Research Article

Received: 20 November 2020 Revised: 18 January 2021 Accepted article published: 28 January 2021 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.6303

An effective 'push–pull' control strategy for European tarnished plant bug, *Lygus rugulipennis* (Heteroptera: Miridae), in strawberry using synthetic semiochemicals

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Abstract

BACKGROUND: European tarnished plant bug, *Lygus rugulipennis* (Heteroptera: Miridae), is a polyphagous pest damaging a range of arable and horticultural crops. Management is reliant upon chemical insecticides for control. These studies developed a synthetic semiochemical push–pull control strategy to reduce numbers of *L. rugulipennis* and subsequent fruit damage in UK strawberry crops. Using a series of small field experiments and testing in commercial strawberry crops we explored the efficacy of hexyl butyrate (HB) as the push element and female sex pheromone combined with phenylacetaldehyde as the pull element.

RESULTS: HB dispensers placed 1.0, 3.5, 5.0 and 7.0 m from all-green Unitraps baited with *L. rugulipennis* female sex pheromone significantly reduced male catches by 99%, 54%, 44% and 20% compared with untreated control, respectively. Subsequently, in commercial crops, HB dispensers at 2-m intervals along the crop row (the push) combined with a perimeter pull reduced numbers of adult and nymph *L. rugulipennis* by up to 80% in organic strawberry crops compared with the untreated control. Finally, the push–pull system halved fruit damage (8%) compared with untreated areas (16%) in conventional crops. In organic strawberry crops, 90% of untreated strawberries had some mirid damage compared with only 41–51% in push–pull-treated areas.

CONCLUSION: To our knowledge, this is the first demonstration of a push–pull approach using synthetic semiochemicals giving a significant reduction in crop damage by mirids and paves the way for non-pesticide control of a range of mirid species on multiple crops.

Keywords: capsid; cat-facing; horticulture; integrated pest management; Miridae; traps

1 INTRODUCTION

The European tarnished plant bug, *Lygus rugulipennis* Poppius (Heteroptera: Miridae), is a polyphagous herbivorous pest,[1] widespread in Europe.[2] It is typically distributed in northern and central Europe but can also be found in southern Europe and Iran.[3] At least 402 host plant species have been reported,[3] including cultivated crops, such as alfalfa, potato, cereals, sugar beet and strawberry.[4]

To supply year-round demand, European strawberries are produced in greenhouses and polyethylene-clad tunnels from February.[5] In the UK, short-day or day-neutral (everbearer) cultivars, which flower and fruit continuously through summer and into the autumn, have enabled the cropping season to be extended to October.[6]

Under UK conditions, *L. rugulipennis* overwinters as male and female adult stages that become active from around February.[7,8] Female adults lay eggs in the spring and the resulting generation is a major pest of everbearing strawberry varieties from late July to early August.[9] Both *L. rugulipennis* nymphs and adults feed on strawberry flower buds, flowers and early fruitlets[6,10] using piercing and sucking mouthparts (stylets), which they insert into the plant tissue[11] injecting digestive enzymes. These salivary enzymes damage the plant tissue and prevent development of achenes around the strawberry seeds.[4] This results in small and deformed fruit, sometimes referred to as 'cat-faced'.[4,6,10] *L. rugulipennis* is the major cause of fruit malformation in late-season strawberry crops in the UK[6] with one insect per 40 plants considered enough to cause economic losses.[12] Unsprayed strawberry crops can have over 50% fruit downgraded.[13]

Chemical plant protection products are usually applied as foliar sprays to control *L. rugulipennis*.[14] However, recent changes to pesticide approvals have seen registration withdrawal for key

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Funding information: Agricultural and Horticultural Development Board, Grant/Award Number: SF 156; Department for Environment, Food and Rural Affairs, Grant/Award Number: HL0191.

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mirid-controlling products in the European Union, including the broad-spectrum organophosphate chlorpyrifos,[15] and more recently, the neonicotinoid thiacloprid. Hence, there are fewer effective active ingredients available to control this pest and alternative approaches for mirid pest control, which reduce dependency on chemical plant protection products, are required.[16] Currently, lambda-cyhalothrin is the only active substance approved specifically for *L. rugulipennis* control in UK protected strawberry, but this product is broad-spectrum and damaging to biological control agents,[17] which control other pests such as thrips and phytophagous mites.

In the UK, natural enemies such as *Orius laevigatus* (Say) (Hemiptera: Anthocoridae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) have shown some predation of *L. rugulipennis*,[18] and *Nabis ferus* (L.) (Hemiptera: Nabidae) has been observed feeding on *L. rugulipennis* nymphs.[19] However, *O. laevigatus* and *C. carnea* effectiveness is less than optimal when more than one pest species is present in a crop,[18] indicating that *L. rugulipennis* is not a preferred food source for these predators. None of these generalist predators prevents *L. rugulipennis* reaching damaging levels in strawberries.[19] *Peristenus* spp. (Hymenoptera: Braconidae) parasitoids have shown promise against *L. rugulipennis* in Italy, with up to 30% parasitism in nymphs.[20] However, these levels are not observed in UK *L. rugulipennis* populations (Pers. obs. M. Fountain). In addition, *L. rugulipennis* nymphs continue to feed and develop after parasitism, so crop damage may not be reduced.[19]

Vacuuming trap crops such as alfalfa adjacent to strawberry (or the strawberry crops themselves) has shown efficacy for supressing other *Lygus* species in the USA, particularly in organic crops where insecticide options are not available.[21,22] The attractant trap crop can be used in combination with a push element to repel or deter the pest from a crop. Similarly, trap cropping with alfalfa, which is subsequently treated with insecticides, has demonstrated some efficiency for managing *L. rugulipennis* in lettuce.[23]

Originally developed using companion cropping,[24] the 'push–pull' strategy has been demonstrated most notably against lepidopterous stem borers and the pea leaf weevil, *Sitona lineatus* L.,[24–27] and is rarely effectively employed in horticultural crops (see review by Eigenbrode *et al.*[28]). The stem borer push–pull strategy includes wild grasses around the perimeter, which act as a trap crop for stem borers and a reservoir for natural enemies, coupled with repellent plants inside the crop.

