Project title:	Enhancing the monitoring and trapping of protected crop pests by incorporating LED technology into existing traps
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Project leader:	Dr Andy Evans, SRUC (formerly SAC)
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Location of project:	SRUC Edinburgh
Industry Representative:	Alan Davis
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

A system to take advantage of the attraction of crop pests to specific light spectra is being developed using LED's and sticky traps, to allow early detection and better timing of pest management.

Background

Pest management is a high priority for growers and insecticide use is discouraged where possible. Efficient and effective pest management requires precise timing of biological and/or chemical applications to the crop and an assessment of their effectiveness post-application, to determine whether any further applications are required.

Currently, sticky traps (often coloured) are used to detect the presence of many pests (e.g. thrips, whitefly, various aphid species, leaf miners, fungus gnats) and a decision on whether to begin application of biological control agents (BCA's) and/or insecticides is often based on whether pests are being found on the traps. Traps rely on their attractiveness to these pests, and exploit the behavioural attraction of the pests to their colour. It has been known for many years that specific colours are attractive to specific pests, such as blue for thrips, yellow for whitefly, white for mushroom flies. Recent research has indicated that traps can be made more effective through the use of light emitting diodes (LED's) incorporated within the trap. For example, the capture of tobacco whitefly (*Bemisia tabaci*) was enhanced by 100% through the addition of a lime-green LED (530 nm wavelength) to the trap. Similarly, a 250% increase in trapping efficiency for Western flower thrips (*Frankliniella occidentalis*) was obtained on blue sticky traps that had a blue LED (465 nm wavelength) incorporated with the trap.

Various researchers have looked at the use of LED's to enhance the efficacy of insect trapping, particularly of biting pests such as mosquitoes, but there is relatively little work on exploiting this on a commercial scale to enable growers to incorporate these traps into their IPM programmes.

To determine what colour of LED will enhance attraction of specific pests to a coloured (yellow or blue) sticky trap, the sensitivity of the insect eye to a range of light wavelengths (i.e. 'colours') needs to be determined, coupled with behavioural studies to confirm that they are attracted to that specific wavelength of light. To determine the colour sensitivity of the eye of a range of pests, a technique called 'electroretinography' is being used, which involved detecting the response of receptors in the insect eye to flashes of different light wavelengths. Using this approach we can determine a specific light wavelength that the eye is sensitive to (e.g. green, red, blue etc). Behavioural tests will confirm whether the insect under study is attracted to that particular ‘colour’. LED’s that emit that specific ‘colour’ can then be attached to standard yellow or blue sticky traps and evaluated in protected crops to assess whether they enhance the capture of specific pests compared to traps without LED’s.

This project aims to bring together expertise in insect behaviour and response to visual stimuli at SRUC and the Organic Research Centre (both research and knowledge transfer organisations) to develop and evaluate the potential use of LED’s with existing colour traps used for pest monitoring.

Specifically, the project aims to identify the light spectra that are most attractive to a range of protected crop pests and their biological control agents; screen LED’s of specific light wavelengths that can be used with traps to enhance the attractiveness of traps to pests; evaluate the efficacy of LED/trap combinations for their use in trapping pests under protected crop conditions with a small group of growers; supply prototype LED traps to a wider group of growers to evaluate under commercial conditions for their effectiveness in contributing to enhanced IPM of specific pest/crop situations; and devise IPM approaches that utilise the novel LED traps for use against specific pests.

Summary

Much of the first year has involved developing the electroretinogram set up, as well as the behavioural chamber experiments and trap design. Trap comparisons were run at 4 commercial sites and showed promise for the attraction of sciarid fly, and diamond back moth to green (540nm) LEDs clipped to yellow sticky traps. There was no increase in the capture of *Encarsia formosa.*

