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Giving it our best shot in the war against soldier flies – future research directions

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Abstract Soldier flies are economically damaging pests of sugarcane, particularly in central and southern Queensland. Despite decades of research on soldier fly control, the search for an effective management approach, except for cultural control, remains elusive. Trials were conducted from 2015 to 2017 to identify potential management solutions for soldier flies by assessing insecticide efficacy and varietal tolerance in field conditions. Five field trials were established to determine whether applying insecticide at plant cane would reduce the build-up in soldier fly larvae in subsequent ratoons. Ten products, comprising seven active ingredients, were field tested at high application rates. Overall, as in most previous studies, none of the insecticides tested reduced the number of larvae in field-trial conditions. The inefficacy of insecticide treatments could be due to products failing to come into contact with soldier fly larvae or simply lack of an effective active. In addition, three field trials, using up to 14 varieties, were conducted, to assess varietal tolerance. Some varieties tended to host fewer larvae than others, suggesting some resistance, in two trials established in southern Queensland. Any future insecticide and varietal screening trials will need to be conducted in both controlled laboratory and field conditions. However, before such trials can be undertaken, a standardised laboratory rearing method and improved field sampling strategy for soldier flies needs to be developed. Soldier fly outbreaks are also unpredictable and developing methods to forecast them (e.g. using climatic data or identifying preferential soil properties) will also be highly beneficial to inform growers of the potential risk of soldier fly establishment in their paddocks and for selecting field-trial sites. Additionally, recent DNA barcoding and morphological studies have revealed that at least six species of soldier flies are found in sugarcane, not two as previously identified. That finding highlights that the distribution of soldier fly species in Australia and the relative damage to sugarcane varieties needs to be resolved to enable the development of targeted species-specific management approaches.

Key words *Inopus*, pest control, insecticide, root-feeding pest, sugarcane, variety

INTRODUCTION

Sugarcane soldier flies (*Inopus rubriceps* and *I. flavus*) are economically damaging pests of sugarcane in central and southern Queensland and northern New South Wales (Hitchcock 1970) and also more recently in the Atherton Tablelands in far-northern Queensland (K. Powell, pers. obs.). These pests are native to Australia and their host plants include a wide range of grasses, including Guinea grass (*Megathyrsus maximus*), kikuyu (*Pennisetum clandestinum*) and molasses grass (*Melinis minutiflora*) (Allsopp & Robertson 1988). *Inopus rubriceps* has also established in New Zealand and California (USA) since the 1940s, where it can cause severe damage to pasture grass and lawns (Wilcocks 1974; Robertson *et al.* 1981). Soldier flies are slow-growing insects and complete their development in 1-2 years, spending most of their life cycle as larvae (Hitchcock 1976). The egg and pupal stages last for 1-2 weeks and 3 weeks, respectively. Adult females mate, oviposit and die within 24 h of hatching, while males live for up to 1 week (Hitchcock 1976). Most individuals reach the adult stage within a year after developing through 8-9 larval instars. Larvae that fail to pupate in March to June develop over 2 years and go through a total of 10-12 larval instars (Hitchcock 1976; Samson & McLennan 1995).

Soldier fly larvae are root-feeders and even moderate levels of feeding damage can lead to reduction in sugarcane growth and ratooning ability (Samson 2001). Larvae may inject inhibitory substances into sugarcane plants while feeding, which may explain the long-term effect of soldier fly feeding on sugarcane ratooning ability (Samson 2001). Upregulated venom proteins have been found in the salivary glands of soldier flies that may be transmitted to the plant during feeding (Etebari *et al.* 2020). Soldier flies damaged 1.1% and 1.7% of harvested areas in Bundaberg and Mackay, respectively, during the 2013-2014 season (J. Matthiessen pers. com.). Farmers and local productivity services also regularly report significant economic losses to individual farms due to soldier fly infestations. Because of the ability of soldier flies to affect ratooning ability, the economic loss to sugarcane growers is caused by both yield reduction and premature plough-out of affected blocks.

