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Enhancing the monitoring and trapping of protected crop pests by incorporating LED technology into existing traps

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Dedication

To Patricia, my inspiration.

Declaration

I certify that:

(a) This thesis has been composed by me, and
(b) Either that the work is my own, or, where I have been a member of a research group, that I have made a substantial contribution to the work, such contribution being clearly indicated, and
(c) That the work has not been submitted for any other professional degree or professional qualification except as specified.

Kevin McCormack

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Lay Summary

The use of coloured light-emitting diodes (LED) attached to sticky traps has been shown to enhance the capture of some insect pests of protected crops to allow for more effective monitoring. For example green (540 nm wavelength) LEDs attached to yellow sticky traps tended to catch more fungus gnats than yellow sticky traps alone, and green (540 nm) or blue (480 nm) LEDs attached to yellow sticky traps caught significantly more diamondback moths than yellow sticky traps alone. The use of LEDs did not have a negative effect on the use of biological control agents such as *Encarsia formosa* (used for whitefly management). A naturally occurring parasitoid wasp (*Kleidotoma psiloides*) of shorefly was caught in fewer numbers when green LEDs (540 nm) were attached to yellow sticky traps.

The potential for LEDs to enhance the monitoring of certain pests in protected crops without any effect on biological control agents has been demonstrated and warrants further development to make the use of LEDs with sticky traps more practical within protected cropping systems.

Protected crops require significant pest management inputs in many cases, particularly with edible crops where insecticide use is discouraged where possible, and the use of biological control agents (BCA) is most often undertaken (e.g. tomatoes, cucumbers, peppers). To obtain the most efficient pest management using insecticides or BCAs (or in combination) requires precise timing of application 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, sciarid flies) and a decision on whether to begin application of insecticides and/or introduction of BCAs is often taken based on whether pests are being found on the traps. The efficacy of traps relies on their attractiveness to these pests, and exploits 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 sciarid flies. Recent research by others has indicated that traps can be made more effective through the use of light emitting diodes (LEDs) incorporated with the trap. For example, the capture of tobacco whitefly (*Bemisia tabaci*) was doubled through the addition of a lime-green LED (530 nm wavelength) to the trap. Similarly, a 2.5 times more western flower thrips (*Frankliniella occidentalis*) were captured on blue sticky traps that had a blue LED (465 nm wavelength) incorporated with the trap.

Various researchers have looked at the use of LEDs 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 intergrated pest management (IPM) programmes.

This project aimed to identify the light spectra that are most attractive to a range of protected crop pests and their biological control agents; screened LEDs of specific light wavelengths that can be used with traps to enhance the attractiveness of traps to pests; and evaluated the efficacy of LED/trap combinations for their use in trapping pests under protected crop conditions with a small group of growers.

Abstract

Management of pest species is ordinarily required in the production of protected crops. Integrated pest management (IPM) is commonly used when controlling insects. The European Union Sustainable Use Directives states that "*integrated pest management' means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment. 'Integrated pest management' emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.*" Effectively monitoring pests is a key component of IPM, with decisions to use biological control agents (BCA) and insecticides often based on the presence of pests in traps. A commonly used monitoring tool is the sticky trap; these traps are coloured and rely primarily on their visual attractiveness to the pest.

The capture efficiency of sticky traps can potentially be increased with the addition of light emitting diodes (LEDs). The objective of this project was to use LEDs to enhance the efficacy of yellow sticky traps for trapping a range of insect pests, to enable more effective timing of pest management by optimising pest monitoring. The addition of LEDs may also enable more effective mass trapping via yellow sticky traps, and minimize the trapping of beneficial insects.

Comparisons between standard yellow sticky traps and those equipped with green (540 nm) or blue (480 nm) LEDs were carried out at four commercial growing facilities. Green (540 nm) LED equipped traps were compared with standard yellow traps in a mass release of the biological control agent *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), to determine if there are negative consequences to the addition of green (540 nm) LEDs when using this biological control agent. Relative spectral preferences of western flower thrips (*Frankliniella occidentalis* Pergande (Thysanoptera: Thripidea)) and Glasshouse whitefly (*Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae)) were determined using a choice test comparing a range of wavelengths in 20 nm steps against a control wavelength.

Green (540 nm), and blue (480 nm) LED equipped traps captured significantly more dark-winged fungus gnats (*Bradysia difformis* Frey (Sciaridae: Diptera)) and diamondback moths (*Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae)) than those without. No significant

differences were found between green (540 nm) LED equipped traps and those without for *E. formosa*, and a significant decrease in the capture of the shore fly parasitoid *Kleidotoma psiloides* Westwood (Hymenoptera: Figitidae) was observed. In behavioural experiments *F. occidentalis* showed a peak spectral preference at 360, 420, and 480 nm, and *T. vaporariorum* at 320, 340, and 380 nm.