The most common push–pull strategies rely on establishing and maintaining extra plants/crops, in addition to the focus crop requiring protection. An alternative approach would be to deploy synthetic insect- and plant-produced semiochemicals, mimicking insect and plant natural interactions to deter pest incursion into crops and attract pests away from crops. To our knowledge, there are no push–pull approaches in agricultural systems that employ only synthetic semiochemicals.

Synthetically deployed hexyl butyrate (HB) has been shown to have a repellent effect on *L. rugulipennis* under field-trapping conditions, whereby significantly fewer *L. rugulipennis* females were captured in traps baited with synthetic semiochemical blends.[29] This suggested that HB might have potential as the push component of a push–pull strategy. In addition, Groot *et al.*[30] observed a similar response in another mirid, *Lygocoris pabulinus* L. (Miridae). The metathoracic scent gland contained mostly HB, which was hypothesised as an alarm pheromone released by females. Indeed, undisturbed males and females and disturbed males released less than 100 ng h^{-1} HB, compared with disturbed females which released 25 ng h^{-1} to >1 mg h^{-1}. When HB was included in the field tests, the trap also caught fewer male *Lygocoris pabulinus*. Interestingly, HB is also a component of the female sex pheromone and is required for the interspecific attraction of males to females in both *Lygocoris pabulinus* and *L. rugulipennis*.[31] Groot *et al.*[30] suggested that male *Lygocoris pabulinus* were not repelled by HB, but rather it inhibited sex pheromone release in females.

In previous research we identified and developed a synthetic female-produced sex pheromone for *L. rugulipennis*,[31,32] which combined with an all-green (cross-vane and bucket) bucket trap (Unitrap; Agralan), attracted males for use as a monitoring tool.[33,34] The pheromone is a specific blend of HB, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal impregnated onto a cigarette filter in a polypropylene pipette tip.[31] The synthetic blend captured more males than traps baited with virgin females, with all three compounds required for maximum attractiveness. Additionally, males of the common green mirid *Lygocoris pabulinus* were attracted to the same blend.[31] *Lygocoris pabulinus* is also a pest of strawberry, and more frequently cane fruits such as raspberry and blackberry, and tree fruits (e.g. apple, pear and cherry).[7] Furthermore, a plant floral volatile component, phenylacetaldehyde (PAA), was shown to be attractive to female *L. rugulipennis*[35,36] and was added in an additional dispenser next to the sex pheromone to attract both male and female *L. rugulipennis* into one trap.[35]

The aim of these studies was to develop and test a synthetic, semiochemical, push–pull control method for *L. rugulipennis* in strawberry crops that would reduce the numbers of pest in the crop, the number of fruits with 'cat-facing' damage caused by *L. rugulipennis* and the requirement for insecticide sprays to control mirids in high-value crops. The benefits of this would be a control strategy that is more conducive to integrated pest management of crops, application of fewer insecticides and reduced residue levels in fresh produce. We utilised a series of field experiments to test the efficacy of this approach.

2 MATERIALS AND METHODS

2.1 Semiochemicals and dispensers

All synthetic semiochemicals were formulated at the Natural Resources Institute (NRI), University of Greenwich. The HB (Sigma Aldrich) 'push' component was dispensed from heat-sealed polyethylene sachets (Transpack Ltd). The standard dispensers contained 1 ml (860 mg) HB on a cellulose dental roll in a polyethylene sachet (50 mm × 50 mm × 120 μm thick) with release rate of 18 mg d^{-1} at 22°C. To double the release rate of dispensers, two dental rolls each with 1 ml HB were sealed in a polyethylene sachet (100 mm × 50 mm × 120 μm thick) to give a release rate of 36 mg day^{-1} at 22°C.

The synthetic *Lygus* sex pheromone 'pull' component was formulated in 1-ml disposable pipette tips containing 10 mg hexyl butyrate + 0.3 mg (*E*)-2-hexenyl butyrate + 2 mg (*E*)-4-oxo-2-hexenal + 1 mg Waxoline Black (ICI) in 100 μl sunflower oil on a cigarette filter, with a release rate of HB 0.93 ± 0.05 (SE) μg h^{-1} at 27°C, as described by Fountain *et al.*[31]

An attractant for female *Lygus*, PAA, was formulated in polyethylene sachets (0.5 ml on dental roll in a polyethylene sachet 50 mm × 50 mm × 120 μm thick), with a release rate of 6.7 mg d^{-1} at 22°C.[35]

Semiochemical attractant (pull) and repellent (push) dispensers were renewed at least every 4 weeks in all experiments in the following trials.

2.2 Ability of hexyl butyrate to reduce pheromone trap catches

In 2011, three field experiments were undertaken to test whether the addition of standard HB dispensers could reduce numbers of *L. rugulipennis* males caught in sex pheromone-baited traps and thus act as inhibitors of attraction or repellents.