Cultural control is the only recommended option to control soldier flies in sugarcane, as research into other methods of control has been largely unsuccessful. Current control recommendations by Sugar Research Australia (SRA) include taking out the affected block early in the season, fallowing the block to give it a grass-free break from sugarcane (e.g. short fallow followed by a non-grass crop), and planting the next sugarcane crop late in the season after the mating period of soldier flies (Moller 1968; Samson *et al.* 1991; Morris & Samson 2006). There is currently no insecticide registered in Australia for the control of soldier flies despite many insecticide trials conducted over decades (Moller 1968; Samson 1992, 2002, 2015; Samson & Harris 1994, 1997). The entomopathogenic fungus *Metarhizium anisopliae* naturally infects *I. rubriceps* larvae and was found to be somewhat pathogenic to them in bioassays (Samuels *et al.* 1989; Samson & McLennan 1993). However, this method of control did not reduce soldier fly numbers in field trials and was not developed further (Samson *et al.* 1994, 2000). There is also no sugarcane variety that has been identified as resistant or tolerant to soldier fly damage despite evidence that some varieties can withstand soldier fly pressure better than others or may inhibit larval development (Samson *et al.* 1991, 2004).

We established trials from 2015 to 2017 in southern and central Queensland to identify potential management solutions for soldier flies by assessing insecticide efficacy and varietal tolerance in field conditions. Our two aims were to: (i) assess the efficacy of different insecticide products applied at plant cane for soldier fly; and (ii) identify tolerant varieties to soldier flies. Here, we discuss the results from these trials, as well as opportunities for future research in soldier fly management.

METHODS

Insecticide field-trial design

We established five insecticide field trials from 2015 to 2017 each in a randomised complete-block design with four replicates per treatment (including controls) in each trial. We selected two plant-cane sites with a history of soldier fly infestation in Central Queensland and three in Southern Queensland. Each treated plot was four rows wide and 15 m long. Treatment application took place in September to December depending on the site. Insecticides were applied once at plant cane with handheld knapsack sprayers on both sides of each row and by walking at an appropriate pace to ensure even application. The treatments were covered by a tractor and tine following application. Ten commercially available insecticide products, including four in-confidence products, were tested across all trials (Table 1). The products comprised seven different active ingredients belonging to five different mode-of-action groups (Table 1). The rates used in the trials were higher than maximum recommended label rates for most products (1.4-4 times higher) to determine product efficacy on controlling soldier fly larvae.

Variety field-trial design

We established four variety field trials in 2015 and 2017 each in a randomised complete block design. We selected one site with a history of soldier fly infestation in Central Queensland near Mackay and three in Southern Queensland near Bundaberg. Each plot was three rows wide and 15 m long. A 1-m gap was left unplanted at the end of each plot. One-eye setts of one clone per plot were planted in August or September depending on the sites. Fifteen clones were tested across the trials (Table 2), with four replicates per variety. The clones selected were already grown by cane farmers in the region or were being developed for that region.

Table 1. Insecticide treatments applied in trials in the Central (EC) and Southern (ES) regions.

Product	Active ingredient (ai)	Mode of action groups*	Max. rate sugarcane (g ai/ha)	Rate applied (g ai/ha)	EC15-01	ES15-04	ES15-05	EC17-08	ES16-07
Confidor®	Imidacloprid	4A: Neonicotinoid Blocks nicotinic acetylcholine receptor (nAChR) competitive modulators	504	1000	+	-	-	+	-
			504	2000	+	-	-	-	-
Nuprid® 700WG	Imidacloprid	As above	720	1000	-	+	+	-	-
			720	2000	-	+	+	-	-
Phenyle** + Confidor®	Cresylic acid + imidacloprid	As above	504	1000	-	-	-	+	-
Shield®	Clothianidin	As above	500	750	-	-	-	+	-
			500	1000	+	+	+	+	+
			500	2000	+	+	+	-	+
Regent®	Fipronil	2B: Fiprole GABA-gated chloride channels (GGCC) antagonist	71	30	+	+	+	-	-
			71	60	+	+	+	+	+
Venom®	Bifenthrin	3A: Pyrethroid	37.5	75	+	-	-	-	-
		Voltage gated sodium channel (VGSC) modulators	37.5	150	+	-	-	-	-
Webzone®	Bifenthrin	As above	n/a	75	-	+	-	-	-
			n/a	100	-	-	-	-	-
			n/a	150	-	+	+	-	-
Product 1	Undisclosed	30: Meta-diamides & isoxazolines GGCC allosteric modulators	n/a	50	+	+	+	-	-
Product 2	Undisclosed	As above	n/a	200	+	+	+	-	-
Product 3	Undisclosed	28: Diamides Ryanodine receptor (Ry-R) modulators	n/a	200	+	-	+	+	-
			n/a	400	-	-	-	+	-
Product 4	Undisclosed	As above	n/a	200	+	-	-	+	+
			n/a	400	-	-	-	+	-