The addition of LEDs to yellow sticky traps enhanced their capture efficiency for some key pests in commercial protected crop growing environments, and has the potential to enable pest detection at an early stage, consequently optimising the timing of pest management options.

Contents

Chapter 1 General Introduction

Integrated Pest Management

Management of pest species is ordinarily required in the production of protected crops. Crop pest is a broad term encompassing arthropod pests, weeds, pathogens, and non-arthropod pests. Here the focus will be arthropod pests, these pests cause damage in numerous ways, for example via direct feeding (Moorhouse *et al.,* 1992), oviposition, (Allsopp, 2010) and the spread of crop diseases, e.g. tomato spotted wilt (German *et al.,* 1992; Culbreath and Srinivasan, 2011) and *Verticillium albo-atrum* (Kalb and Millar, 1986). Currently North America and the majority of countries within northern Europe apply some form of integrated pest management (IPM) when controlling insects (Kogan, 1998; Finch and Collier, 2000; Puente *et al.,* 2011). The European Union Sustainable Use Directives states that "*integrated pest management' means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment. 'Integrated pest management' emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.*" (Directive 2009/128/EC).

This has not been the case in developing countries, where uptake of IPM is hindered by many factors. For example, local farming practices are often quite dissimilar to those found within developed countries, and IPM practices designed to fit these systems are poorly suited (Sinzogan *et al.,* 2004). Steps towards an improved knowledge of local practices, to better enable the creation of IPM strategies in developing countries are being undertaken (Midega, *et al.,* 2012).

One of the goals of IPM is to minimise the risks of chemical pest control to human health and the environment (Kogan, 1998; Directive 2009/128/EC). This is particularly desirable in edible crops, where unnecessary or improper use of pesticides is discouraged, as there are potential financial penalties on the grower if residues exceed Government regulations, and supermarkets may reject crops if they deem residues to high (Garthwaite *et al*., 2009; Chemicals Regulation Directorate, 2012). While social and moral concerns are of importance to crop growers (Mzoughi, 2011), there is the additional incentive of the reduction in costs associated with the implementation of IPM. This reduction in costs can come as a result of the direct reduction in the use of pesticides, for example Filipino growers trained in IPM spent~PhP5,000 (~£74) less on chemical pesticides per ha than untrained growers (Yorobe *et*

al., 2011). IPM can also result in an increase in net profit; experimental celery plantations by Trumble *et al.* (1997) compared standard chemical pesticides practices against IPM. The plantations using IPM generated net profits US$600-$1400 greater per hectare than those using standard chemical pesticide practises. The appropriate use of pesticides as part of an IPM system, for example by rotating between chemicals with different modes of action, can reduce or delay the development of resistance to a particular insecticide (Georghiou, 1994), potentially averting large economic losses like those seen in Californian celery crop in the 1980's, where the leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) developed a resistance to all available chemical pesticides, resulting in a loss of around US$20 million (Reitz *et al*., 1999).

A key component of IPM is the effective monitoring of pest species within the crop and in particular detection of the initial infestation. 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 traps, the most common of which is the sticky trap, (Fig. 1) which are usually coloured and rely primarily on their visual attractiveness to the pest.

Figure 1. Yellow sticky trap with LED attachment.

Insect Trapping

Introduction

The high economic cost of insect crop pests (e.g. Reitz *et al.*, 1999; Adkins, 2000; Parella *et al.*, 2003) necessitates insect populations be monitored as part of a management strategy. Traps can be an effective tool for this, and usually consist of two main components, an attractant, and a mechanism to retain and/or kill the pest. Attractants are chemical or visual in nature, for example pheromones or coloured surfaces (Hoddle *et al.*, 2002; Broughton and Harrison, 2012). Retaining and killing is typically performed using a sticky substance (Vernon and Gillespie, 1990), electricity (Frick and Tallamy, 1996), or liquid (Döring *et al.*, 2009).

