'Good Vibrations' – Developing and testing the efficacy of biotremology as a control strategy to disrupt mating/reproductive success in *Lygus rugulipennis* and *Drosophila suzukii* in strawberry

Introduction

The UK Soft Fruit industry faces several challenges, including uncertain chemical pesticide approvals, the loss of actives (and associated insecticide resistance), emerging and invasive pests and climate change, which can result in higher insect pest populations as well as unpredictable outbreaks. Effective alternative approaches to pest control are therefore required to prevent a reliance on chemical intervention.

The invasive Spotted Wing Drosophila (SWD, *Drosophila suzukii* Matsumura), and capsid European Tarnished Plant Bug (ETPB, *Lygus rugulipennis* Poppius) are both serious pests of commercial strawberry in the UK, having the potential to reduce marketable yields of fruit by 50% and more, if left uncontrolled (AHDB, SF 174 report 2021).

Specifically, SWD causes damage to fruit through larval feeding and pathogen infection via the oviposition (egg-laying) hole. It is particularly detrimental to crops due to the female's serrated ovipositor, which allows it to lay eggs in ripening fruit, whereas other fruit fly species are only able to lay in overripe, decomposing fruit. ETPB feeding causes damage to developing fruits and results in severe deformation known as 'cat-facing'.

Biotremology, the study of mechanical vibrations and their effect on organism behaviour, has revealed that some insects, including ETPB, use vibration signalling at species-specific frequencies in their close-range courtship, in addition to strategies such as semiochemical, visual and audial communication. Interfering with these signals could reduce mating and reproductive success and therefore pest population growth.

Trial C-300164 (AHDB funded, summary included below) developed and pilot tested equipment that could be used to send vibratory signals, perceivable by insects and detectable by frequency receptors, through several platforms. Laboratory trial results were promising: SWD oviposition was impacted by the vibrations in four of six replicates, but further analysis found these differences not to be significant.

A field-based trial (C-300159 WCoF funded) was subsequently designed to test the efficacy of vibration signalling to disrupt SWD and ETPB behaviour in commercial strawberry crops. This transferred the equipment designed and tested in the C-300164 project from the laboratory into a semi-field trial. The impact of biotremology on SWD egg laying and ETPB cat-facing damage was assessed to investigate whether this approach could be adopted in a commercial table-top strawberry crop for IPM of these two pests.

This report summarises the methods and results from both the laboratory (AHDB funded C-300164) and semi-field (WCoF funded C-300159) projects.

Aims

The aims of the laboratory trial were:

- to explore methods of sending vibrations through different platforms to ensure these signals can be detected by the pest insects.
- to identify how to power the equipment for future use in the field, not connected to mains power.
- to execute replicated laboratory trials to assess the impact biotremology has on SWD oviposition.

The aims of the semi-field based trial were:

- to determine whether a method of biotremology can be employed in commercial tabletop growing systems
- to determine whether biotremology could reduce the incidence of capsid feeding damage (cat-facing) in strawberry.
- to determine whether egg-laying and larval counts of the soft fruit pest SWD may also be reduced using the same methodology.

Laboratory trial

Design, production, and equipment testing

Platform Design: The project commenced in January 2021, at NIAB EMR, Kent, with desk-based research into methods of vibration production, and the identification of suitable motors and power sources. At the time of writing, no biotremology devices were available commercially. Several methods to send vibratory signals were uncovered by this research including the use of sound and movement. The use of sound was quickly dismissed as we believed this method would not transfer into a field situation as well as the use of movement.