*Sparks *et al*. (2020).

**Phenyle is a disinfectant/cleaning/deodorising product not used for insect control.

Table 2. Clones grown in sugarcane variety trials in the Central (EC) and Southern (ES) regions in 2015 and 2017.

Clone	EC17-07	ES15-01	ES15-02	ES15-03
SRA1[Φ]	-	+	+	+
SRA2[Φ]	-	+	+	+
SRA4[Φ]	-	+	+	+
SRA9[Φ]	+	-	-	-
SRA22[Φ]	-	-	+	-
Q208[Φ]	+	+	+	+
Q232[Φ]	-	+	-	-
Q238[Φ]	-	-	+	+
Q240[Φ]	+	+	+	+
Q250[Φ]	+	-	-	-
Q252[Φ]	+	+	+	+
Q253	+	-	-	-
SP80-1816	+	-	-	-
KQ228[Φ]	+	-	+	-
QS03-206	-	-	+	-

Soldier-fly sampling

All trials ran for three harvest seasons (from plant cane to third ratoon) for trials established in 2015 (EC15 and ES15 trials), two seasons for trials established in 2016 (ES16 trials) and one season for trials established in 2017 (EC17 trials). Four soil cores (65 mm diameter and 120 mm deep) were sampled in the middle two rows of each plot once a year from the end of October until early March. Soil cores were brought back to the laboratory and larvae wet sieved from the samples following the method described by Robertson (1984). Live soldier fly larvae were counted.

Due to their relatively high abundance and known occurrence, earthpearls were also counted in the Southern region trials. They were extracted during the same wet sieving.

Data analysis

We analysed the count data in R version 3.6.1 (RStudio Team 2020) and used functions from the ASReml-R package (Butler *et al*. 2018). We used a generalized linear mixed model accounting for residual correlation followed by Wald tests. We used a Poisson distribution for all models unless the data was overdispersed, in which case we used a negative binomial distribution.

For each yearly count, the response variables were the average number of soldier fly per soil core for each insecticide trial and soldier fly or earthpearl counts for each variety trial. Chemical treatments or varieties were treated as fixed factors and replicate as random. Likelihood ratio tests were used to compare models and included spatial correlation terms (column and/or plot) if the model including these terms was significantly different from the model without them. We also analysed the effect of insecticide treatment or variety on counts of earthpearls in trials that had enough earthpearls for model building using the same methods as described above.

For insecticide trials, a model was built using counts of soldier flies for all three seasons for trials established in 2015. The response variable was the average number of soldier fly, the fixed factors were the ratoon number, chemical treatment, and the interaction term. The correlation terms were ratoon number, column and plot and were included following the same method as for the other models.

