Received: 3 May 2023 | Revised: 22 August 2023 | Accepted: 26 August 2023
DOI: 10.1111/jen.13184

ORIGINAL ARTICLE

JOURNAL OF APPLIED ENTOMOLOGY WILEY

A novel protein-based fruit fly trap in melon flies *Bactrocera cucurbitae* for effective pest control management

Andrew W. McCracken[1] | **Nurah Niazy**[2] | **Samu Turi**[2] | **Vijeth Arya**[3] | **Vivek Kempraj**[3] | **Juliano Morimoto**[2]

[1]Department of Evolution, Ecology, and Behaviour, Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, UK

[2]School of Biological Sciences, University of Aberdeen, Aberdeen, UK

[3]Bioorgo Innovation Centre, Kempmann Bioorganics LLP, Bengaluru, India

Correspondence
Juliano Morimoto, Institute of Mathematics, Fraser Noble Building, University of Aberdeen, Elphinstone Rd, Aberdeen AB24 3UE, UK.
Email: juliano.morimoto@abdn.ac.uk

Present address
Vivek Kempraj, USDA-ARS, Daniel K Inouye Pacific Basin Agricultural Research Center, Hilo, Hawaii, USA
Juliano Morimoto, Institute of Mathematics, University of Aberdeen, Aberdeen, UK

Abstract

Agriculture remains a major source of subsistence for local communities in India. However, agricultural yield can be strongly affected by agricultural pest outbreaks. This can result in economic losses for small-scale farmers who already experience socio-economic challenges, such as lack of appropriate infrastructure and subsides. Sophisticated pest management techniques (e.g. sterile insect technique) are less accessible to small farmers in developing countries, and therefore, alternative cost-effective approaches for pest management are needed. Here, we report our findings of a 3-year field trial (2018–2020) in India, which was designed to test for the potential effectiveness of a novel, slow-release formulation protein-based trap, compared to standard Cuelure traps against melon flies *Bactrocera cucurbitae* (Diptera: Tephritidae). Protein-bait traps can attract flies from both sexes (as opposed to male-only, chemical traps), bearing the potential to have a greater long-term impact on pest populations by decreasing future reproductive potential of trapped individuals. We found that, despite uneven sampling efforts, Cuelure had overall higher trapping performance, while protein-bait traps, despite trapping at lower efficiency, were equally effective for trapping males and females. Simulations with our field data revealed that protein-bait traps can have an 'inclusive' advantage by trapping females and thereby preventing future individuals. Overall, our study highlights the potential benefits of using this alternative trapping technique to supplement pest management in developing countries.

KEYWORDS
agriculture, innovation, IPM, pest control, SDGs

1 | INTRODUCTION

Tephritid fruit flies pose a major threat to global food security (Qin et al., 2015), particularly the melon fly, *Bactrocera cucurbitae*, which infests various crops in Africa, Asia Pacific sub-continent and Southeast Asia (Koyama et al., 2004). Recurring crop infestations by *B. cucurbitae* can have devastating effects for agricultural yield: from 30% to 100%, depending on crop and season (Laskar, 2013). For small farmers in emerging economies such as India, which ranks as the second largest producer of vegetables and fruits in the world,

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Authors Vivek Kempraj and Juliano Morimoto are equal supervisory contributions.

the economic and agricultural consequences can be irreparable and have long-term socio-economic implications for local and global food security (Dastagiri et al., 2013).

Melon fly infestation begins when females oviposit eggs 2–5 mm deep into the flesh of ripe fruit (Patel & Patel, 2018) and where, upon hatching, larval feeding leads to early-onset of fruit decay while still on the plant (Mukherjee et al., 2007). This poses two key problems for farmers. First, once the larvae are feeding on the fruit, pesticide control is inviable since farmers lack access to chemicals that penetrate the fruit flesh, narrowing pest management options once an infestation has started (Dhillon et al., 2005). Second, the product loses its commercial value, resulting in loss of agricultural capital and broader economic sanctions, such as quarantines (Vargas et al., 2016).