Experiments were in or adjacent to a purpose sown weed field (~0.25 ha, 29 April 2010) at NIAB EMR, UK, (51.285173N, 0.461774E), comprising *Tripleurospermum inodorum* (L.) (Asteraceae) and *Chenopodium album* L. (Amaranthaceae). The field had a high *L. rugulipennis* population. Traps were all-green bucket traps (Unitrap, Agralan) consisting of a bucket with a funnelled entrance and square green cross-vanes between the bucket and the roof (bucket 16 cm diameter, 12.5 cm high with a 3-cm diameter opening; cross-vanes 10 cm high, cover 16.5 cm diameter). A single sex pheromone dispenser was hung under the roof of the trap and traps contained a drowning solution of 250 ml water with a drop of detergent to enable the insects to sink. Traps were hung ~30 cm from the soil surface, spaced 8–20 m apart in a line or a grid design, and secured to the ground with a metal pin. Each test used a randomised block design with five replicates of each treatment.

In the first experiment, catches of male *L. rugulipennis* were compared for: (i) traps baited with the pheromone alone, and (ii) pheromone combined with a sachet dispenser containing HB. The experiment was repeated three times in April and June 2011 and catches were recorded and discarded approximately every 7 days.

In the second experiment there were four treatments, an individual trap with: (i) pheromone only, (ii) pheromone and HB dispenser, (iii) pheromone and six HB dispensers at a distance of 1 m, and (iv) pheromone with six HB dispensers at a distance of 5 m. HB dispensers were attached to canes at crop height in a hexagonal arrangement around the pheromone trap. Trap catches were compared between treatments. The experiment started on 7 July 2011 and catches were recorded and discarded after 5, 7, 12 and 14 days.

In the third experiment, there were four treatments, an individual trap with: (i) pheromone only, (ii) pheromone and HB dispensers 0.7 m from the trap, (iii) pheromone and HB dispensers 3.5 m from the trap, or (iv) pheromone and HB dispensers 7.0 m from the trap. The four HB dispensers were arranged in a square around the trap. Trap catches were compared between treatments. The experiment started on 11 August 2011 and catches were recorded and discarded after 5, 7 and 13 days.

2.3 Push–pull field trials

In 2017, a trial was carried out in commercial strawberry crops to test the application of: (i) push only, (ii) pull only, or (iii) combined push–pull strategies in comparison with (iv) an untreated control. In 2019, a similar trial tested whether the push could be further enhanced by doubling the amount of HB in the crop.

2.3.1 *Experimental sites and layout*

Field experiments were undertaken in polythene-clad high tunnels of commercially grown strawberry. In 2017, all replicate fields were in Kent (south-east England) on cvs. Amesti and Sweet Eve 2 with plants grown on a tabletop system. In 2019, three organic strawberry fields (cvs. Serena and Eve 2) in the West Midlands, UK were used, and plants were grown in soil in raised beds on the ground.

Randomised block designs were employed with four and three replicate blocks in 2017 and 2019, respectively. In both years, each block (field) had four plots and the minimum distance between plots was 50 m. All plots were 24 × 24 m, that is three or four polytunnels wide depending on polytunnel width (6 or 8 m) at each block. Each plot was set up on the corner or edge of a field adjacent to non-crop habitat and contained no fewer than 2000 strawberry plants, and no fewer than 75 HB dispensers per plot.

2.3.2 *Treatments*

The push treatment consisted of a central 15 × 15 m area with repellent sachets stapled to the coir bags or plastic mulch that the strawberries were grown in along eight rows at regular intervals dependent on deployment rate. The pull was 12 green bucket Unitraps with green cross-vanes (as above) baited with synthetic *Lygus* sex pheromone and synthetic female *Lygus* attractant, plus PAA. Traps were positioned around the plot perimeter, ≥4.5 m from the HB sachets to prevent interference between repellent HB and attractive *Lygus* sex pheromone. Traps were spaced at 8-m intervals and secured between strawberry plants or row ends between the metal support and the first plant.

In 2017, the four treatments were: (i) push, (ii) pull, (iii) push–pull, or (iv) an untreated control. HB sachets in the push were spaced at 2-m intervals (64 per plot). In 2019, treatments were: (i) the push–pull tested in 2017, (ii) HB sachets every 1 m (120 per plot), (iii) a double HB release rate sachet at 2-m intervals, and (iv) untreated control.

2.3.3 *Assessments*

In both years, field trials were set up in June and ended in early September and insect numbers and fruit damage were assessed every 2 weeks. In 2017, field trials assessments were carried out on four occasions for each category, which included arthropod counts from plant tap samples, sex pheromone trap catches, and fruit damage assessments. In 2019, field trials between three and five arthropod assessments and two and four fruit damage assessments were done in each block in response to number of mirids and crop stage, that is, attention was focused where more mirids were present.

Assessments compared numbers of *L. rugulipennis* and other arthropods in each plot by tap sampling individual plants, striking each plant rapidly at least three times over a white tray (460 × 356 × 25 mm) in the central 15 × 15 m of each plot. In 2017, 60 plants were tap sampled per plot on each visit. In 2019, 50 plants were tap sampled. Insects were returned to the crop.

To compare numbers of *L. rugulipennis* and other arthropods caught in perimeter traps, all 12 traps per plot were emptied every 2 weeks and arthropods recorded. Pest mirid species, sex and life-stage, and beneficial arthropods were identified in the field using a hand lens. If required, samples were brought back to the laboratory to confirm identification under a microscope.

Strawberry flowers were tagged with coloured tape in the central 15 × 15 m of each plot at each visit to time subsequent fruit damage assessments. The timing of the first assessment was determined by following tagged flowers through to fruit ripening. Fruit at the same development stage were assessed to prevent bias. This was normally just before ripening, with a pink blush to red colouration. Approximately 100 fruits were assessed per plot and categorised according to mirid damage: 0 (zero), 1 (slight), 2 (moderate) and 3 (severe) (Figure 1).