Sticky Traps

Sticky traps are the most common used insect trap, and rely primarily on their visual attraction to pests (Vernon and Gillespie, 1990), although they can be enhanced with chemicals (Broughton *et al.*, 2012). 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* Trybom (Thysanoptera: Thripidae) (Yaku *et al.*, 2007). Yellow traps are attractive to many species, for example multiple species of whiteflies and aphids (Byrne *et al*., 1986; Moreau and Isman, 2011) (Table 1). 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 the case. For example, in a comparison between clear, white, yellow, and blue sticky traps, Hoddle *et al.* (2002) found avocado thrips (*Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae)) to very strongly prefer yellow, despite the white trap reflecting more strongly within the green area of the spectrum, at around ~90% versus ~80%. This may be due to a colour opponent mechanism, where light in the UV and blue regions of the spectrum inhibits the excitatory response caused by light in the green/red region. A yellow sticky trap reflects light strongly in the green/red region, and weakly in the UV and blue regions, whereas the white trap used by Hoddle *et al.* (2002) reflected strongly in all three regions (Hoddle *et al.*, 2002; Döring and Chittka, 2007b).

Table 1. Sticky trap colour preference of notable insect crop pest species.

Species	Family	Trap colour	Reference
Bemisia tabaci	Aleyrodidae	Yellow	Lu *et al.*, 2013
Trialeurodes vaporariorum	Aleyrodidae	Yellow	Premalatha and Ranjangam, 2011
Plutella xylostella	Plutellidae	Yellow	Sivapragasam and Saito 1986
Ceratothripoides claratris	Thripidae	Blue, UV-reflective	Leelananda *et al.*, 2007
Frankinothrips orizabensis	Thripidae	White	Hoddle *et al.*, 2002
Franklinelle occidentalis	Thripidae	White, blue	Hoddle *et al.*, 2002; Broughton and Harrison 2012
Scirtothrips perseae	Thripidae	Yellow	Hoddle *et al.*, 2002
Thrips tabaci	Thripidae	Blue	Broughton and Harrison 2012

Rectangular shaped traps are the most common form of sticky trap, although a range of different shapes (e.g. squares, cylinders) have been tested with varying success (Byrne *et al.*, 1986; Kim *et al.*, 2011; Idris *et al.*, 2012). This is presumably due to commercial availability, practicality, or there being no significant differences when compared with the standard rectangular yellow sticky traps, as with the square shaped trap (Quiring, 1986). For example, in cotton fields Naranjo *et al* (1995) found that cylindrical shaped traps captured significantly more sweet potato whiteflies (*Bemisia tabaci* (Gennadius)) ($P<0.001$) than vertical traps placed at canopy height, and Byrne *et al.*, (1986) captured 55% more (total) banded winged whiteflies (*Trialeurodes abutilonea* (Haldeman)) and *B. tabaci* than horizontal and vertical traps. It should also be noted that while altering the traps shape may increase the capture efficiency for some pests, it may reduce the capture efficiency of another. For example, standard yellow sticky traps were shown to be more effective for capturing almond moths (*Ephestia cautella* (Walker) (Lepidoptera: Pyralidae)) than cylindrical traps (Bowditch *et al.*, 1994). Similarly, although Kim and Lim (2011) found sticky traps with two yellow circles on a black background to be more effective than

standard yellow sticky traps, these are not commercially available and would be costly and time consuming to produce. The lack of uptake of these trap modifications may be indicative of a barrier in the future uptake of LED enhancements, particularly as a power source is required. These barriers to entry should reduce overtime, as LED crop lighting facilities become more common owing to their advantages over high pressure sodium lighting (e.g. lower power consumption, longer functional life, numerous available light wavelengths) (Goto, 2012; Hernàndez and Kubota, 2012). These facilities should provide easier access to mains power, and will require improvements to the standard yellow sticky trap due to their reduced effectiveness under the limited wavelength range used in LED crop lighting, which focus on wavelengths in the blue and red regions of the spectrum (Choi, Moon, and Kang, 2015; Piovene et al., 2015; Davis, undated).

The placement height and position of sticky traps may influence their effectiveness. In cucumber glasshouses, it has been recommended that traps be placed 15-30cm below the canopy level to maximise the capture efficiency for *B. tabaci* (Shen and Shunxiang, 2003; Hou *et al*., 2006). When monitoring *B. tabaci w*ithin cotton fields a trap height of 25cm was found to be more effective then 30cm, and more *B. tabaci* were captured on traps placed 60cm above then ground when compared with 80cm, 100cm, and 120cm (Gencsoylu, 2007; Yathom *et al*., 1988). The orientation of the trap with respect to the ground can alter the efficiency of sticky traps, and Gencsoylu (2007) found vertically placed traps captured more *F. occidentalis* than horizontally placed traps with cotton fields, but no difference was found for *B. tabaci*. Hallet (1986) found more *Plutella xylostella* were captured by horizontally placed traps (3.4±0.9) when compared with the trational verticle placement (north-facing: 0.3±0.2; south –facing: 0.0±0.0). The cardinal direction of sticky traps has not been thoroughly investigated, Hou *et al.* (2006) have stated that cardinal direction does not appear to influence *B. tabaci* catch; however, more recently work has demonstrated greater capture efficient in vertically placed East-West facing traps when compared with North-South facing traps placed in cotton fields (Gencsoylu 2007). Similarly, more *Frankliniella occidentalis* were captured in East-West facing traps when compared with North-West facing traps (Gencsoylu 2007).