Various methods have been developed to manage melon fly and other tephritid fruit fly outbreaks, including sterile insect technique (SIT), field sanitation and pest-resistant crops (Dhillon et al., 2005). However, these sophisticated techniques are costly and inaccessible for small-scale farmers in developing countries who lack the financial means to implement them. Instead, farmers often resort to mass trapping methods due to their low set-up and operating costs (Verghese et al., 2002). A commonly used, cost-efficient trap is the Cuelure, which exploits parapheromones to attract male flies (Shelly & Villalobos, 1995). By trapping males, it is possible to skew sex ratio towards females and lead to a scarcity of mating opportunities, in turn decreasing population size of the pest. As an adjunct to Cuelure, traps employing food-derived attractants have also been created to trap individuals of both sexes (Jang & Light, 2020). These alternative, both-sexes traps are promising, but our understanding of their effectiveness in field trials has been limited due to the lack of large-scale multi-year field data.

Here, we conducted large-scale field trials in India from 2018 to 2020 with a novel slow-release formulation protein-bait trap, and compare its efficacy in capturing *B. cucurbitae* males, relative to Cuelure. We further used simulations to estimate the number of F1 adults protein-bait traps would prevent, considering their ability to trap fertile female flies. These simulations were informed by parameters derived from the published literature on melon fly life-histories and offer insights into the long-term potential of protein-bait trapping for *B. cucurbitae* control in developing regions. Overall, this study provides a basis for further research on the role of protein-bait trapping in pest control; this in turn may aid in future developments of more effective single and combined pest management programmes, ensuring food security at local and global levels.

2 | MATERIALS AND METHODS

2.1 | Traps

The protein-bait traps used in this study (FruitClean®FTS) are of proprietary design, developed by the Bioorgo Innovation Centre (Kempraj et al., 2023a). The traps are a delivery system-embedded device for attracting fruit flies of both sexes for up to 180 days, in all-weather conditions. The delivery system ejects 0.2–0.3 mL/day of protein bait (FruitClean®PB; Kempraj et al., 2023b) – a proprietary kairomone-based formulation specifically designed for attracting *Bactrocera cucurbitae* of both sexes. The trap device has three main parts: a reservoir for the protein bait, a transparent upper hemisphere and a yellow-coloured lower hemisphere. The round shape of the trap and yellow colour synergises with the odour of the protein bait to increase the attraction of flies to the trap (Piñero et al., 2006). Cuelure traps used to capture male flies were also developed by Bioorgo (FruitClean®MLS) but additionally contained a proprietary slow-release formulation of Cuelure, which extends its release beyond 120 days.

2.2 | Field trials

We tested our protein-bait traps against a well-established trapping system – Cuelure. To ensure minimal influence on the likelihood of male capture in protein-bait traps, we minimised the use of control Cuelure traps. Ten protein-bait traps and one Cuelure trap per acre were placed in the borders of cucumber fields in each of the 16 locations across Karnataka, India (N = 11 traps × 16 locations × 3 years = 528). Field areas ranged from 1000–4000 m^2. Traps were installed at a height of 1.5 m, on wooden poles affixed to soil near non-host border crops, with a distance of at least 100 m between traps. Traps remained in the field for 2 months (December to January) each year for the three consecutive years (2018–2020), with deaths recorded every 15 days. Given the slow release of attractants, they remained within traps for the duration of the field trial, without replenishment. We enumerated the total number of male and female flies captured per year, per location, by totalling recorded deaths.

2.3 | Statistical analysis

For all analyses, we standardised the number of trapped male individuals at each location per year with the following formula:

$$s = \frac{(m / t)}{\mu_f}$$

where m denotes the total number of male flies per location; t is the total number of traps per location; and μ_f represents the mean number of flies per trap type across all 3 years. To estimate the relative trapping effectiveness of protein-bait traps, we compared the number of males trapped in Cuelure and protein-bait traps (as Cuelure only traps males). To account for the uneven deployment of the two trap types, we standardised the number of flies within each trap by dividing the total number of trapped flies by the number of traps deployed in a given location. All data were analysed in R version 4.1.0 (R Core Team, 2013). We used linear mixed models from the 'lme4' (Bates et al., 2014) and 'lmerTest' (Kuznetsova et al., 2017) packages to test for differences in the standardised number of trapped males between trap types (i.e. Cuelure vs. protein-bait traps), year and the interaction between these factors. We fitted the standardised number of male flies trapped per trap type (controlling for uneven sampling) as the response variable, location as random effect, and trap type and year as fixed effects. This was followed by an analysis of the differences in the number of females captured in the protein-bait trap using the same approach but this time, comparing between years only (as Cuelure traps are male-specific). Data visualisations were carried out using the 'ggplot2' package (Wickham, 2016).