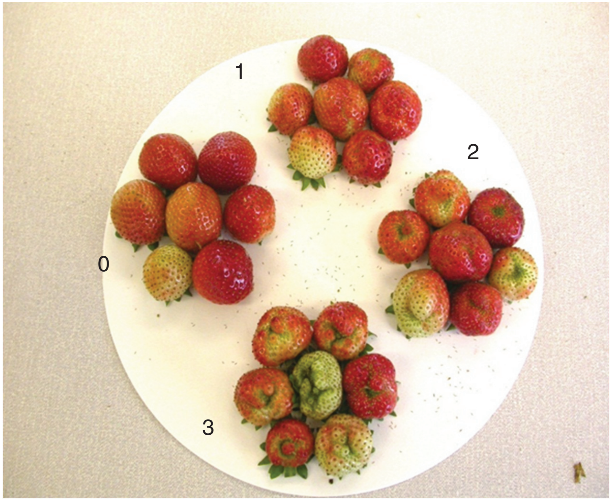

FIGURE 1. Categories of mirid damage for strawberry fruits; clockwise from left: 0 = no damage; 1 = slight damage; 2 = moderate damage; 3 = severe damage.

2.4 Statistical analyses

All statistical analyses were carried out using R 3.6[37] with the significance threshold set at $P < 0.05$.

For the small-scale HB experiments the effect of treatment on insect catches was estimated by fitting a generalised linear model (GLM) with Poisson family and log link function. The dependent variable was the number of insect counts per day or the total (thus multiple models were fitted), and independent variables block and treatment. A χ^2 analysis of deviance was used to test whether there was a significant effect of treatment. To test for differences between treatments, estimated marginal means and contrasts were calculated for experiments with more than two treatments (experiments 2 and 3) as implemented in the R emmeans package.[38] Contrasts used Tukey adjustment to control the familywise error rate.

Assessments in the push–pull trials in 2017 and 2019 were repeated measures and therefore used a generalised linear mixed model (GLMM) approach to account for non-independence between assessments. For several of the models variance within the repeated measures random effect was effectively zero, or counts were too low to support a GLMM, and these models were therefore refitted with a GLM. For the 2019 trap catch data, a GLM approach with Poisson family and log link function was used. Where over-dispersed, the models were refitted with the Quasi-poisson family. Statistically significant effects of treatment, assessment and their interaction were calculated using analysis of deviance. The 2017 tap assessments and trap catch and 2019 tap assessments of beneficials used a GLMM with Poisson family and log link. For the 2019 tap assessments, the effect of treatment and assessment date on mirid numbers was estimated using a GLMM with the negative binomial family and a log link function due to large over-dispersion. Statistically significant effects of treatment, assessment and their interaction were calculated using a likelihood ratio test. For all models post-hoc marginal means and contrasts were calculated using the R emmeans package. GLMMs were fitted using the R lme4 package[39] and the negative binomial family used the R MASS[40] package implementation.

In 2017 and 2019, data for fruit damage were analysed by first calculating a damage score using the formula (%0*0 + %1*1 + %2*2 + %3*3)/3. Values ranged from 0 if all the fruits are in the '0' category, to 100 if all the fruits are in the '3' category. Although this did not relate directly to the mean % damage, it allowed data between plots to be compared statistically and transformed for analysis; in this case, the data were transformed using an angular transformation (arcsine(square root(count)) * (180/pi)) prior to analysis of variance. Overall effects of the respective 'push–pull' treatments and interactions were examined. Results were back-transformed from the transformed scale for presentation. The number of strawberries in each category was also analysed separately using mixed model logistic regression (GLMM with binomial family and logit link) as implemented in lme4. Test for significance used likelihood ratio tests, and post tests were performed with emmeans.[38]

3 RESULTS

3.1 Ability of hexyl butyrate to reduce pheromone trap catches

3.1.1 Experiment 1

Trap catches of male *L. rugulipennis* were significantly lower where a HB sachet was added to the sex pheromone dispenser in the trap, compared with where there was no additional HB sachet (Table 1).

3.1.2 Experiment 2

Catches of male *L. rugulipennis* were lower in traps with HB in the trap (0 m), or with HB placed in a hexagon pattern at 1 or 5 m distance around the sex pheromone traps compared with those in the control traps (Table 2). When HB dispensers were placed 5 m from traps, numbers captured were reduced by 44% compared with 99% if HB was placed inside the trap (0 m) or only 1 m from the trap.

3.1.3 Experiment 3

Catches of male *L. rugulipennis* were significantly fewer in traps surrounded by HB dispensers at 0.7 or 3.5 m from the trap, but not 5 m, compared with control traps with no HB dispensers (Table 2). When surrounding HB dispensers were placed in 3.5 or 5 m from the trap, numbers captured were significantly higher than when HB was at 0.7 m distance ($z = -7.214$, $P < 0.001$ and $z = 9.207$, $P < 0.001$, respectively). When HB dispensers were placed in 5 m squares, numbers captured were significantly higher than when HB was at 3.5 m distance ($z = 4.411$, $P < 0.001$).