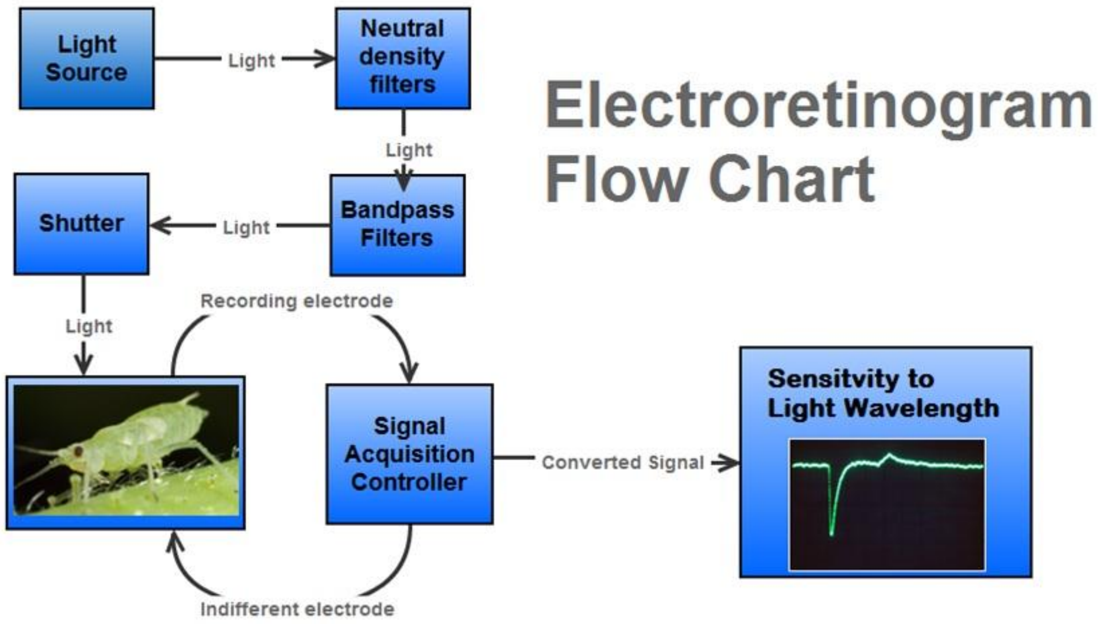

Figure 1. The subject's eye is pierced by a recording electrode; an indifferent electrode is placed into the body. An output is then obtained by exposing the eye to short flashes of light across a range of narrow wavelengths. By measuring the magnitude of the response to these wavelengths, the spectral sensitivity of the insect can be determined.

Financial Benefits

LED's are now relatively cheap (~10-30p per unit, depending on wavelength and output) and have a very long life - >50,000 hours. If powered from the mains within a protected crop, the cost is estimated to be in the region of £0.08 per LED, per week, as the LED's do not require much power to work. In the absence of mains power, LED's can be powered by batteries, but this does increase the cost.

If the LED traps can be shown to enhance the monitoring of specific pests within protected crops, particularly by early detection, the improvement in timing of use of insecticides and/or release of biological control agents would be of economic benefit to the grower.

Action Points

There are no grower recommendations at this early stage of the project.

SCIENCE SECTION

Introduction

A key component of integrated pest management (IPM) is the effective monitoring of pest species. The detection of these pests is either direct, e.g. the presence of insects on traps, or indirect, e.g. damage to crops as a result of pest activity. The decisions to use chemical pesticides or biological control agents (BCA) are often based on the presence of pests within/on traps, the most common of which is the sticky trap; these are coloured and rely primarily on their visual attractiveness to the pest. Certain trap colours are known to be more attractive to specific pests, for example blue are typically used to attract thrips (Vernon and Gillespie, 1990), although red was demonstrated to be more successful in the common blossom thrips (*Frankliniella schultzei*) (Yaku *et al.,* 2007). Yellow traps are attractive to a myriad of species, for example multiple species of whiteflies, aphids (Byrne *et al*., 1986; Moreau and Isman, 2011). Yellow is frequently used as a general purpose colour, as many phytophagous insect species show a preference for yellow over other colours (Bernays and Chapman, 1994). This may be due to a super-normal foliage-type stimulus, i.e. the green wavelength (~520-570 nm), which would be expected to attract phytophagous insects, is reflected at a greater intensity by the colour yellow than by green (Prokopy and Owens, 1983). This does not fully account for this yellow preference, as a white sticky trap will also project more strongly in the green wavelength and thus would also be expected to preferentially attract phytophagous insects, which is not this case. This may be due to a colour opponent mechanism (Döring and Chittka, 2007).

Despite the wide, and successful, use of coloured sticky traps as a method of monitoring insect pests, vision has been assumed to be of little importance in host-finding in insects when compared against chemical cues (Reeves, 2011). There are undoubtedly numerous factors behind this, but the most important are likely the assumptions that: 1. Insects have poor visual acuity, and; 2. Insects are unable to differentiate plant species using visual cues.

The capture efficiency of a trap can be increased with the addition of an active light source. The Centre for Disease Control (CDC) have long used incandescent bulbs in the field to attract disease vectors for monitoring, although over the past ten years they have been undertaking a switch to light-emitting diode (LED) bulbs (Cohnstaedt, 2008).

This increase in capture efficiency has been demonstrated with sticky traps; for example Chu *et al.* (2003) were able to increase the capture of *Bemisia tabaci* by 100% by equipping plastic cup traps with a lime-green (530nm) LEDs. A greater increase in trap capture efficiency (250%) of *Frankliniella occidentalis* was found when equipping blue sticky traps with blue LEDs (465nm) (Chen *et al*., 2004), with later work by Chu *et al.* (2005) demonstrating that UV (398nm) is even more effective than blue (465nm). It should be noted that these studies do not appear to have accounted for the spectral sensitivity of the subject species where it is known, for example Chen *et al.* (2004) appear to have made no use of the previously determined spectral sensitivity of *F. occidentalis* (Matteson *et al.,* 1992). Rather, with the exception of Nakamoto and Kuba (2004), previous studies appear to have either used a green LED (530nm) (Chu *et al.*, 2003; Nombela *et al*., 2003), perhaps to simulate the colour of plants, or used the colour previously found effective as a trap colour (Chen *et al.,* 2004).