2.4 | Simulations

2.4.1 | Population size estimate

We estimated the population size of melon flies based on trapped data. This was done in an attempt to characterise whether or not the trapping methods deployed here could have an impact at population level, given the importance of knowing population sizes for appropriate integrated pest management (Binns & Nyrop, 1992). However, precise estimates of population size are difficult to obtain for melon flies and insects more generally (Binns & Nyrop, 1992). To do this, we relied on removal trapping as a method of population density estimation developed by Hayne (Hayne, 1949). Hayne proposed that the number of animals taken during any trapping period may be represented by the following equation:

$$y = p\,(P - x) \text{ or } y = pP - px$$

where P = original population, p = probability of capture, y = number captured during the period (whole year), and x = number previously captured and removed before beginning of period in question. We rearranged the equation to solve for the original population size P, given that the empirical dataset contained measurements for the total number of flies captured during each year. We used estimates of x as the previous years' trapping average (which was 0 for the first trapping year), y as the current years' trapping average and p as a variable for which we simulated values from 0.01 to 1 (i.e. very low trapping probability to perfect trapping probability). This allowed us to estimate P as:

$$P = \frac{px + y}{p}$$

2.4.2 | Simulating the removal of F1 adults from the population

To understand the potential long-term efficacy of the protein-bait trap, we used simulations to estimate the number of viable offspring prevented from introduction into the population, by considering the number of eggs traps that would prevent from being laid (via the trapping of female flies). A set of assumptions for the simulation was generated by collecting life-history information on *B. cucurbitae* from a review of existing literature (Balas et al., 2018; Gaddanakeri & Rolania, 2020; Harwood et al., 2013; Kaur & Rup, 2002; Laskar, 2013; Mir et al., 2014; Mukherjee et al., 2007; Patel & Patel, 2018; Pradhan et al., 2020; Rahaman et al., 2015; Sohrab et al., 2018; Vayssières et al., 2008; Yang et al., 1994). Focusing on laboratory studies using fly populations of Indian origin, we collated a master table (Table 1), which was used to extract values relating to length of each life stage, fecundity and lifespan of *B. cucurbitae* females. A standard arithmetic mean was used on the collated life-history traits, to determine the relevant simulation parameters. We simulated the distribution of lifespan for females in the population using a Poisson distribution, since studies show that it approximates age distributions of flies in nature (Tasnin et al., 2021). Our simulations operated under a number of assumptions:

(i) The lifespan of flies within the hypothetical population follows a Poisson distribution of $\lambda = 56.2$.
(ii) There is a uniform likelihood of capture across all ages for flies within the population (e.g. flies are as likely to be captured at age 10 days as at age 40 days).
(iii) The age structure of the population corresponds to the survival likelihood of a population with a lifespan distribution of $\lambda = 56.2$. This assumption may be violated if extrinsic sources of mortality are not applied equally to all age groups.
(iv) Simulated traps capture 34 female flies, which is the average number of female flies per trap from our field trials.
(v) Females reach sexual maturity at 12.1 days and have average maximum fecundity of 154.8 eggs with a standard deviation of 7.1 and either:
  A meet 70% of their lifetime reproductive output prior to mean lifespan of 56.2 days, thus averaging a daily reproductive rate of ~2.5 eggs/day (Figure S1).
  B cease reproduction at age 30.7 days, thus averaging a daily reproductive rate of ~8.3 eggs/day
(vi) Egg to adult viability is 80% (±2%; Vayssières et al., 2008).

Future studies should test the extent to which these assumptions are violated using large-scale population ecology of melon flies, and how violations of these assumptions affect the estimates of our simulations. Nevertheless, the approach adopted here provides a first step towards an understanding of the long-lasting effects of female trapping in insect pest populations. To generate the capture and hypothetical death age of the flies in the population, the functions 'survfit' and 'rtpois' were used from the 'survival' and 'extraDistr' packages, respectively (Therneau, 2023; Wolodzko, 2019). The simulation in Figure S2 assumed a sample size of $n = 10{,}000$ flies at each age interval.