RESULTS

Insecticide trials

There was no significant difference in numbers of soldier fly larvae between untreated plots and any of the treated plots across all trials ($p>0.05$ for all *post-hoc* tests between treated and untreated plots, Figure 1). There was no effect of any of the treatments on the average number of soldier fly larvae per core for EC15-01 and ES15-04 ($p>0.05$ from first to third ratoon). There was a significant effect of insecticide treatment ($p<0.01$) on the average number of soldier fly larvae per core for EC17-08. However, the average number of soldier fly did not differ between

the untreated plots and any of the treated plots (p>0.05 for all *post-hoc* tests between untreated and each treatment). The low number of soldier flies for two trials in the Southern region (ES15-05 and ES16-07) prevented model building for these trials. We only counted 14 larvae over 176 cores at the second ratoon for ES15-05, while a single larva was counted for ES16-07 at the first ratoon. Numbers of soldier flies were also low for ES15-04 and EC15-01, but significantly increased as ratoon aged regardless of treatment (p<0.001 for ratoon number, p>0.05 for chemical treatment and the interaction term in both trials, Figure 2). For ES15-04, we found 0.75±1.43 larvae per soil core (average ± SD) at the first ratoon, 1.5±1.14 at the second ratoon, and 2.55±2.53 at the third ratoon, while we found 0.92±1.96 larvae per soil core at the first ratoon, 2.33±3.19 at the second ratoon, and 7.57±6.7 at the third ratoon for EC15-01. Soldier flies were only counted at first ratoon for EC17-08, but the average number of larvae per soil core (15.39±10.63) was higher than in all other trials regardless of ratoon number.

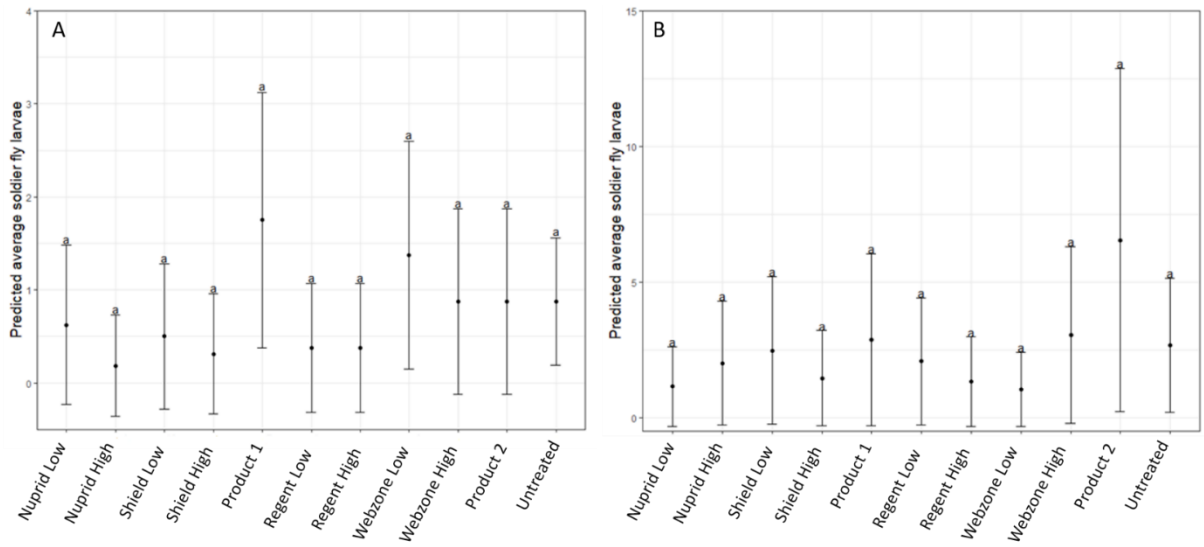

Figure 1. Predicted (back-transformed) average number of soldier fly larvae per soil core with confidence intervals for each treatment in the trial ES15-04 2017. We present a single figure from one representative trial as results were similar in other trials: A: first ratoon, B: third ratoon. Low: low rate, High: high rate (Table 1). There was no significant effect of insecticide treatment (p>0.05 at first ratoon and third ratoon) on the average number of soldier fly larvae per soil core (p>0.05 for all *post-hoc* tests).