Nakamoto and Kuba (2004) performed a preference test to determine which LED light wavelength to equip their traps with to attract the West Indian sweet potato weevil (*Euscepespost fasciatus)*; however, this relied on the simple presentation of four different light wavelengths of varying broadness. In order to more effectively determine LED colour for enhancing the capture efficiency of traps, as well as for acquiring a better understanding of why these colours are attractive to the pest species, it is important that the spectral sensitivity of these species be determined prior to preference testing.

Spectral sensitivity, the efficiency at which light is detected by the photoreceptors in the insect eye, can be determined using an electroretinogram (ERG) (Kirchner *et al.* 2005) (Fig. 1). The ERG can be defined as a graphic record of the retinal action potential, reflecting the summed mass response of photoreceptors and higher order neurons (Brown, 1998; Lindsay *et al.,* 1999). The ERG works by detecting the action potential which occurs in response to the detection of light by the rhodopsin in the rhabdomes. Within mammals this electrical response is detectable via an electrode placed on the surface of the eye. Due to the structure of the insect compound eye this is not possible, and the electrode used to detect the action potentials (recording electrode) must be placed inside of the eye, this is usually achieved by piercing the eye with a tungsten electrode (Matteson *et al.,* 1992; Brown and Anderson, 1996; Kirchner *et al.,* 2005). In order to complete the circuit a second, indifferent, electrode must be placed into another part of the insect’s body. This circuit is to be connected to a signal acquisition controller which converts the signal to a visual representation of the response to the detection of light (Fig. 1).

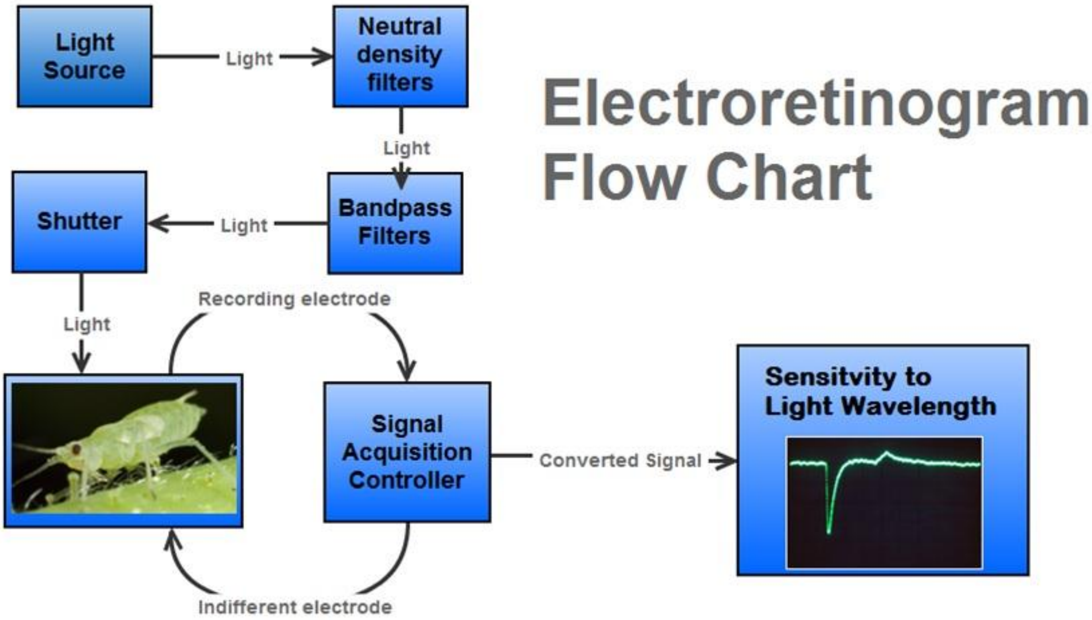

Figure 1. The subject's eye is pierced by a recording electrode; an indifferent electrode is placed into the body. An output is then obtained by exposing the eye to short flashes of light across a range of narrow wavelengths. By measuring the magnitude of the response to these wavelengths, the spectral sensitivity of the insect can be determined.

A response to a particular light wavelength does not imply patterns of behaviour will alter. In order to better determine which wavelengths of light may be used to attract, or repel, a particular insect the ERG should be supported by a behavioural study which makes use of their spectral sensitivity (Brown *et al,* 1998).

The main aims of this project are to:

- Determine the spectral sensitivity in proposed subject species where this has not already been achieved (Table 1). This will be achieved using an electroretinogram (Fig. 1), following the protocol laid out by Kirchner *et al*. (2005).
- Determine the relative attractiveness of light wavelengths to the subject species (Tables 1 & 2)
- Compare the capture efficiency of sticky traps with and without LED attachments, as well as between different LED wavelengths.
- Determine the behavioural response of biological control agents to light wavelengths.