3 | RESULTS

3.1 | Cuelure traps are more effective in trapping male flies compared with protein-bait traps

We detected a significant effect of trap type ($F_{1,1891} = 21.2$, $p < 0.001$) and year ($F_{1,1891.6} = 141.1$, $p < 0.001$) on the number of *B. cucurbitae* males trapped (Table 2; Figure 1a). There was also a

TABLE 1 Master table comprised of *B. cucurbitae* life-history traits used in simulations.

Food source	Lifespan (days)	Fecundity (±SD or *SE*)	Pre-oviposition period (days)	Oviposition period (days)
Sugar + yeast hydrolysate		72.9*	16.3	
Sugar + Protinex + pumpkin	87.7	412.5 (±*6.1*)		
Sweet gourd	23.5	52.8 (±*6.8*)	11.3	9.8
Sugar + yeast hydrolysate/cucumber	182	456*	10	
Sugar + yeast hydrolysate /pumpkin	237	219*	12	
Sugar + yeast hydrolysate/squash	217	132*	10.6	
Bitter gourd	33.2	138.4 (±44.1)		
Pumpkin	30.6	135.6 (±33)		
Sugar + yeast hydrolysate (no host)	76.2			
Sugar + yeast hydrolysate (host 0 days)	44.4	465.1 (±*33.6*)		
Sugar + yeast hydrolysate (host 7 days)	39.7	404.4 (±*36.6*)		
Sugar + yeast hydrolysate (host 14 days)	49.9	371.8 (±*31.9*)		
Cucumber		75.8 (±12.5)	12.4	18.2
Water + honey	23			
Water + Protinex	34.4			
Water + molasses	31.6			
Water + honey + Protinex	33.2			
Water + molasses + Protinex	48.6			
Water + molasses + honey	44.2			
Water + sugar + glucose + yeast	27.67			
Water + honey	25.3	74.8 (±11.69)	12.6	21.4
Bitter gourd	15.1	33.4 (±1.3)	11	2.8
Bottle gourd	17.2	44.4 (±1.5)	11.7	3.7
Watermelon	16.9	53 (±2)	11.6	3.3
Water + sugar/ridge gourd		225 (±25)	13.5	18
Honey/bitter gourd	40.4	87.8 (±8.61)		16.3
Ash gourd	49.7	60.2		34
Bitter gourd	40	77.5		23
Bottle gourd	37.3	48.1		22.6
Cucumber	41	57.1		24.7
Pumpkin	41.7	48.6		24
Ridge gourd	44	59.4		26.1
Snake gourd	54.2	63.9		31.7

*Net reproductive rate reported.

significant interaction between trap type and year on the number of male flies trapped ($F_{1,1891} = 21.2$, $p < 0.001$; Table 2), driven by the progressive decline in males trapped in Cuelure traps, with relative consistency of males captured in protein-bait traps (Figure 1a). Cuelure traps captured a significantly higher number of males than the protein-bait trap across all years (estimate = 257.5, LCI = 147.9, UCI = 366.9, $p < 0.001$; Table 2). Likewise, we observed a significantly higher number of males captured in our protein-bait traps, per year, than female (mean estimates: male = 71, female = 34; paired $t_{(50)} = 6.7$, $p < 0.001$).

3.2 | Estimated long-term benefits of protein-bait female trapping

Despite lower trapping incidences in protein-bait traps, we sought to estimate inclusive benefits to this approach. We began by modelling *B. cucurbitae* population sizes as a function of trapping probability and year, to discern the success of protein-bait traps, relative to the estimated population size (Figure 2). Even low trapping probabilities could potentially translate into notable reductions of population sizes, although it is impossible to estimate real

TABLE 2 Complete outputs of the linear mixed-effects model to test the effect of trap type, year and the interaction between the two on the number of *B. cucurbitae* male flies trapped.