3.2 Push–pull field trial, 2017

In 2017, a trial in commercial, conventionally managed strawberry crops tested whether the HB (push) alone, sex pheromone + PAA alone (pull), or a combined push–pull system could reduce fruit damage compared with an untreated control. The effect of treatment (HB, PAA, combined) on the number of *L. rugulipennis* nymphs or adults in tap catches was not significant ($P > 0.16$), although it is of note that the total *Lygus* count was low in this experiment (Table 3). Therefore further analysis of pull/no pull, push/no push and their interaction was performed which indicated that there were fewer *L. rugulipennis* nymphs (Figure 2A) and significantly fewer adults (Figure 2B) in strawberry plots where HB (push) was applied compared with plots without HB (untreated control and pull only) ($\chi^2 = 3.4239$, df = 1, $P = 0.06$ and $\chi^2 = 4.189$, df = 1, $P = 0.04$, respectively).The effect of the pull treatment and the interaction on numbers of either nymphs or adults was not significant ($P > 0.18$).

TABLE 1. Experiment 1: mean numbers of male *Lygus rugulipennis* caught in Unitraps baited with sex pheromone with or without hexyl butyrate ($N = 5$)

		Mean numbers of males (SE)				
Start date	Duration (days)	Pheromone	Pheromone + hexyl butyrate	χ^2	df	*P*-value
18 April 2011	24	25.8 (4.9)	0	1267.4	1	$P < 0.001$
20 April 2011	22	4.2 (0.6)	0	137.0	1	$P < 0.001$
20 June 2011	15	11.0 (1.7)	0.2 (0.2)	127.0	1	$P < 0.001$

TABLE 2. Mean numbers of male *Lygus rugulipennis* caught in traps baited with sex pheromone alone or surrounded by hexyl butyrate (HB) dispensers ($N = 5$)

Distance of HB from pheromone trap (m)	Mean catch (SE)	% Reduction in catch compared with control	*z* ratio from control	Significance from control
Experiment 2 (7–21 July 2011): six HB dispensers in hexagon formation				
0	0.2 (0.2) a	99	4.839	$P < 0.001$
1	0.2 (0.2) a	99	4.839	$P < 0.001$
5	8.8 (3.7) b	44	3.049	$P = 0.012$
None (control)	15.6 (7.1) c	–	–	
Experiment 3 (11–24 August 2011): four HB dispensers in square formation				
0.7	2.4 (0.8) a	95	10.055	$P < 0.001$
3.5	21.4 (4.3) b	54	6.543	$P < 0.001$
7.0	36.6 (6.5) c	20	2.309	$P = 0.096$
None (control)	46.0 (13.6) c	–	–	

Within each experiment, mean catches followed by different letters are significantly different at $P \leq 0.05$.

TABLE 3. Mean numbers of *Lygus rugulipennis* nymphs and adults pooled from tap sampling in all plots (adjusted to 50 plants per plot) over the whole season, during two years of push–pull trials in commercially grown strawberry. Unidentified nymphs were early instars

		Mean number/50 plants *L. rugulipennis*		Unidentified
Year	Growing system	Nymphs	Adults	Nymphs
2017	Conventional	0.3	0.2	0
2019	Organic	10.9	5.6	23.3

When comparing mirid catch in perimeter 'pull' traps, fewer were captured in the pull only treatment compared with the push–pull treatment (mean = 2.19 and 1.43, respectively), but the difference was not significant at $P \leq 0.05$ ($\chi^2 = 3.5946$, df = 1, $P = 0.06$).

When strawberry fruit damage score was analysed, push (HB) and pull (sex pheromone + PAA) treatments alone did not significantly reduce fruit damage compared with the control ($t = 1.500$, df = 3, $P = 0.447$ and $t = 1.916$, df = 3, $P = 0.238$, respectively), but the combined push–pull treatment did ($t = -3.103$, df = 3, $P = 0.018$) (Figure 3A). Comparing mean per cent of strawberries with zero mirid damage, the push or pull treatments alone did not significantly increase undamaged fruit compared with the control ($t = 1.653$, df = 3, $P = 0.3617$ and $t = 2.052$, df = 3, $P = 0.1868$, respectively), however the combination of push–pull did reduce numbers of undamaged fruits ($t = 3.117$, df = 3, $P = 0.0173$) (Figure 3B).

3.3 Push–pull field trial, 2019

In the 2019 trial, different release rates and spacing of HB repellent sachets in combination with the pull, perimeter traps with sex pheromone plus PAA traps, were compared relative to an untreated control in commercial organic strawberry crops.

There were significantly fewer nymphs per 50 plants in all push–pull treatment plots (standard HB, double HB deployment and double HB release rate) compared with the untreated control ($z = 5.526$, $P < 0.001$; $z = 4.118$, $P < 0.001$; and $z = 4.983$, $P < 0.001$) (Figure 4A). Mirid nymphs were grouped for statistical analysis because early instar nymphs could not be accurately identified to species in the field. Late instar nymphs were predominantly *L. rugulipennis* (Table 3). There were also significantly fewer *L. rugulipennis* adults in all push–pull treatments compared with the untreated control ($z = 3.565$, $P = 0.002$; $z = 3.468$, $P = 0.003$; and $z = 3.567$, $P = 0.002$, respectively) (Figure 4B).

More than half of all beneficials, per 50 plants, were Araneae spp. (61%), followed by parasitoids (Hymenoptera spp.) (13%), and Anthocoridae (10%). These three groups, along with Neuroptera spp. nymphs (4%) were present in sufficient numbers for statistical analysis, but there was no significant difference between treatments and control ($\chi^2 = 5.0784$, df = 3, $P = 0.17$; $\chi^2 = 1.9566$, df = 3, $P = 0.58$; $\chi^2 = 0.11762$, df = 3, $P = 0.42$; and $\chi^2 = 0.03167$, df = 3, $P = 0.30$, respectively).