Table 1. Pest species with previously determined spectral sensitivity.

Group	Common Name	Scientific Name
Whiteflies	Glasshouse whitefly	*Trialeurodes vaporarium*
Aphids	Peach-potato aphid	*Myzus persicae*
Thrips	Western flower thrips	*Frankliniella occidentalis*

Table 2. Pest species which have an unknown spectral sensitivity.

Group	Common name	Scientific name
Whiteflies	Tobacco whitefly	*Bemesia tabaci*
Aphids	Cotton aphid	*Aphis gossypii*
Flies	Shore flies	*Scatella spp*
	Sciarid flies	*Bradysia spp*
Thrips	Onion thrips	*Thrips tabaci*
Leaf miners	Leaf miners	*Phytomyza spp*

Materials and methods

Spectral sensitivity determination

The spectral sensitivity of certain pests (Table 1) is determined using an electroretinogram (Fig. 1), following the protocol laid out by Kirchner et al. (2005). Using this approach the spectral sensitivity of an insect to a range of different light wavelengths can be determined.

The light source is a 100 W xenon arc lamp (Osram XBO100W/2 OFR) housed inside a Xe-100 housing device (UV-Grobel, Errlinger, Germany). Light is filtered to be near monochromatic using narrow bandpass filters in 10nm steps ranging between 320-640nm (Ealing Davin Optronics, Watford, UK). The amount of light is varied over approximately 2.5 log units using combinations of neutral density filters (Ealing Davin Optronics, Watford, UK). A modified camera (T70, Canon) is used as a manual shutter to allow brief (0.5s) flashes of light to emit at a point around 1.5cm from the insects eye (distance differs between species). The optical system is calibrated by measuring the amount of light for all neutral density filter combinations (see Figs. 2 & 3 for images of the optical rail set-up).

Using a dissection scope (80x magnification) subjects are secured to a platform using double sided sticky tape, or wax, depending on the size of the species (sticky tape is not strong enough to restrain the larger species effectively). Recording and indifferent electrodes are constructed of electrolytically sharpened tungsten. Using micromanipulators

(Syntech, Germany) the recording electrode is inserted into the subjects left eye and the indifferent electrode is inserted into the abdomen. The subject's eye is exposed to light and the output is displayed on a laptop via a signal acquisition controller (IDAC 2, Syntech, Germany), using EAG pro software (Syntech, Germany). Spectral sensitivity is investigated under three different light conditions 1. Dark adapted; 2. White light adapted; 3. Yellow light adapted. An additional light condition may be used in species which appear to possess a red photo receptor. Pre-adaptation takes around 30 minutes (depending on species), and the duration of the test series for each subject takes around 40-50 minutes.