Fixed effects	numDF	denDF	*F*-value	*P*-value
Trap type	1	1891.000	21.224	<0.001
Year	1	1891.600	141.101	<0.001
Trap type*Year	1	1891.000	21.221	<0.001

		c. 95% CI		
Fixed effect	**Estimate**	**Lower**	**Upper**	***p*-Value**
Intercept	2.082e+02	175.980	240.499	<0.001
Cuelure	2.575e+02	147.925	366.924	<0.001
Year	−1.027e−01	−0.119	−0.087	<0.001
Cuelure × Year	−1.275e−01	−0.182	−0.073	<0.001

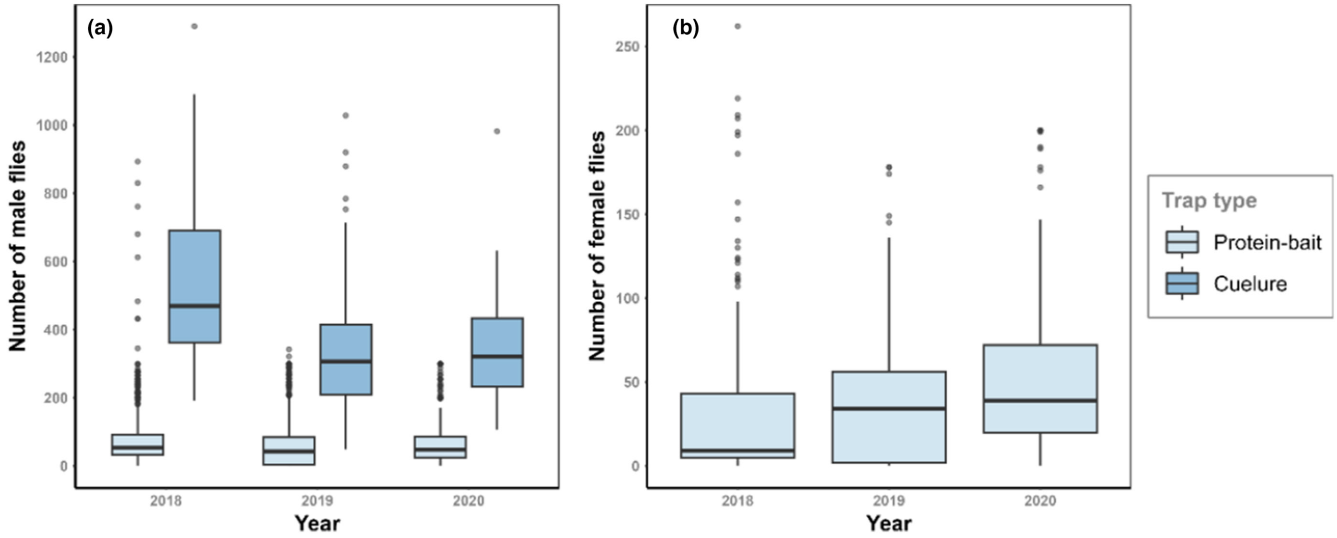

FIGURE 1 (a) Number of *B. cucurbitae* males trapped in protein-bait and Cuelure traps per year. (b) Number of females trapped in protein-bait devices per year.

effect sizes due to the lack of knowledge of true population sizes of the wild population.

We further assessed how the trapping and removal of female flies from the population could reduce individuals incorporated into the F1 generation, due to the removal of viable eggs. We simulated this benefit by using parameters of life-history traits derived from the published literature (Table 1; see 'Materials and Methods' for details) and generated an estimate of 1613.5 adults (SD = 314.6) prevented, per trap. By comparison, our most effective Cuelure trapped fewer males (*n* = 1290, 2018; Figure 1a), indicating the promise of a female capture approach. Consequently, in a field trial with 160 protein-bait traps, there is potential to prevent 259,941 (SD = 3930.5) newly eclosed adults per year.

Our literature-derived parameters determined a cessation to reproductive output at age 30.7 days and a mean lifespan of 56.2 days; since only a modest proportion of captured flies were still reproducing ($\hat{p} = 0.56$, SD = 0.08), we suspected our estimates may have represented a lower-bound on the number of adults prevented. To corroborate our estimates in a more realistic context, we simulated a scenario where all captured females were capable of future reproductive output and achieved 70% of their lifetime reproductive output by mean lifespan of 56.2 days. Here, we found our estimates increased to 1824.5 adults prevented per trap (Figure S1; SD = 174.9). Assuming similar capture rates across in traps across our field study, we estimate 290, 934.7 adults (SD = 2305.7) could be prevented from introduction into the F1 generation, per year.