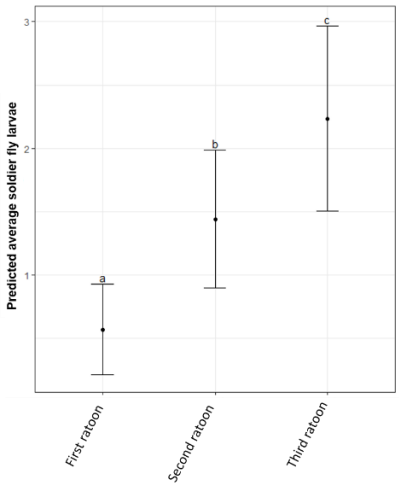

Figure 2. Predicted (back-transformed) average number of soldier fly larvae per soil core with confidence intervals in ES15-04 from first to third ratoon. We present a single figure from one representative trial as results were similar in EC15-01. The number of soldier flies increased over the three years of the trial (p<0.001, p<0.05 for all *post-hoc* tests).

Variety trials

We found that some varieties tended to host fewer soldier fly larvae than others in two Southern region trials but the low number of larvae in the Central region trial and the third Southern region trial prevented model building for these two trials. There was a significant effect of variety on the number of soldier fly larvae at third ratoon for ES15-01 and ES15-02 ($p<0.05$, Figure 3). The number of larvae was low at the third ratoon with an average of 0.63±1.42 (±SD) larvae per soil core for ES15-01 and 0.57±1.26 for ES15-02. None of the *post-hoc* tests were significant, but the confidence intervals did not overlap for some varieties which indicate that some varieties may host fewer larvae than other ones (Figure 3). For example, Q232$^{\phi}$ and SRA2$^{\phi}$ tended to host lower numbers of soldier fly larvae than Q208$^{\phi}$ in ES15-01, while SRA1$^{\phi}$ and QS03-206 tended to host fewer larvae than SRA2$^{\phi}$ in ES15-02. There were too few larvae for ES15-01 and ES15-02 at the first (0 and 1 larva counted, respectively) and the second ratoon (2 counted for ES15-01 and 0.17±0.58 larvae per core on average for ES15-02) to build models. The data from the other two trials (EC17-07 and ES15-03) could also not be analysed because of the low numbers of soldier fly larvae.

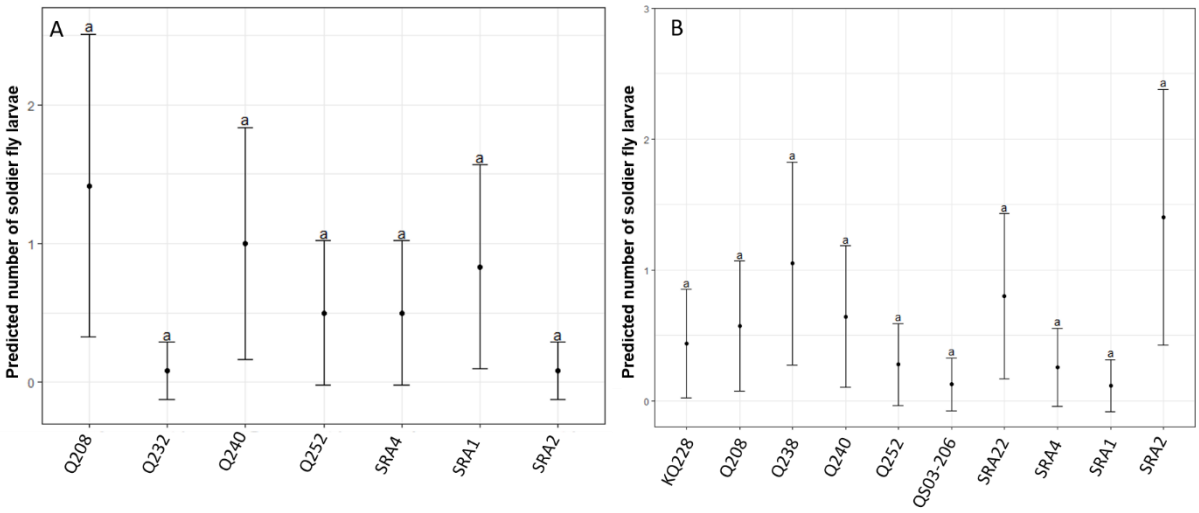

Figure 3. Predicted (back-transformed) number of soldier fly larvae per soil core with confidence intervals for each variety at third ratoon for two trials: A: ES15-01, B: ES15-02. There was a significant effect of variety ($p<0.05$ for both trials) on the number of soldier fly larvae per soil core, but the *post-hoc* tests were not significant ($p>0.05$ for all *post-hoc* tests).

