also observed on carrot umbels as an adult.

Order	Family	Species	Carrion	Manure	Carrot
Diptera	Calliphoridae	*Calliphora stygia**	12		1
		Calliphora sp.			2
		*Lucilia sericata**	1		
Diptera	Sarcophagidae	*Oxysarcodexia varia**	4		
Diptera	Muscidae	*Australophyra rostrata**	7		
Diptera		*Muscina stabulans**			1
Diptera		*Musca vetustissima*		2	
Diptera	Sciaridae	*Bradysia pallipes*		4	
Diptera	Anisopodidae	sp.			6
Diptera	Sepsidae	*Parapalaeosepsis plebeia**			1
Coleoptera	Histeridae	*Saprininae* sp.	1		

were Diptera, while the remaining one species was a Coleopteran (Table 1). The carrion and carrot resource both had five different larval species, while the manure only had two species. There was only one blowfly species, *C. stygia*, that was found on both the carrion and carrot resource. All other larval species were only collected from a single resource.

We found the abundance of insects on umbels was not significantly different between treatments (GLMM: $p > 0.05$, Appendix A: Table S3). There was a large amount of variation in insect abundance around each treatment, with several outliers of high abundance (Fig. 1 (a)). Average abundance on umbels around the carrot treatment was the highest, while the lowest was around the control. We found that insect abundance on umbels was significantly affected by ambient temperature, with a positive linear effect (GLMM: estimate = 0.925, std. error = 0.126, z value = 7.343, $p < 0.001$) and a significant negative quadratic effect (GLMM: estimate = −0.021, std. error = 0.003, z value = −7.021, $p < 0.001$) (Fig. 1 (b)).

We found treatment did not significantly influence the insect community composition on umbels (PERMANOVA: $F = 1.11$, $p = 0.065$). The insect community on umbels around each treatment did not differ, and there was a large amount of community overlap between the treatments (Fig. 2). As for individual species, most species observed on umbels were not significantly affected by the treatments (Fig. 3). However, *L. sericata*, *A. rostrata* and *O. varia* had significantly higher abundance on umbels around the carrion resource compared to the control. In addition, *L. sericata* also had a higher abundance on umbels around the carrot resource compared to the control. Only the abundance of one species, larvae of the lady beetle *Hippodamia variegata* (Goeze, 1777) was reduced on umbels near the manure resource compared to the control.

We harvested 20 primary umbels around each replicate treatment and found the average clean, dry seed yield from the primary umbels was not significantly different between any treatments (GLMM: $p > 0.05$, Appendix A: Table S4). Average yield around the manure treatment was found to be the highest, while the other three treatments had similar yield (Fig. 4).
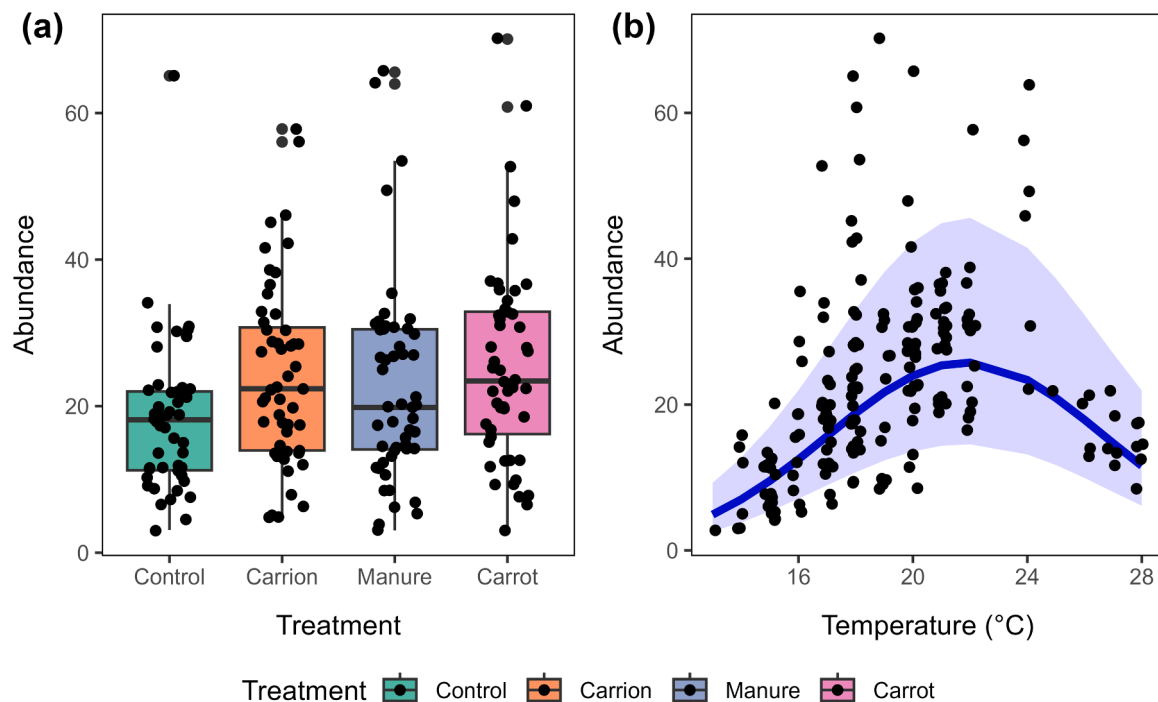