Research progressed into the design and manufacture of a prototype vibrating platform in February 2021. Following initial testing, the platform design was refined until it consisted of a rectangular timber frame (51 x 36 x 11cm, timber dimensions 4.3 x 4.3cm), raised on four rubber feet (Figures 1-5). Eight metal springs were positioned on the upper surface of this frame (four on each longest side) and these springs supported a three-sided timber (2 x 2cm) frame of the same dimensions, on to which a sheet of clear Perspex (0.5cm thick) was secured with screws. This formed the platform that would vibrate (Figs. 1 & 2). The rubber feet and springs were to prevent vibration signals being transferred to the tabletop where the equipment was being tested as this could cause the unintentional application of biotremology to the untreated controls which were also on the same tabletop. A single motor was bolted to one of the shorter sides of the frame, through the Perspex into the wood (Fig. 3). To the opposite side two motors were clipped directly to the Perspex sheet (Figs. 1 & 2). The platform could be used to support potted strawberries (Fig. 4) or Perspex boxes (Fig. 5) for testing.

Motor selection: Motors were obtained that required minimal power and that were able to produce consistent movement over several days when connected to a power source. Several motor types were tested over a period of three weeks and of those tested the DC 3V 8100RPM High Speed Motors were identified as suitable, with adequate longevity. Several of the other motors tested did not last more than a few days, even when connected to mains power, and were deemed unsuitable for long term use which was a requirement for use within this project. The DC 3V 8100RPM High Speed Motors were adapted to produce vibrations by securing a metal bolt and nut to the end of each axle and reinforcing this with hot glue. These off-centre weights (Fig. 3) caused vibrations as the motors spun. Additionally, the soldered connections of the motors were reinforced with hot glue to aid waterproofing and to make them more resilient to movement.

Speed and frequency control: Motor speed controllers were used to adjust the voltage (3V - 8100 RPM) in order to create vibration at the same low frequency range as L. rugulipennis' mating signal (0-200Hz). These were found to be more accurate, with a greater range, than potentiometers which were initially used. Piezoelectric acoustic pick-up diaphragms were connected via an audio interface to a laptop (Fig. 4) where Visual Analyser 64 software was used to visualise the frequency of vibration in real-time (Fig. 6). This software was used to identify the frequency travelling through various substrates including plant material and SWD egg laying media placed on the platform. The frequency controls on the motors were adjusted to ensure the correct range of 0-200Hz was detected in these different substrates, which is what the insects would detect when alighting on them. This software was also used to ensure that untreated controls were not transmitting the biotremology signal unintentionally.

Power supply: In initial motor tests, mains power was used to ensure any issues with the duration of movement was not caused by problems with power. Once the motors had been identified, each motor was powered by a rechargeable solar battery pack (Pealiker Solar Charger 25000mAh) (Figs. 1, 2 & 5) for SWD assessments. Prior to migrating into the field trial, motors were trialled for longevity using Halfords Leisure batteries HLB678, which was able to power the motors consistently for eight days on one charge (Fig. 15).

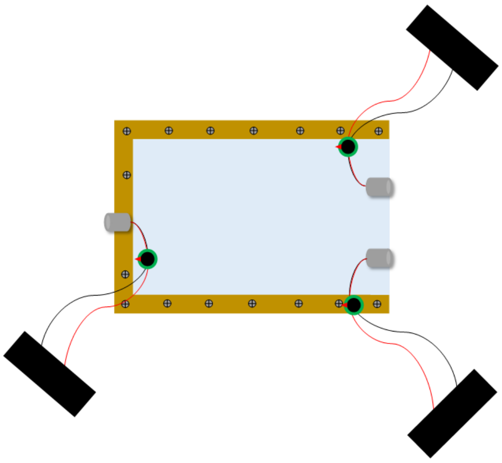

Figure 1: Overhead view of prototype platform showing 3-sided upper timber frame supporting Perspex sheet secured with screws, 3 motors, powered by rechargeable solar batteries, each regulated by speed controllers.

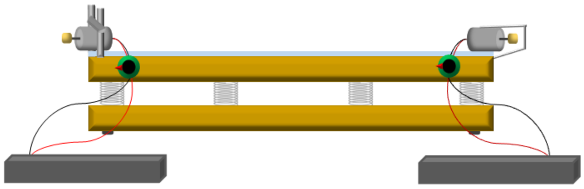

Figure 2: Side view of prototype platform: lower timber frame resting on rubber feet, springs supporting 3-sided upper timber frame with attached Perspex sheet, motor (L) bolted through Perspex into wood, motor (R) secured to Perspex using metal clip, each powered by rechargeable solar batteries, and regulated by speed controllers.