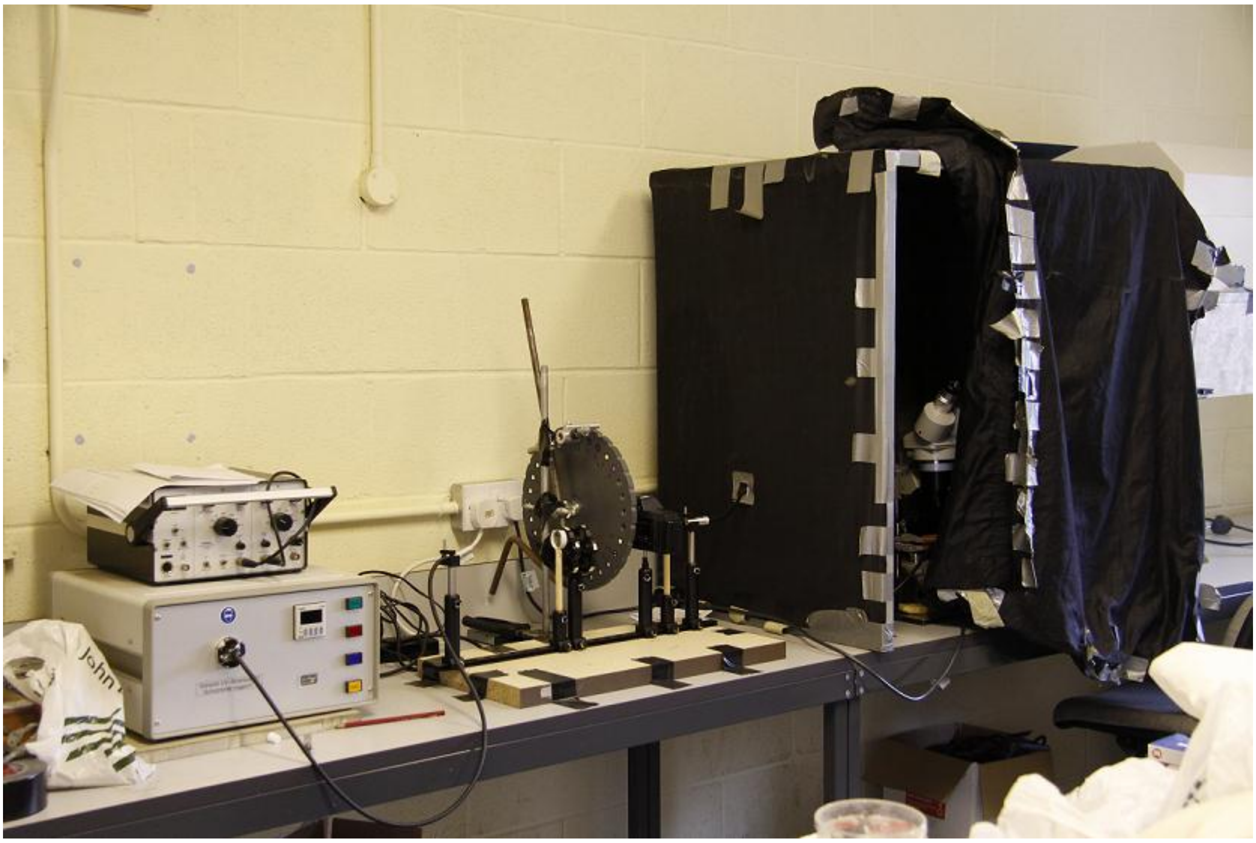

Figure 2. The optical rail set-up.

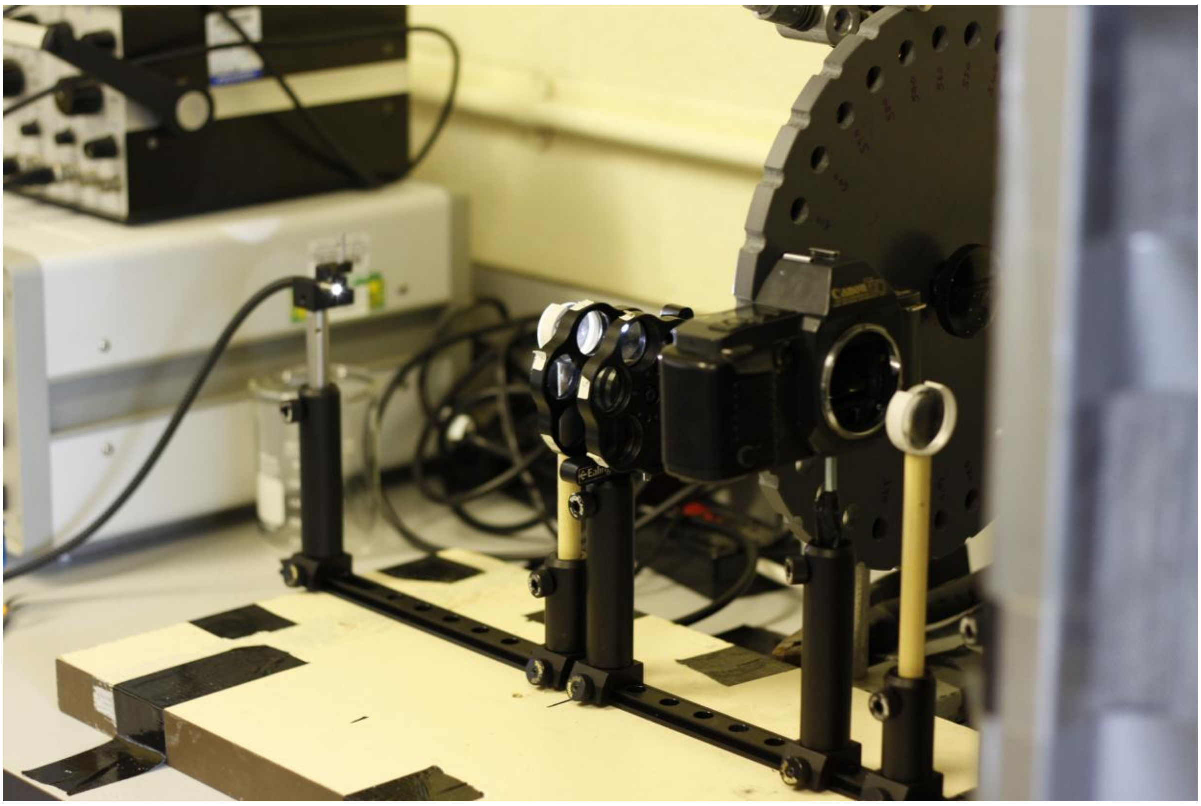

Figure 3. Optical rail set-up showing light path from the left through bandpass filters and neutral density filters, with light exposure duration controlled by the camera shutter.

Behavioural response to different light wavelengths

The relative attractiveness of light wavelengths to the subject species (Tables 1 & 2) will be determined using a simple choice test (Fig. 4). The subjects will be introduced into the centre of a chamber, at either end of which is a light source. The sources of light will be filtered to a narrow wavelength using bandpass filters, one of which will remain the same wavelength as a control. The amount of light (mmol) in the middle of the chamber will be equal. The subjects are left in the chamber for a period of time (differing between species under test) and their choice, i.e. the wavelength they move towards, will be considered their preference.