Fig. 2. NMDS plot comparing insect community composition on carrot umbels around the treatments (control (no resource), carrion, manure, and carrot material).

Discussion

We aimed to determine if non-floral resources could support fly pollinator life history requirements in a crop landscape, and whether this would lead to increased insect activity on carrot umbels. Each resource supported the larval development of unique species, thereby highlighting the potential for non-floral resources to maintain biodiversity. Although these resources could be present in the surrounding environment, adding these types of non-floral resources to agroecosystems could increase species distributions and connect habitats given the patchy nature of these resources (Butterworth et al., 2022). Our results are similar to other studies that demonstrate how increasing agroecosystem heterogeneity can promote insect biodiversity (Benton et al., 2003; Fijen et al., 2022) and possibly pollinator activity; however, further examination of the larval species reared in this study is needed to determine if they are able to effectively pollinate carrot crops. Not only

Fig. 1. (a) Predicted abundance of insects on carrot umbels around the treatments (control (no resource), carrion, manure, and carrot material). (b) Abundance of insects on carrot umbels compared to ambient temperature (°C). Smoothed line represents predicted abundance and shaded region represents 95 % confidence intervals. Predicted values and confidence intervals are derived from the GLMM.
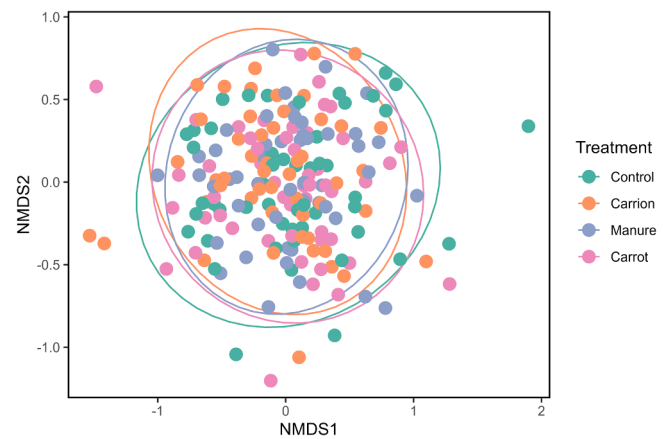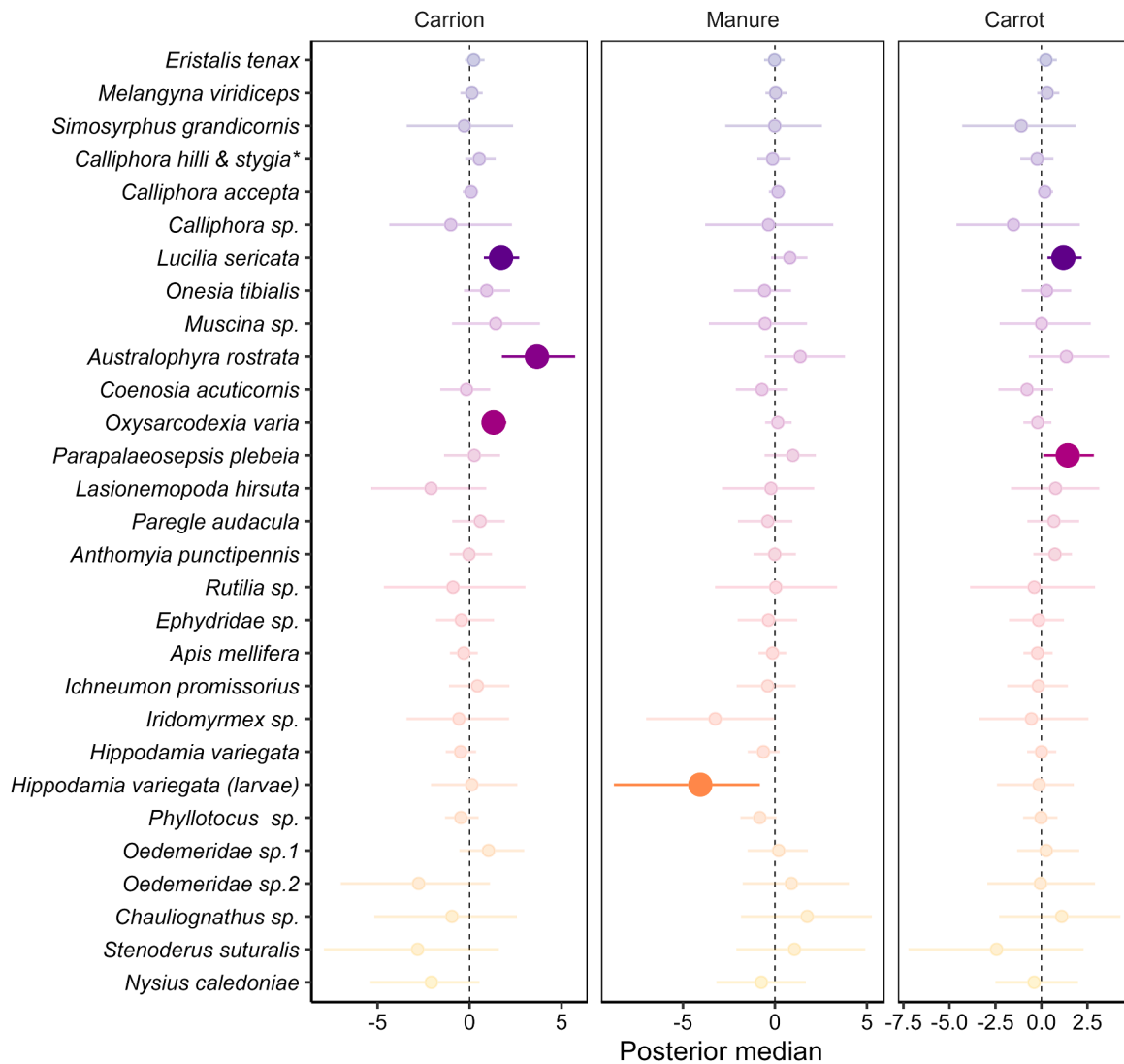
Fig. 3. Bayesian ordination and regression analysis results of the effect of each treatment (carrion, manure and carrot material) compared to the control (grey dotted line) on the abundance of each insect species on carrot umbels. Credible intervals (95 %) which do not cross the zero-posterior median (control line) and displayed as not faded are significant. If significant (not faded), the side of the posterior median where the dot falls represents the