Mean fruit damage (score) was significantly lower in all push–pull treatments (standard HB, double HB deployment and double HB release rate) compared with the untreated control ($t = 4.163$, df = 6, $P = 0.02$; $t = 3.615$, df = 6, $P = 0.04$; and $t = 4.163$, df = 6, $P = 0.02$, respectively) (Figure 5A). Correspondingly mean per cent of strawberries with zero mirid damage was significantly

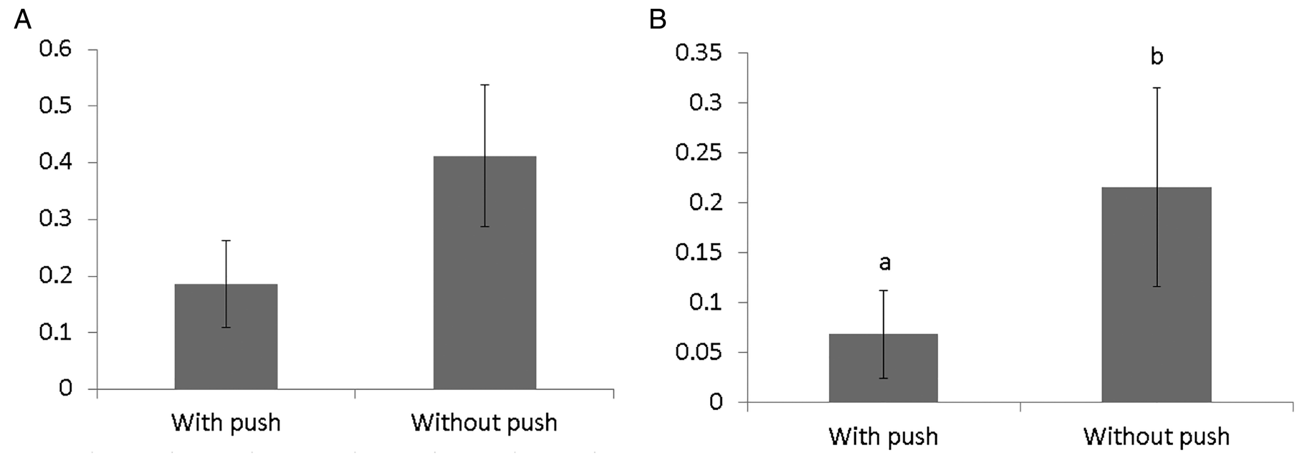

FIGURE 2. Mean numbers (± SE, $N = 4$) of *Lygus rugulipennis* (A) nymphs and (B) adults recorded in tap samples of 60 strawberry plants where the push treatment (hexyl butyrate dispensers alone or in combination with pheromone traps) was applied compared with no push (pheromone trap or control treatments), in conventional strawberry in 2017. Different letters indicate significant differences between treatments at $P \leq 0.05$.

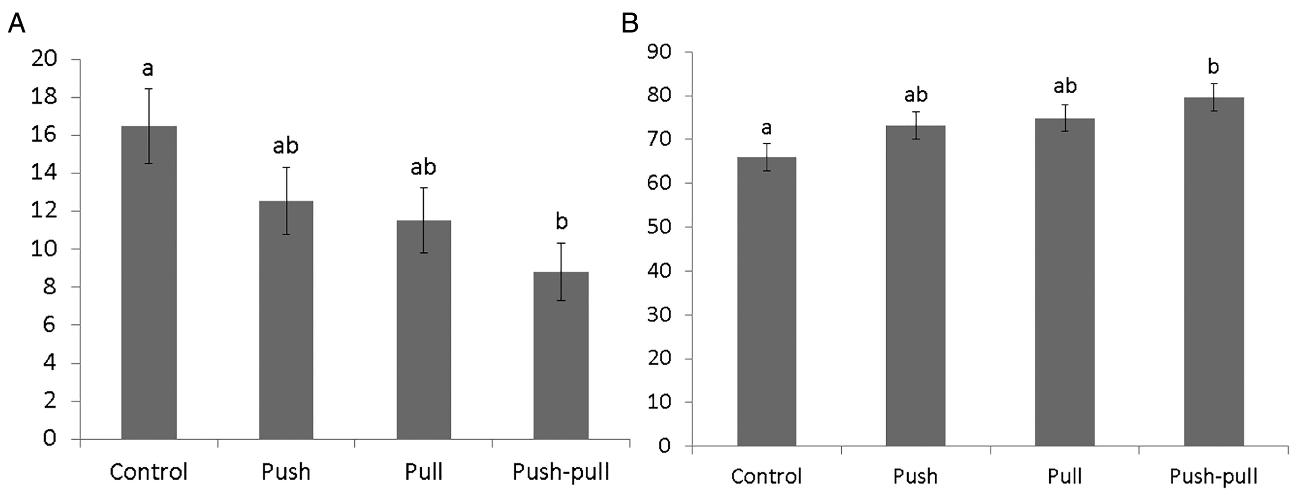

FIGURE 3. (A) Mean fruit damage score (back transformed ± SE; N= 4) of ~100 strawberries sampled, and (B) mean percentage ± SE ($N = 4$) of ~100 sampled strawberries with zero mirid damage (category 0) in the untreated control, push (hexyl butyrate), pull (perimeter sex pheromone and phenylacetaldehyde traps) and push–pull treatments, in conventional strawberry crops, in 2017. Different letters indicate significant differences between treatments at $P \leq 0.05$.