Two chambers have been devised, one smaller one for small insects (e.g. thrips), and a larger one for insects such as whitefly.

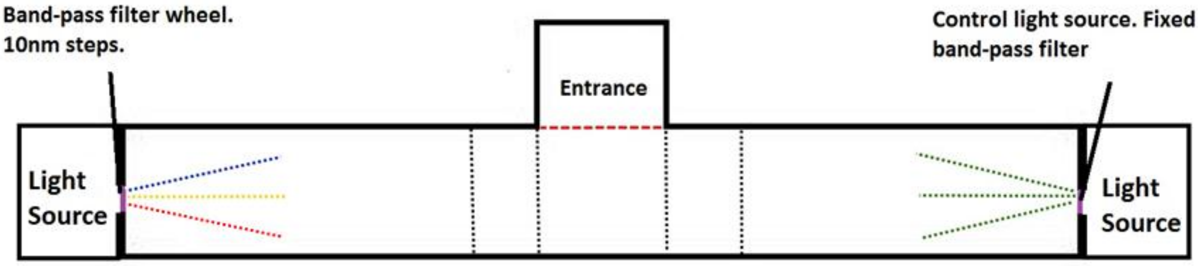

Figure 4. Choice test chamber.

Effective sticky trap LED wavelength combinations

Using the information gained from the ERG and behavioural studies, and/or the literature, the capture efficiency of sticky traps with and without LED attachments (Fig. 5), as well as between different LED wavelengths is being assessed in protected crops. Comparisons are between the capture efficiency of the traps, i.e. total number of insects per species captured. Study sites are located around the UK and sticky traps are returned for identification via post. There are currently four study sites (Table 3). Sticky traps are returned via post for assessment.

LED attachments are simple clip on devices (Fig. 5), which are currently powered by four D cell batteries or mains power where available.

Table 3. Study sites

Study Sites	LED wavelength	Trap colour	Crop
Site A	540nm	Yellow	Varies
Site B	540nm	Yellow	Poinsettia
Site C	540nm	Yellow	Poinsettia
Site D	480nm	Yellow and blue	Mint

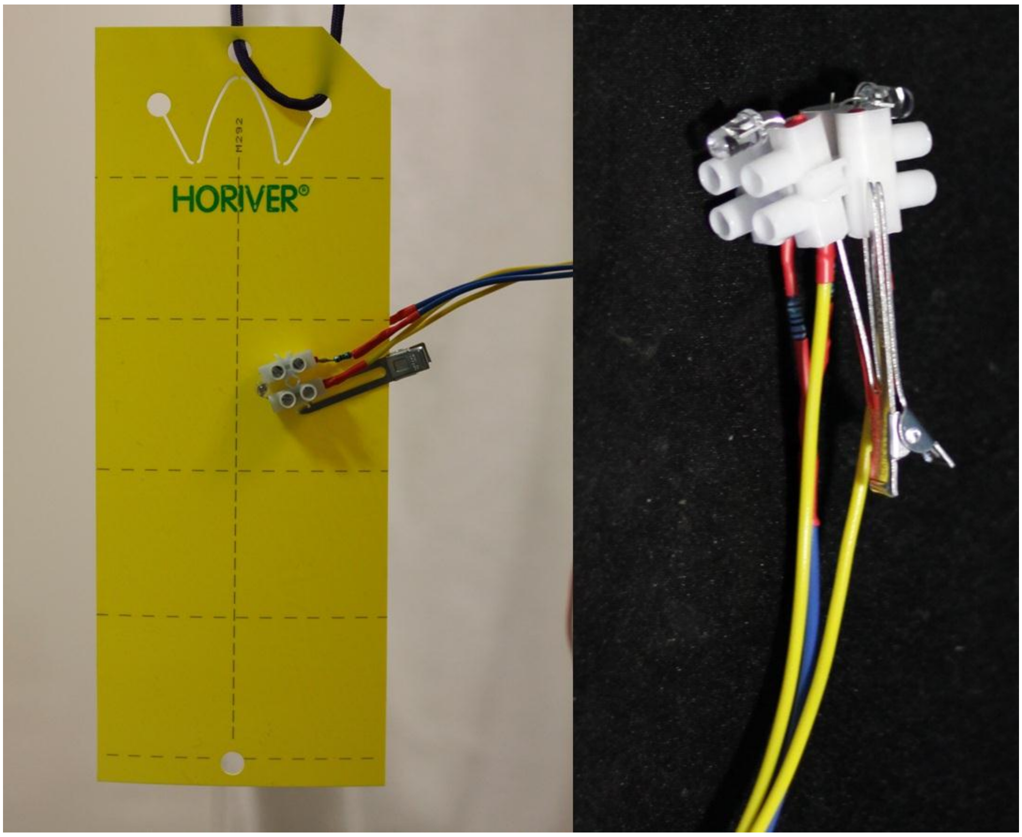

Figure 5. LED attachment on a yellow sticky trap.