Earthpearls

We found that some varieties hosted fewer earthpearls than others in two Southern region variety trials at the third ratoon, but the number of earthpearls was too low in a Southern region insecticide trial to build a model. We analysed earthpearls data for ES15-01, ES15-02, and ES15-04 at the third ratoon. We only found white earthpearls in these trials. Numbers of earthpearl were high for ES15-01 and ES15-02; we counted 7.94±8.37 earthpearls per soil core (average ± SD) in ES15-01 and 28.05±30.36 in ES15-02. There was a significant effect of variety on the number of earthpearls for ES15-01 and ES15-02 at third ratoon ($p<0.05$, Figure 4). In ES15-01, Q252$^{\phi}$ hosted fewer earthpearls than Q240$^{\phi}$ and SRA2$^{\phi}$ (Figure 4A, *post-hoc* tests<0.05), while Q208$^{\phi}$ hosted fewer earthpearls than Q238$^{\phi}$ and SRA2$^{\phi}$ for ES15-02 (Figure 4B, *post-hoc* tests<0.05). The number of earthpearls for ES15-04 was too low to build a model (0.18±1.42 per core on average ±SD).

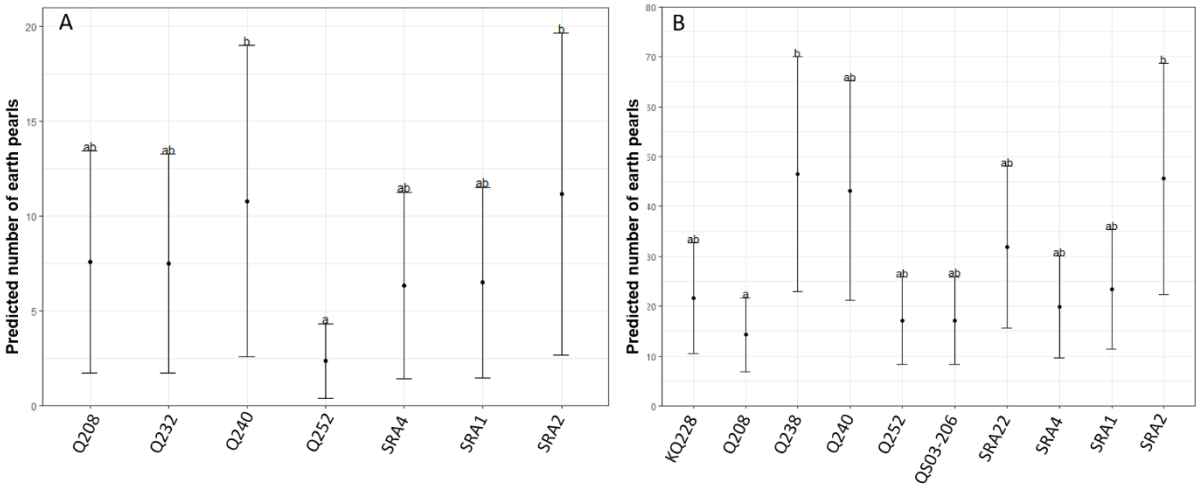

Figure 4. Predicted (back-transformed) number of earthpearls per soil core with confidence intervals for each variety at third ratoon for two trials: A: ES15-01, B: ES15-02. There was a significant effect of variety ($p<0.05$ for both trials) on the number of earthpearls per soil core and some varieties hosted fewer earthpearls than others (see significance letters for significant *post-hoc* tests).