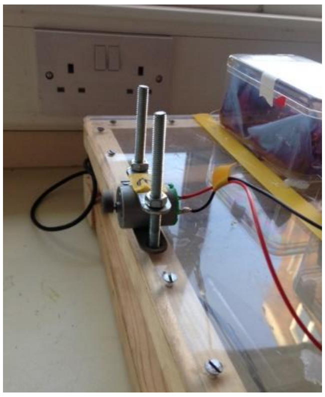

Figure 3: Left hand side of platform showing motor with off-centre weight on axle (L) (in motion), bolted to upper frame through Perspex sheet.

Figure 4: Laboratory set up showing vibrating platform supporting 6 potted strawberry plants (L) with microphone and audio interface (R) connected to laptop (out of shot).

Figure 5: Apparatus testing. Treatment (top), control (bottom), each with Perspex box containing dampened blue roll and 5 blueberries in dish. SWD to be added and lids replaced.

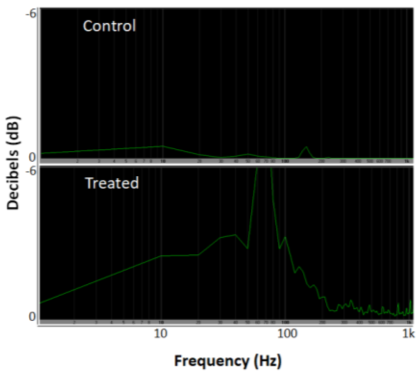

Figure 6: Screenshot of Visual Analyser 64 display for apparatus test. Top graph shows control where biotremology was not applied and bottom graph biotremology treatment.

Methodology testing

The apparatus was assessed using ‘no choice’ experiments carried out in a controlled temperature (20°C) environment at NIAB EMR, Kent. The experiment was subjected to natural light:dark cycles which were roughly 14 L:10 D. No choice tests do not give the test insect an option between the two treatments.

Initial testing involved the introduction of 10 female and five male SWD into two Perspex boxes (22.5 x 12 x 8 cm), each containing five blueberries in a 9cm petri dish, resting on dampened blue roll to maintain humidity levels (Fig. 5). One box was exposed to vibrations on the platform, the other box had no vibration applied. The initial test was run for 24 hours. This test was replicated several times to test the motors and power sources rather than to assess the impact on SWD egg laying. Using this approach, we were able to identify the best motor and power combination to be used in the following efficacy trials.

In the efficacy test the DC 3V 8100RPM High Speed Motors were attached to the platform and were powered by the rechargeable battery packs. Six smaller Perspex boxes (10 x 7 x 5 cm), each containing five female and three male SWD, a dampened sheet of blue roll to maintain humidity, and a 5cm petri dish containing cornmeal fly food medium for egg laying, were placed on the platform. Six Perspex boxes with the same set up were placed on a tray on the surface next to the platform (Fig. 7) and were not subjected to biotremology. Motors were run for nine hours for each replicate. Piezoelectric pick-ups were used to monitor the frequency spectrum produced in petri dishes of medium in both the treatment (to ensure appropriate frequency) and the control (to ensure no vibrations were detected) (Fig. 8). Six replicates of this trial were performed over a three-week period and the total number of eggs in the treatments and controls combined for the analysis.

Results

Vibration treatment resulted in lower SWD egg numbers laid in the blueberries in four of the six replicates when compared to the control treatment (Fig. 9). However, one replicate showed higher egg numbers in the treatment than the control. One replicate showed no significant difference between the two treatments.

Further data analysis showed that the mean number of eggs laid per berry, per rep, was lower in the treatment than the control (Fig. 10), however standard error indicates that this difference was not statistically significant.