4 | DISCUSSION

In this study, we conducted 3 years of field trapping of melon fly *B. cucurbitae* in India to demonstrate how a novel slow-release protein-bait trapping system can fare relative to standard Cuelure trapping. As expected, Cuelure traps proved to be more effective at maximum capture of *B. cucurbitae* males; while protein-bait traps

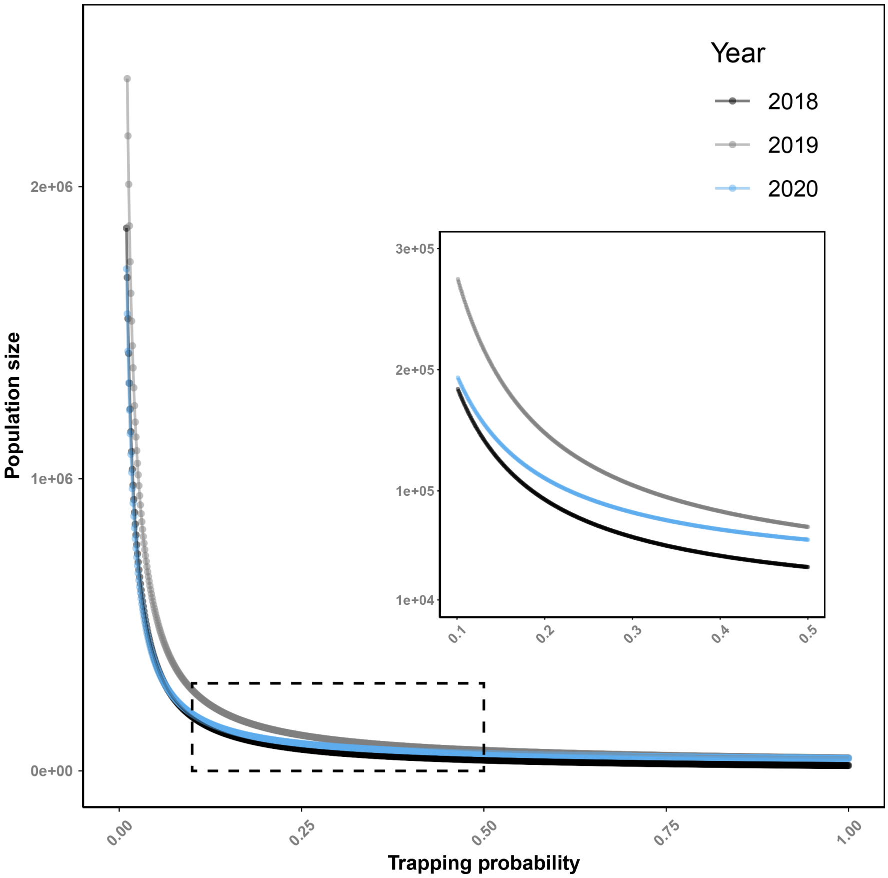

FIGURE 2 Protein-bait trapping probability and F1 adults prevented is relative to population size. Estimated population sizes of field sites as a function of trapping probability and year. Inset (left) shows zoomed view of population size estimates when trapping probability ranges from 0.2 to 1.

showed substantially less trapping power, they were equally efficient in capturing both females and males. A potential benefit of trapping females is the direct removal of egg-laying individuals from the population, which could have faster, and more robust, impacts to population size and damage caused. Our simulations showed that, with the same number of traps used in the field trials, a high number of eggs – and emerging adults – can be prevented from introduction into the population, despite fewer flies being captured using this approach. In our simulations, we assumed that our populations were age-structured, with the distribution of ages following a Poisson distribution with lambda of 56.2. This value was obtained from the published literature; to our knowledge, there are no detailed studies describing the age structure of *B. cucurbitae* populations in their natural range. A recent study has shown that the age structure of wild populations of a related species, *Bactrocera tryoni*, is bimodal and independent of environmental factors such as temperature and food host (Tasnin et al., 2021). Our simulations assumed a unimodal distribution of age in the population. As a result, approximately 45% of females trapped in our primary simulation had ceased to reproduce (i.e. females at the tail end of the distribution of age). In a bimodal age structure, it is possible to trap females at the front end of the distribution of age for the second peak, thereby curtailing their full reproductive potential. Thus, our simulation likely provides a conservative measure of the number of adults prevented in the F1 generation. Whether the age structure of wild *B. cucurbitae* populations follows uni- or bimodal patterns remains to be studied. Similarly, whether our protein-bait trap preferentially captures (putatively non-protein satiated) virgin females is unknown (Henneken et al., 2022), but such an event would further augment the preventative effect of a female capture approach.