DISCUSSION

The insecticides used in all trials were ineffective against soldier fly larvae when applied to plant cane. There was no effect of insecticide on numbers of soldier flies in any of the trials that were analysed successfully, and numbers of soldier flies built up with ratoon number, as typically observed in infested sugarcane fields (Samson *et al.* 1991). This is despite testing a wide range of products (10 insecticides) comprising seven different active ingredients belonging to five different mode of action groups at high rates (Table 1).

Previous chemical recommendations for the treatment of soldier flies included the use of organochloride such as crude BHC (i.e. lindane), dieldrin and aldrin that are all now banned from use (Moller 1961, 1968; Allsopp & Robertson 1988). Dieldrin was registered for soldier fly control under a permit system until the early 1990s (Samson *et al.* 1991), but no insecticide for the control of soldier flies has been available since its withdrawal due to the absence of proven efficacy of a wide range of products (Moller 1968; Samson 2002a, 2015). Additionally, the use of broad-spectrum insecticides may have killed natural enemies and potentially triggered outbreaks of soldier fly infestations (Allsopp & Robertson 1988). In New Zealand and California, no insecticide was found to be effective against soldier flies in pasture and it is currently considered a minor pest in these areas (East *et al.* 1986). In New Zealand, possible control solutions include accepting temporary losses until predators regulate soldier flies, repeated cultivation during prolonged fallowing and moderate grazing during soldier fly peaks (Wilcocks 1974; Robertson *et al.* 1981; East *et al.* 1986). In California, soldier flies are a pest of lawn turf grasses, but no control solutions exist as their distribution is limited and they are not considered an agricultural pest of importance (Allsopp & Robertson 1988).

Several factors may explain the absence of insecticide efficacy against soldier flies and new approaches are needed to identify efficient chemical control methods for soldier flies. Larvae may be naturally tolerant to insecticides. For example, broad-spectrum insecticides such as the neonicotinoids were ineffective against soldier flies at high application rates (Samson 2015). Only clothianidin significantly reduced the number of soldier flies in a single field trial but only at an application rate more than twice the rate registered for canegrubs (Samson 2015). In addition to being potentially tolerant to insecticides, the biology and behaviour of soldier flies may lower the probability of larvae to come into contact with sufficient insecticide product to trigger control. Soldier fly larvae are small, slow growing insects that are mainly found near the soil surface and can survive without food for several months (Hitchcock 1976; Allsopp & Robertson 1988). Controlling adults with chemicals is not a suitable alternative because adults emerge over several months (March to June) and females emerge, mate, lay eggs and die within 24 h (Hitchcock 1976). Future insecticide trials will have to include controlled bioassays before being tested in the field to ensure efficacy of products, determine the required concentration to control soldier flies, and determine whether this method of control would be commercially viable and satisfy environmental regulations. Field trials could test different application methods of promising insecticides into plant cane and different ratoon stages to increase chances of identifying a successful control method.

We found that some varieties tended to host fewer soldier fly larvae than others, but the number of soldier fly larvae in these trials were too low to make definitive variety recommendations. Other studies have found evidence for variety tolerance or resistance to soldier flies. A survey of damage caused by soldier flies near Bundaberg found that variety had an effect on the frequency of damage but failed to identify which varieties are potentially more tolerant to soldier fly damage (Samson *et al.* 1991). Some varieties hosted fewer larvae than others in other field trials conducted near Bundaberg, but the reduction in larval number was minimal (Samson 2002b). There is some evidence that some varieties may inhibit soldier fly development. In two trials near Mackay, the number and average weight of larvae differed significantly among varieties, with varieties tending to host smaller larvae also hosting fewer larvae (Samson *et al*. 2004). The mechanism of variety tolerance or resistance to soldier fly damage remains poorly understood. Perhaps some varieties are less appetising to larvae or possess an unknown defence mechanism to soldier fly feeding and the venom proteins they potentially transmit to the plant (Etebari *et al.* 2020). Future research into these mechanisms will have to be initiated through pot trials in which variety tolerance and/or resistance to soldier fly feeding can be measured in controlled conditions. Following pot trials, field trials in blocks infested with soldier flies will have to include potentially resistant and susceptible varieties commonly grown in the region in which trials take place as well as new varieties to potentially inform growers on the varieties which may perform better under high soldier fly pressure.