direction of significance (e.g. species on the right side of the control line that are not faded had a significantly higher abundance on umbels around that treatment compared to the control). *Calliphora stygia* and *Calliphora hilli* were grouped for the analysis as they could not be reliably distinguished in the field.

can non-floral resources potentially promote biodiversity, but they may also benefit other ecosystem services as many species provide dual ecosystem benefits such as decomposition or biocontrol (Dangles & Casas, 2019; Dunn et al., 2020). Landscape simplification and habitat destruction are some of the leading causes of insect decline globally, but by altering agroecosystem landscapes with non-floral resources, we may be able to support and bolster insect biodiversity and maintain key ecosystem services (Cardoso et al., 2020).

The addition of non-floral resources increased the abundance of three fly species observed on carrot umbels, however most other species were unaffected by the resources, and subsequent seed yield was unaffected. A similar study in mangos used decomposing fish to attract fly pollinators and found no yield benefits, which they hypothesised was due to pollination saturation by a highly abundant hoverfly (Finch et al., 2023). Yield may have been unaffected in our experiment as flies attracted to the resources were potentially not moving between male and female flowers, unable to carry and transfer pollen efficiently (Gaffney et al., 2018), and/or resources were not out long enough or in a large enough quantity. The yield in our experiment is considered above average compared to yields from previous years and other sites, which

suggests that there might not have been a substantial pollination gap at our sites this year. A pollination saturation by managed honey bees could have occurred at our sites as they were located in an area dominated by commercial agriculture. However, it's important to note that in other locations and seasons, yields are low in part due to a pollination shortfall (Broussard et al., 2017).