Female trapping can have long-term benefits to population control and our simulations aimed at quantifying these benefits on the subsequent generation. This is an important yet often unaccounted feature of female trapping, and can play an important role in the management of pest populations, especially in the light of potential for cumulative, multi-generational impact of female removal. While our simulations indicate protein-bait traps could be highly effective at preventing the laying of melon fly eggs, there are limitations to consider. First, protein-bait traps may erroneously attract and capture non-target species (Galdino & Raga, 2018). Second, we observed high variance for many of our simulation parameters, most likely due to differences in experimental diet or conditions (Table 1). Our estimates should therefore be considered tentative in the absence of further ecological validation in studies of melon flies in the region. Insects can display extensive phenotypic plasticity of lifespan and fecundity in response to a range of environmental factors including nutrition (Jensen et al., 2015; Maklakov et al., 2008) and temperature (Colinet et al., 2015). Furthermore, fitness and the intrinsic rate of ageing will be modulated by the degree of extrinsic mortality (like predation or parasite susceptibility) faced by a population (Stearns et al., 2000). Studies within the relevant ecological environment, specifically in wild populations, may provide more reliable parameters for future estimates of our population size and long-term trapping benefits.

5 | CONCLUSION

Trapping is a cost-effective way in which local small-scale farmers can control and mitigate the socio-economic burdens of insect pest outbreaks. Here, we test a new, extended-release, protein-bait trap for melon flies in India. Our simulations of protein-bait trap efficacy demonstrate an overall reduction in the number of adults incorporated into the F1 generation, despite lower capture success, relative to standard Cuelure trapping. These findings provide evidence for the relative effectiveness of this approach, highlighting the potential of protein-bait traps as an important additional factor for pest management. Future studies should broaden the taxonomic scope of such trapping system and investigate synergy of multiple trapping systems to maximise pest control in infested areas.

AUTHOR CONTRIBUTIONS

Juliano Morimoto: Investigation; funding acquisition; writing – original draft; methodology; writing – review and editing; visualization; formal analysis; project administration; supervision; data curation; resources. **Andrew McCracken W:** Methodology; validation; visualization; writing – review and editing; formal analysis. **Nurah Niazy:** Writing – original draft; methodology; data curation; formal analysis. **Samu Turi:** Writing – original draft; methodology; formal analysis; data curation. **Vijeth Arya:** Conceptualization; investigation; methodology; data curation. **Vivek Kempraj:** Conceptualization; investigation; funding acquisition; writing – original draft; methodology; writing – review and editing; formal analysis; project administration; supervision; resources.

ACKNOWLEDGEMENTS

This study was carried out in collaboration with the Indian Gherkin Export Association (IGEA). We would like to thank anonymous reviewers and the editors for useful comments on the manuscript. NN conducted this work during her NERC QUADRAT REP program (NE/S007377/1) in JM's lab. JM and AWM are supported by a BBSRC grant (BB/V015249/1).

CONFLICT OF INTEREST STATEMENT

Bioorgo® is a brand of Kempmann Bioorganics LLP, which commercialises the protein-bait trap (FruitClean®FTS), bait (FruitClean®PB) and Cuelure trap (FruitClean®MLS) used in this study. VA and VK are employed by Kempmann Bioorganics LLP. The field trials were coordinated and run by Bioorgo®. AWM, NN, ST and JM do not nor have ever had affiliations with the company.

DATA AVAILABILITY STATEMENT

Raw data are available in Dryad: https://doi.org/10.5061/dryad.w6m905qvh

ORCID

Juliano Morimoto https://orcid.org/0000-0003-3561-1920

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: McCracken, A. W., Niazy, N., Turi, S., Arya, V., Kempraj, V., & Morimoto, J. (2023). A novel protein-based fruit fly trap in melon flies *Bactrocera cucurbitae* for effective pest control management. *Journal of Applied Entomology*, *00*, 1–8. https://doi.org/10.1111/jen.13184