We found that white earthpearls (*Promargarodes australis*) were present in soldier-fly-infested blocks at the same time as soldier fly larvae and that some varieties hosted fewer earthpearls than others. White earthpearls were found in high numbers in two trials established near Bundaberg. Earthpearls (or ground pearls) are scale insects belonging to the family Margarodidae. White earthpearls are distributed throughout Queensland, while pink earthpearls (*Eumargarodes laingi*), another pest species of sugarcane, are found in southern Queensland (Hitchcock 1965; Dominiak *et al.* 1989; Allsopp *et al*. 2000). Earthpearl nymphs feed on sugarcane roots and high number of earthpearls may cause similar damage to soldier fly larvae such as yield reduction and ratoon failure (Hitchcock 1965; Allsopp *et al.* 2000). Differentiating sugarcane damage due to earthpearls and soldier flies may be difficult in blocks where both pests are present. It would be interesting to determine whether competition for food resources exist between soldier fly larvae and earthpearls. Other studies have also found that some varieties host fewer earthpearls than others, indicating potential tolerance of some varieties to earthpearls (Allsopp & McGill 1997; Allsopp *et al.* 2000).

Insecticide and variety trials aimed at identifying novel methods for the control of soldier flies have been conducted for decades without success (Moller 1963; Samson 2002, 2015; Samson *et al.* 2004; this study) and new approaches are needed if we are to develop methods of control beyond cultural practices. As outlined above, insecticides should not be trialled in the field prior to being tested against soldier fly larvae in controlled laboratory trials. As well as chemical insecticides, laboratory trials should aim to include newly emerging biorational products and bio-insecticides which may show efficacy against soldier fly larvae as found for *Metarhizium anisopliae* (Samuels *et al.* 1989; Samson & McLennan 1993). Similarly, pot trials with potentially susceptible and tolerant varieties should be conducted before variety trials take place in the field. Rearing methods for soldier flies, including an artificial diet for soldier fly larvae, will need to be developed for such laboratory trials. Site selection and improved sampling protocols for field trials also need to be optimised, as the larval count data from several trials in this study could not be analysed due to the low number of soldier flies. As numbers of soldier flies build up as ratoons age, trials need to run beyond third ratoon, and this calls for research projects running for more than the standard 3 years. Field trials could potentially start with a larger number of sites that would be monitored regularly. Trial sites could be progressively abandoned if the number of soldier flies in the control blocks are too low, so as to only retain trial sites which are heavily infested. Developing prediction models for soldier fly outbreaks would also be useful to conduct trials and inform growers. Soldier fly outbreaks are currently unpredictable and may depend on several biotic and abiotic factors such as climatic conditions (Allsopp 1990), soil properties, and presence of natural enemies. Being able to inform growers on the risk of soldier fly outbreaks in their farms would help identify management options and would help researchers identify suitable blocks for running trials. Additionally, the mechanisms for soldier fly resistance to insecticides could be evaluated by, for example, determining the penetration ability of contact insecticide through the soldier fly cuticle.

The lack of baseline information in soldier fly taxonomy and distribution in Australia potentially impedes research efforts into targeted control methods for these pests. A few soldier fly specimens collected at nine sites in southern and central Queensland were recently barcoded and morphologically identified (Braithwaite *et al.* 2019; B.D. Lessard, pers. com.). Six species were identified in sugarcane instead of two as expected (Braithwaite *et al*. 2019; B.D. Lessard, pers. com.). This newly found diversity of soldier flies highlights the need to improve our knowledge of soldier fly biology, genetic diversity and ecology. That study was only preliminary, and work remains to be conducted to fully identify the species diversity of soldier fly pests of sugarcane across geographical regions. Current research gaps include species distribution, relative soldier fly species-specific damage to sugarcane, interactions with varieties, and insecticide tolerance.

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