Conclusions

Within the laboratory we were able to design and create equipment capable of sending vibratory signals through different substrates. These signals were detectable by Piezoelectric pick ups and could be manipulated through frequency controllers. This ensured the signals were within the desired range to disrupt ETPB. We were also able to identify the optimum combination of motors and power source to ensure equipment functioned consistently for the duration required, up to 48 hours on rechargeable batteries.

While the impact of this technology on SWD egg laying was not consistent (only significant in four of six replicates), it is a promising indication that it could be used to disrupt this pest. This laboratory based trial has enabled us to investigate this novel approach and we have been able to intercept possible issues prior to migrating into the field.

Figure 7: SWD egg laying assessment. Perspex boxes on left of image positioned on vibrating platform. Untreated control (no vibration) on white tray. Audio interface and laptop seen on table along with (disconnected) piezoelectric acoustic pick up diaphragm.

Figure 8: Use of Piezoelectric pick up to monitor vibrations in petri dish containing cornmeal fly food medium in control set up of no choice experiment shown left.

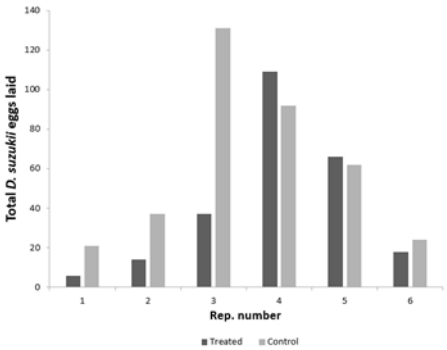

Figure 9: Total number of SWD eggs laid in 9 hrs, treatment (dark grey) versus control (lighter grey). Four of six replicates show lower oviposition in vibration treated blueberries.

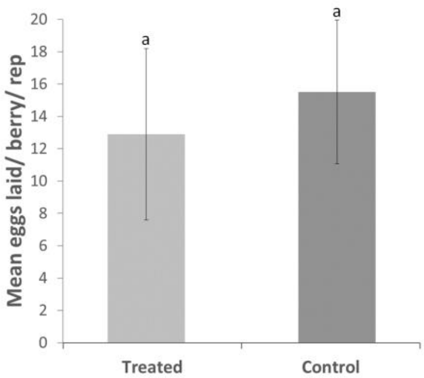

Figure 10: Mean number of eggs laid in 9 hrs, treatment (lighter grey) versus control (darker grey). Fewer eggs were found in treated berries, however difference not statistically significant.

Field trial

Set-up and Plant Husbandry

The field trial ran from July to September 2021, at Ditton Rough, NIAB EMR, Kent. Two commercial-standard, 8m tabletop rows, each containing 50 'Malling Champion' strawberry plants in six Botanicoir substrate growbags, were established within a single, insect exclusion-meshed, 10 x 10m polytunnel compartment (Fig. 15). Each tabletop had tight trellis tapes supported by wire supports.

Automated drip fertigation was employed, and volumetric water content (VWC) monitored using WET sensors. A data logger housed in a Stevenson screen was used to record temperature and humidity within the polytunnel. Regular plant health checks were conducted and any pest control applications e.g. *Amblyseius andersonii* (Agralan), were uniform across both treatment and control rows.

Crop development and fruit quality was monitored. The need for improved pollination was identified and hand-pollination was subsequently carried out weekly, since the addition of bees (Agralan) was ineffective. An additional five trusses per week were hand-pollinated for assessment of impact on ripe fruit shape (after three weeks). Runners and fruit were removed from the plants in the weeks preceding the start of the trial.

System Adaptations

Tests were conducted to determine if it was possible to modify the prototype vibration system in order to make use of the existing tabletop infrastructure to transmit vibrations.