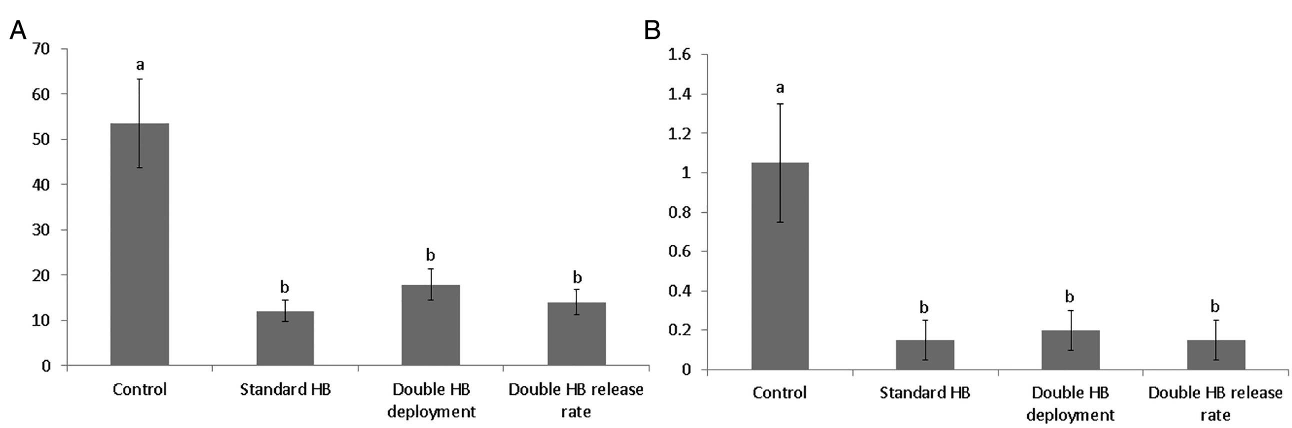

FIGURE 4. Mean numbers (± SE, $N = 3$) of (A) mirid nymphs and (B) *Lygus rugulipennis* adults recorded in tap samples of 50 plants in the organic strawberry crops in 2019 in untreated control, standard HB (hexyl butyrate), double HB deployment and double HB release rate plots. Different letters show significant differences between treatment means at $P \leq 0.05$.

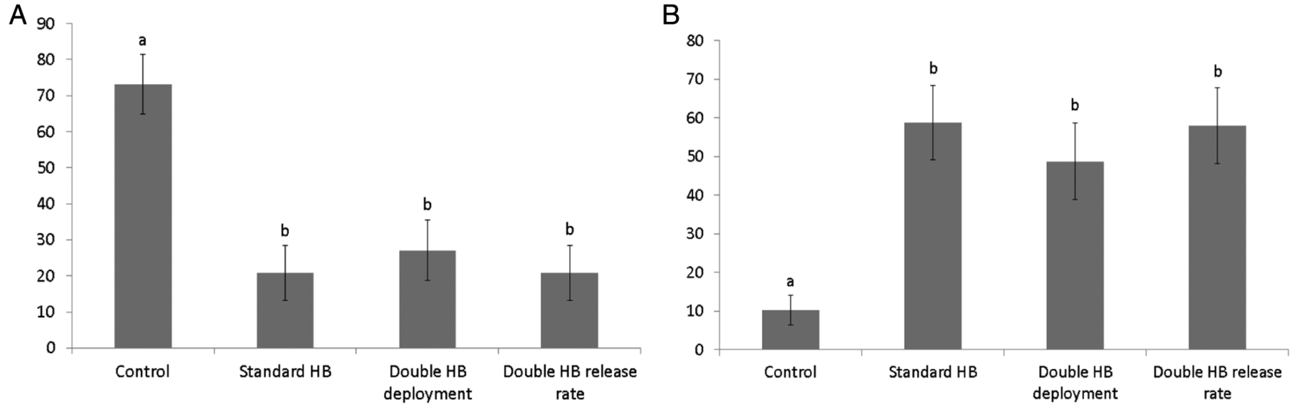

FIGURE 5. (A) Mean fruit damage score (back-transformed ± SE; $N = 3$) of strawberries sampled and (B) mean ± SE ($N = 3$) percentage of ~100 sampled strawberries with zero mirid damage (category 0) in organic strawberry in 2019, in the control, standard HB (hexyl butyrate), double HB deployment and double HB release rate plots. Different letters show significant differences between treatments at $P \leq 0.05$.

higher in all push–pull treatment plots compared with the untreated control ($z = 4.42$, $P < 0.001$; $z = 3.70$, $P = 0.0012$; and $z = 4.314$, $P < 0.001$) (Figure 5B).

When comparing mirid catch in perimeter 'pull' traps surrounding each push–pull treatment (standard HB, double HB deployment and double HB release rate), there was no significant difference in mean numbers of adult *L. rugulipennis* caught ($\chi^2 = 5.001$, df = 2, $P = 0.29$) (mean per trap = 0.08).

There was also no significant difference in numbers of beneficial insects caught in traps between push–pull treatments (no traps in untreated control). These included small numbers of *Apis mellifera* ($\chi^2 = 0.0942$, df = 2, $P = 0.73$), *Bombus* spp. ($\chi^2 = 0.07169$, df = 2, $P = 0.96$) and Coccinellidae spp. ($\chi^2 = 0.27729$, df = 2, $P = 0.28$) (mean per trap = 0.18, 0.04 and 0.06, respectively).

4 DISCUSSION

We have demonstrated a successful push–pull strategy for control of *L. rugulipennis* in conventional and organic strawberry crops using synthetic semiochemicals. The push–pull system reduces the numbers of the pest in the crop, but more importantly reduces the numbers of fruit damaged ('cat-facing') by *L. rugulipennis*, increasing marketable yield and significantly reducing the requirement for traditional insecticide applications, typically two or three per year in UK affected crops.