Behavioural response of biological control agents to LED light

If LED sticky traps show increased capture efficiency for biological control agents (Table 4) then behavioural tests as outlined above will be performed to determine photopositive and photonegative responses to specific light wavelengths.

Table 4. Parasitoids commonly used as biological control agents.

Group	Scientific name
Parasitoids	*Encarsia formosa*
	Diglyphus isaea

Results

Spectral sensitivity determination

Much of the first year of the project has been involved in developing the ERG set up outlined in Fig. 1. This has involved construction of the light delivery apparatus (Figs. 2 & 3). Tests are ongoing using glasshouse whitefly (*Trialeurodes vaporarium*).

Behavioural response to different light wavelengths

Much of the first year of the project has been involved in developing the behavioural choice test chamber (Fig. 4). Tests are currently being undertaken using western flower thrips (*Frankliniella occidentalis*).

Effective sticky trap LED wavelength combinations

Trap catches are still being evaluated but initial results are shown below (Figs. 6 & 7).

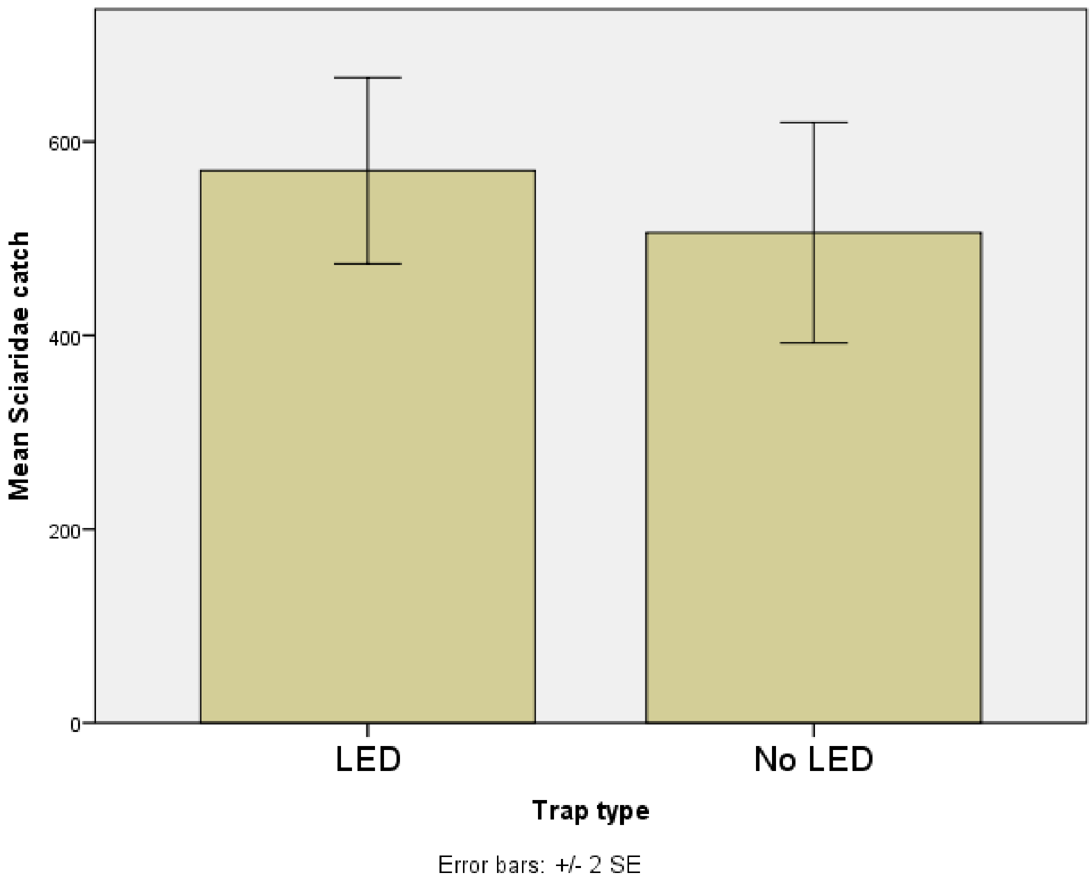

Figure 6. Sciarid fly (*Bradysia* spp.) capture over 4 weeks of trapping at site A using a 540nm green LED.

There does appear to be a small increase in sciarid fly catch size at site A (Fig. 6), and at the other sites also, but this is too small to be significant.

There is a significant increase in capture of the pyralid moth *Scoparia ambigualis* ($P < 0.001$) at site A with the 540nm green LED (Fig. 7). This moth is a potential pest of herbs.

The 540nm green LED yellow traps appear to attract diamondback moths (*Plutella xylostella*) (data to be analysed), and male western flower thrips (*F. occidentalis*) also appear to be attracted, but there is no evidence of this occurring in females (full data yet to be analysed).