Motors, adapted with scotch connectors as off-centre weights, were attached to the trellis tapes and measurements taken using piezoelectric pick-ups in various locations on the plants and tabletop structure (Fig. 11). These tests determined that significant vibrations were conducted throughout the tabletop infrastructure and that low frequency vibrations could be detected in the growbags, and plant material (leaves and fruit) that were not in direct contact with the trellis tapes (Fig. 12).

Consequently, a system of 12 adapted motors (DC 3V 8100RPM High Speed Motors, CPC), calibrated using speed controllers to create vibration at the same low frequency range as ETPB's mating signal (0-200Hz), was deployed on the treatment table's trellis tape and secured using zip ties (Fig. 13).

The system was split into three sets of four motors, with two motors on each side of the row (Fig. 14). Each set of four motors was powered by a Halfords Leisure HLB678 battery (Fig. 15). These were adopted as an alternative to the solar battery packs due to the requirement for a longer running period, which the smaller rechargeable batteries were unable to produce. A fully charged leisure battery was capable of powering a set of four motors continuously for eight days although we only ran them for five days (i.e. Monday morning to Friday evening) during the treatment application period of this trial. Batteries were positioned away from the drip irrigation lines to prevent them getting wet.

The control table was left untreated although was subjected to the same husbandry as the treated table. Vibrations in the plants and trellis tapes were monitored using piezoelectric acoustic pick-up diaphragms on both treatment and control table tops to ensure the correct frequency was achieved in the treatment plants and that no vibrations travelled to the control row (Fig. 16).

Exclusion bags (15 x 15cm) of fine, clear mesh were made using a heat sealer (Fig. 13). These bags were secured to trusses with green twist ties, tight to the stem to prevent ETPB nymphs and SWD accessing fruit prior to the start of the experiment. During the course of the experiment plastic bread bags, with micro-perforation (Sainsbury's) were also used as exclusion bags (Figs. 13 & 15). Any new fruit that set during the period of the experiment was bagged for use in future replicates. All overripe fruit was removed. No fruit was left unexposed to ensure all damage occurred during the treatment application process. Each week all flowers were hand pollinated with a fine paintbrush to ensure cat-facing was the result of ETPB damage and not from poor pollination.

Forty wild ETPB adults, collected from the NIAB EMR site by sweep-netting weeds (e.g. Fat Hen, *Chenopodium album*), were introduced to the tunnel (1:1 male to female ratio) in late July. Additionally, 70 lab-cultured, EMR20 strain, SWD adults (three-seven days old) were introduced (1:2.5 male to female ratio). Both SWD and ETPB were released in the centre of the tunnel, between the two table-tops and were free to travel between rows.

Further introductions of 70 lab-cultured SWD adults (1:2.5 male to female ratio) and 30 lab-cultured SWD adults were made in early August and early September respectively. Four pairs of wild ETPB adults were introduced in late August, and a further three pairs of ETPB adults, plus nymphs, in early September, following low numbers recorded in the table-tops from tap-sampling.

Treatment application

At the start of each replicate the motors were connected to the batteries and switched on (Monday morning). Once the motors were running, exclusion bags were removed from 20 trusses of fruit, exposing ripening fruit to the two insect pests. Trusses were a mixture of ripening stages to ensure there was the availability of green-pink fruit for both pests to target. Fruit was left exposed for five days while the motors were running. After five days, the exclusion bags were reapplied to the green and white fruit and the motors turned off (Friday evening). Trusses were labelled with the assessment week number. Ripe fruit was collected immediately prior to turning off the motors each week, placed in a Perspex box with mesh lid and incubated at 22°C for three days with a 16:8 light dark cycle. This was repeated several times over a two-month period.

Assessments

Sugar-water flotation was used to extract SWD larvae from the strawberries after the three-day incubation period. This was to ensure all eggs would have hatched to larval stage and could then be counted using the sugar-water extraction method. The number of larvae were counted under a microscope at x6 magnification and recorded (Fig. 17).

The green-white fruit that was re-bagged with the exclusion bags was left on the plant for two weeks to develop. It was then removed and assessed for ETPB cat-facing damage using the scale shown in Fig. 18.