Alternatively, other factors may have impacted yield including the timing of resource deployment and the scale of the experimental study. The timing of resource placement should be considered when attempting to attract insect pollinators to crops. Insect abundance around ephemeral resources often fluctuates over time (Butterworth et al., 2022). For example, fly abundance around carrion is generally high during the first few days of decomposition (Payne, 1965). As decomposition progresses, adult flies around the carrion become less abundant as they finish ovipositing and the carrion changes in quality and quantity (Dawson et al., 2022). Abundance increases again later in decomposition as a new generation emerges from pupae if the resource is large enough to support larval development. Recently emerged flies may also be more likely to visit flowers as females require a protein source just after emergence to develop their ovaries, which they can acquire from
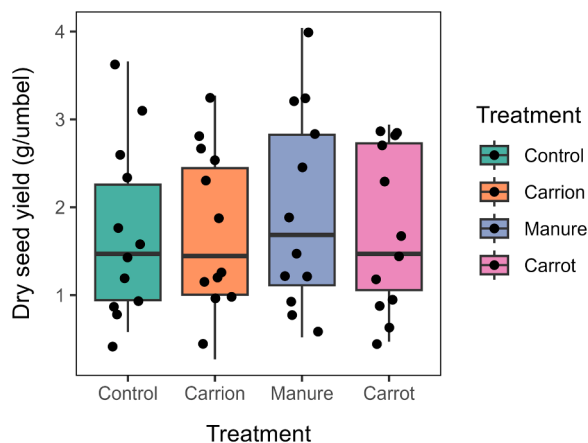
Fig. 4. Dry seed yield (g/umbel) in a 10 m radius around the treatments (control (no resource), carrion, manure, and carrot material). Each data point represents the average yield ($n = 20$ primary umbels per replicate) around each replicate.

pollen (Mackerras, 1933). In our experiment, the resource quantity and duration of resource deployment might not have been sufficient for widespread emergence or alter local fly population abundance and diversity. Future experiments should incorporate a time series-based approach to determine the optimal length of time for resource deployment, and measure fly populations in surrounding landscapes to assess whether pollination services are already sufficient.

The resources in our experiment facilitated the reproduction of several species, and therefore biodiversity benefits in a generally homogenous environment. However, caution must be used when choosing non-floral resources and taxa to target as some species have costly negative impacts. For example, blowfly larvae of *L. sericata* were found in the carrion resource, and adult abundance of *L. sericata* on umbels was found to have increased around the carrion and carrot resource. This species of blowfly can cause myiasis in livestock which can result in the death of the host animal if left untreated (Bambaradeniya et al., 2022; Phillips, 2009). Species like hoverflies which have additional positive impacts such as biocontrol agents should be targeted in future non-floral resource addition experiments (Dunn et al., 2020; Wotton et al., 2019). There are also some potential health risks that need to be examined before some non-floral resources are implemented into agricultural systems. It currently remains relatively unknown if harmful pathogens from decomposing organic matter can be carried by flies, transferred to flowers, and persist from flower to fruit (Förster et al., 2007; Fotedar et al., 1992).

Conclusions

Overall, we have shown there are potential biodiversity benefits in adding non-floral resources to localised crop landscapes as flies were able to utilise the resources for larval development. However, any benefits the resources provide to crop yield require further investigation and is likely dependent on several factors such as the biology and management of the crop system, the non-floral resource used, and the timing of non-floral resource placement. Future experiments should consider these factors carefully, and consider other management practices such as mass releasing reared flies simultaneously to placing resources (Cook et al., 2020b). This would ensure released flies do not disperse quickly out of open crops as they have the necessary resources within the vicinity to fulfil their life history requirements. Understanding fly behaviour, biology, and life history requirements is key to utilizing flies as managed pollinators to supplement honey bees.

CRediT authorship contribution statement

Blake M Dawson: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Nikolas P Johnston:** Writing – review & editing, Methodology, Formal analysis. **Stephanie Cerato:** Writing – review & editing, Investigation. **Raylea Rowbottom:** Writing – review & editing, Methodology, Investigation. **Cameron Spurr:** Writing – review & editing, Methodology, Investigation. **Abby Davis:** Writing – review & editing, Methodology, Conceptualization. **Romina Rader:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2025.01.007.

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