Prototype polyethylene sachets loaded with 1 ml HB (push) repelled 99%, 54%, 44% and 20% of males from sex pheromone baited traps when placed at distances of approximately 1.0, 3.5, 5.0 and 7.0 m from the trap. When HB dispensers were placed at 2-m intervals along strawberry rows in combination with perimeter traps (baited with sex pheromone and PAA; push–pull), numbers of damaged strawberry fruit were reduced by approximately 50%; from approximately 16% (untreated) to 8% (push–pull) damaged fruits in conventional crops. Application of either the push or the pull alone reduced fruit damage by around a quarter, but this was not statistically significant.

Subsequent commercial field trials in organic strawberry crops, where there are few chemical options for mirid control that are not damaging to natural enemies, reduced numbers of *L. rugulipennis* adults and nymphs in the crop by approximately 80%. As a result, there were five times more 'cat-face' damaged fruit in the untreated control plots compared to the plots with the push–pull system. Indeed, only approximately 10% of strawberries in organic crops had no damage from mirids in untreated organic strawberry plots, with approximately 49–59% of fruits being damage free in the plots which employed the push–pull control strategy.

The push–pull control was deployed in crops before the first invasion of the summer generation of adults in June. It is likely that if the push–pull treatment was applied earlier in the season to disrupt overwintered *L. rugulipennis*, from March, there would be a greater season-long impact. As fewer, highly mobile, adult *L. rugulipennis* were observed in crops with the push–pull system it is hypothesised that mated females were deterred from entering the crop to lay eggs, resulting in fewer *L. rugulipennis* nymphs. Nevertheless, HB was also a deterrent to male *L. rugulipennis*, as evidenced from the earlier small-scale experiments in which the addition of HB to the sex pheromone traps significantly reduced catches. The mechanisms behind these responses deserve further investigation, but studies by Fountain *et al.*[31] found that headspace collections from groups of *L. rugulipennis* females elicited a higher relative release rate of HB than collections made from single females, indicating that crowding may be one trigger for higher HB release. Groot *et al.*[30] showed that undisturbed male and female *Lygocoris pabulinus* and disturbed males released less than 100 ng h^{-1} HB, whereas disturbed (shaking in a bottle) females released a highly variable amount (ranging from 25 ng h^{-1} to >1 mg h^{-1}). Groot *et al.*[30] proposed that males were not repelled by HB, but that this compound inhibited sex pheromone release in females. For *L. rugulipennis*, we did not differentiate between males and females tapped from strawberry plants during the field assessments, so it is difficult to conclude whether the latter is the mechanism of action of the HB in these mirids. HB has been identified in previous research as a potential repellent agent for *Lygocoris pabulinus*[30] and *L. rugulipennis*[29,31] and is part of the sex pheromone blend of these two species; hence it is potentially a specific alarm pheromone in the mirid family. Minimal effect was observed on strawberry pest natural enemies in our study, but a more rigorous set of tests would be needed to show no effect. However, findings from our study suggest this strategy would be applicable to integrated pest management and organic systems.

Increasing the frequency of the HB sachets to every 1 m in the strawberry rows or doubling the release rate of the HB from the sachets in the push areas of the crop did not further improve mirid control or fruit damage. However, more research is needed to investigate, on a larger scale, whether HB dispensers could be spaced further apart, especially earlier in the season or in conventionally managed plots where *L. rugulipennis* numbers in are lower. In addition, no studies have been done to optimise the spacing of sex pheromone + PAA baited traps (pull) and this may be dependent on the pest numbers in the surrounding landscape. The combination of the push and the pull was more effective than push or pull alone, but the combination was not synergistic.[28,41]

The first attempt at using semiochemicals to control a mirid bug investigated small-scale mating disruption of *Campylomma verbasci*[42] and there has been little progress since. In recent years, advances have been made in the identification of a range of mirid sex pheromones (see El-Sayed[43]), opening up opportunities to manipulate mirid behaviour in crops akin to Lepidopteran damage limitation by semiochemicals in past decades (Knight *et al.*[44]).

5 CONCLUSION

To our knowledge, our approach is the first to significantly reduce crop damage by mirids using synthetic semiochemicals, potentially reducing the need for alternative approaches, including chemical insecticides. Advantages of a synthetic semiochemical approach compared with a plant-based push–pull system are that it is not necessary to maintain an additional crop or give up an area of land to the pull.[27,28] However, Cook *et al.*[25] recognised that the cost of registration of semiochemicals is often high, and this is currently a potential barrier to their application, particularly for horticultural crops where market size and area of crop may be relatively small compared with arable crops. Another barrier to adoption is the cost of labour to deploy and maintain the traps and dispensers, hence investigation into reduced dispenser densities is warranted.

Future studies could optimise the female attractant for *L. rugulipennis* with specific blends of plant compounds.[45–47] More understanding of the cues and modalities (including short-range, spatial and contact repellence) of the semiochemical insect interaction, and fine-tuning the deployment of geometry and spacing[28] are needed to optimise the system for further exploitation.

ACKNOWLEDGEMENTS

We would like to thank the Agricultural and Horticultural Development Board (projects SF 156 and 94) and the Department for Environment, Food and Rural Affairs (project HL0191) for financial support and the growers for hosting the field trials. We would particularly like to thank the NIAB EMR field assistants for their help with data collection including J. Bubb, M. Perry-Clarke, J. Lowe, C. Silva and C. Conroy.

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