Efficacy of the biotremology treatment was assessed by quantifying the amount of damage caused by ETPB to fruit and by the number of SWD larvae in the fruit. Results from fruit collected from the untreated control were compared to those collected from the biotremology treatment.

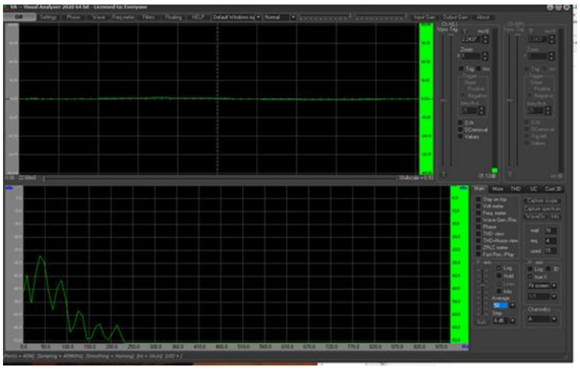

Figure 11: Piezoelectric pick up located on grow bag, between leg poles (i.e. not directly above a leg).

Figure 12: Screenshot of Visual Analyser 64 display, illustrating frequency detected in grow bag Fig. 11.

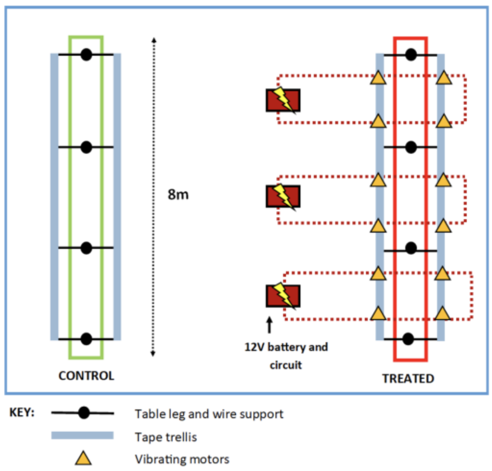

Figure 13: Adapted motor attached to treatment trellis tape using zip tie. Please note off-centre weight not visible on the axle as it is in motion. Fine mesh exclusion bag visible (above right of motor) and microperforated plastic bread bag containing ripening fruit truss to right of image. Orange labels indicate assessment number.

Figure 14: Birds-eye-view diagram of trial set up, illustrating three sets of four motors (each powered by a battery), attached to the trellis tape of the treatment row. No motors on control row.

Figure 15: Trial set up showing treatment row, including insect exclusion mesh and polytunnel structure in background, batteries, data logger in a white Stevenson screen and exclusion bags on plants.

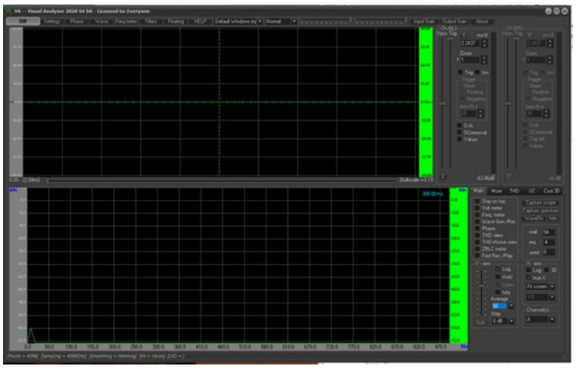

Figure 16: Screenshot of Visual Analyser 64 display, illustrating no vibrations detectable in control row (leaf not in contact with tape). Please note small peak at low frequency the result of slight movement/wind/tremors etc.

Figure 17: Counting of SWD larvae following sugar-water flotation extraction from fruit.

Figure 18: Capsid damage (cat facing) scale used to score assessment fruit.

Results

In the combined analysis, biotremology treatment had no significant effect on the number of marketable fruits (Grade 0) compared to the untreated control treatment (Fig. 19) in relation to ETPB cat-facing damage.