The parasitic wasp of glasshouse whitefly (*Encarsia Formosa*) does not appear to be attracted to the 540nm green LED in preference to the standard non-LED yellow trap.

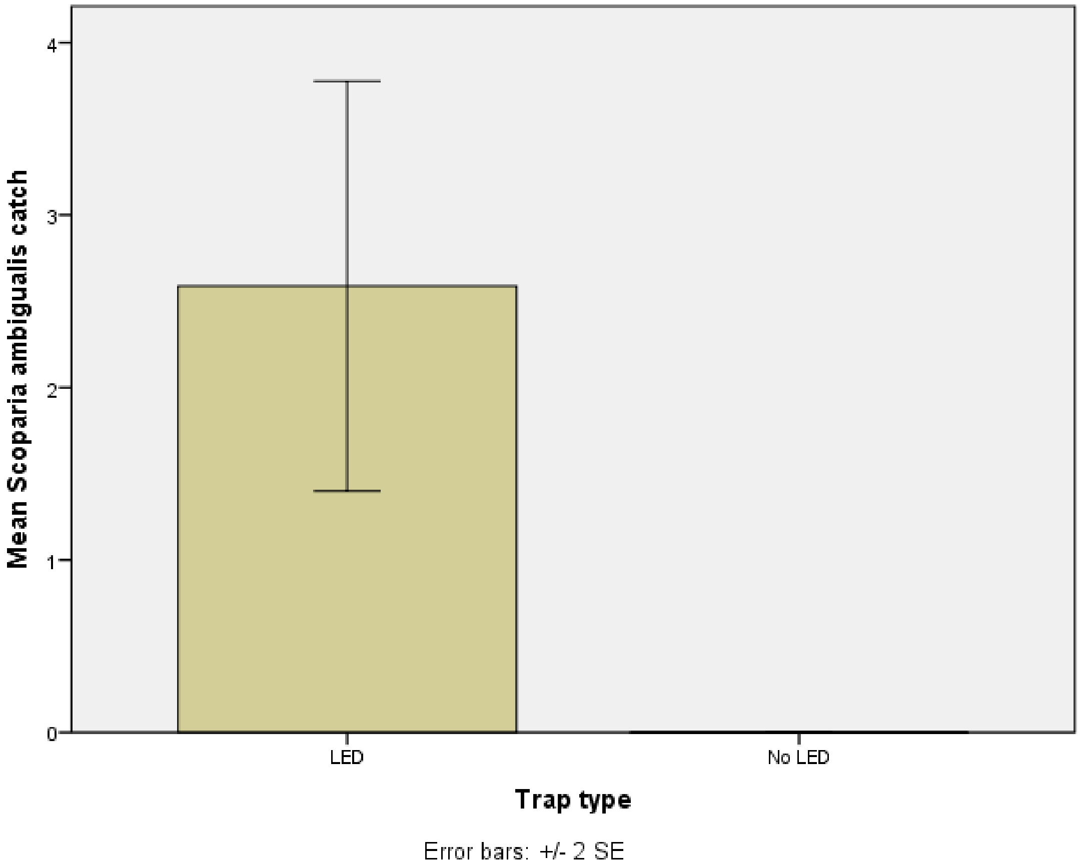

Figure 7. Capture of *Scoparia ambigualis* over 4 weeks of trapping at site A.

Discussion

Experiments and analysis of the results from trap catches are ongoing, and a fuller picture of the spectral sensitivity and behaviour will be forthcoming over the next few months as experiments continue.

For the 2013 season, it is planned to expand the trials of LED sticky traps to additional sites, aiming to capture a wider range of pests. One issue from the 2012 trapping season was the relative lack of pest species at the trapping sites, possibly due to effective pest management by the growers.

An issue that has arisen is the supply of pest species for the laboratory work, particularly sciarid fly and glasshouse whitefly.

Conclusions

- LED attachments have been developed and distributed to a selection of growers.
- Identification of species captured by traps in grower facilities is ongoing
- ERG set up has been complete and experiments are ongoing
- Behavioural experiments set up has been developed and experiments are ongoing

Knowledge and Technology Transfer

Event description	Date
SAC corporate induction	October 2011
BioSS: Basic Statistics	11-12/11/2011
Demonstrator induction (UoE)	27/11/2011
BioSS: Getting Started in R	09/01/2012
SAC Postgraduate Conference (Presentation)	21-22/03/2012
Meeting with Koppert representative	20/03/12
BioSS: Experimental Design and Analysis of Variance	21-22/03/2012
Presentation to crop growers (to obtain volunteers for field work)	26/03/2012
Physics training with Prof. J. Allen (St Andrews)	30/04/12
Use of physics equipment at St Andrews	05/05/12
HDC studentship conference 2012 (poster presentation)	04-05/06/2012
Koppert Entomology Course 2012	05-07/07/2012
HDC Focus on Light Spectrum for Horticulture	04/12/2012

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