The mean number of SWD larvae was significantly higher in two out of eight assessments in the control fruit but was significantly higher in three out of eight assessments in the biotremology treated fruit (Fig. 20).

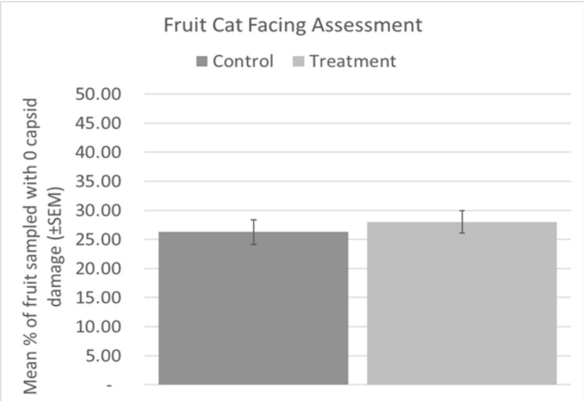

Figure 19: Mean percentage of fruit with 0 capsid damage i.e. marketable.

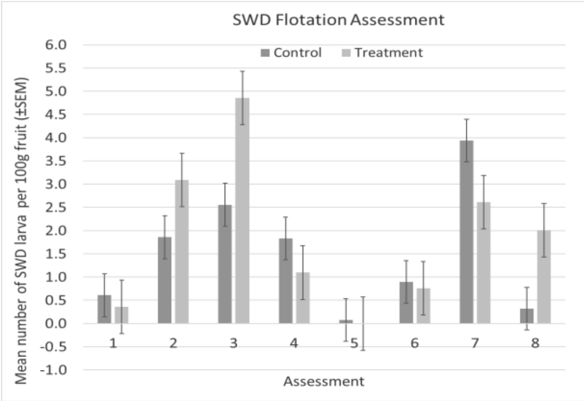

Figure 20: Mean number of SWD larvae per 100g fruit.

Conclusions from both trials

Unfortunately, we were not able to deter ETPB or SWD feeding or egg laying in the fruit during these field trials, however several positive outcomes have been obtained in both the laboratory and field. A method for applying biotremology to strawberry crops through the tabletop strawberry truss support tapes was successful. The equipment developed in the laboratory, after several minor modifications, was found to be reliable and consistent throughout the two-month period in which it was deployed. The leisure batteries were able to power the motors throughout the treatment application process.

Within the field trial, the vibratory signals were transmitted through the plants via the tapes which supported the strawberry plant trusses. While only a small amount of contact between the tapes and the trusses occurred, we were able to detect the vibratory signals in other parts of the plant, not in contact with the tapes. We were able to detect the vibrations were within the frequency range associated with ETPB (0-200Hz) on different parts of the plant material and the coir growing bag. It is worthy of note that the furthest distances away from the point of contact with the trusses were subjected to lower frequencies and so the signal was not uniform throughout the plant. This could be mitigated by sending vibrations through the table-top itself, however, engineering would be required to ensure the table-top structure could withstand constant vibrations. In addition, due to the inconsistency in the vibratory signal itself, it is possible that the range of vibration was not appropriate for deterrence of these insect pests.

Future work should focus on optimising:

- vibration emission method
- transmission of vibrations through the crop
- battery life for extended use

Other areas of interest include:

- Testing other crops e.g. pear using wire and post system to transmit vibrations
- Testing other substrates
- Determining if efficacy is dependent on pest density
- Investigating the control of other pest insects that communicate through vibration signals e.g. pear psylla
- Assessing possible impacts on beneficial insects

Acknowledgements

We greatly appreciate the funding provided to enable us to execute this novel investigation. Due to the funding provided by the AHDB and the WCoF, we have been able to gather pilot data which will be used to support subsequent funding applications. Thanks must be given to the support of the technical team who performed the trial work and design and whom without this project would not have been possible.

AHDB