The combination of trap height and colour influences the effectiveness of sticky traps, and Gillespie and Vernon (1990) found a blue sticky traps placed at 2.4m above the ground (crops were grown on 2.1m high trellises) captured a greater number of female *F. occidentalis*, while yellow sticky traps captured more males. This effect was not observed at

numerous other distances between 0.6-3m above the floor. This suggests a mix of trap colours may be appropriate for some species.

Chemical attractants can be used in increase the effectiveness of sticky traps. For example, the addition of the aggregation pheromone Thripline$_{ams}^{®}$ increased the number of *F. occidentalis* capture on both blue and yellow sticky traps (Broughton *et al.,* 2012; Broughton *et al.,* 2015). Similar results were seen when using Lurem-TR (kairomone), an attractant derived from host plans and related compounds (Teulon *et al*, 2008) (Broughton *et al.,* 2012; Broughton *et al.,* 2015; Teulon *et al*, 2008). Traps can be baited with multiple pheromones to attract multiple species simultaneously (Kim *et al.,* 2015). Chemical attractants should be used with caution, as these may attract, or interfere with the foraging of, biological control agents (Broughton *et al.,* 2012).

Natural oils have been successfully used to increase the attractiveness of yellow sticky traps to *T. vaporariorum*, with sandalwood oil, basil oil, and grapefruit oil increasing the number of *T. vaporariorum* captured by 487.64%, 483.20%, and 333.09% respectively (Górsk, 2004). Yellow card (18×18cm^2) hung vertically (25cm above the ground) and coated with castor oil were found to be more effective than yellow sticky traps (hung horizontally 25cm above the ground) for capturing *T. vaporariorum*; however, these traps were described as being triangular shaped with sticky card inside, so are not representative of the standard positioning used to capture whitefly with sticky traps (Premalatha and Rajangam, 2011). Conversely, the use of natural oils may decrease the capture of Scariadae flies on yellow sticky traps, with a broad range of oils (basil, clove, juniper, sage, spruce, sweet flag, tea-tree) showing a decrease in attraction, while ginger, cinnamon and pine-needle oil showed a small increase, although none of these results were statistically significant (Górski 2004). A significant increase in the number of trapped in sects on both blue and yellow sticky traps was found when applying lemon oil and patchouli oil (Górski, 2004).

When using chemical attractants consideration should be given whether the trap capture can be used to give an accurate representation of the population, particularly when using chemicals which attract a particular sex. For example, *Plutella xylostella* males can be attracted to traps using pheromones; however, it is unusual for the relationship between moth catch and larval density to be statistically significant, suggesting this is not an effective means of measuring *P. xolostella* population sizes with the intention of applying control measure once a threashold is reached (Miluch *et al.* 2013).

Man-hours should be a consideration when devising a pest management strategy. The identification of insects can be challenging and time consuming, particularly when

identifying those captured by sticky traps (Murphy, 1985; Knodel and Agnello, 1990). In some circumstances identification may not be possible unless the specimen is removed from the trap using a solvent, a time consuming process which is unlikely to be economically viable if used frequently (Miller *et al.* 1993). With this in mind, methods to reduce the number of required man-hours should be considered. As there is a negative relationship between the size and number of insects captured by sticky traps (Zhang and Yu, 2009), and it has been suggested that small traps should be used to reduce the man-hours devoted to insect counts (Park *et al.*, 2011). This is provided there is a correlation between the insect population and trap counts, which appears to be variable between pest and crop type. In the case of thrips, sticky traps were found to be an effective monitoring tool in mango orchards (Aliakbarpour and Rawi, 2011) and hydroponic strawberries (Steiner and Goodwin, 2005), but ineffective in greenhouse cucumber production and cotton fields (Boone, 1999; Slosser *et al.*, 2005).

Further reduction in trap counting man-hours may be achievable using a presence-absence model to estimate the number of insects on a trap (Binns and Nyrop, 1992; Sileshi, 2007), or by using an image processing system to aid in identification (Qiao, *et al.*, 2008). Presence-absense models attempt to estimate the population of a particular species in a location without performing a complete count, here just the presence or absence of a pest is recorded (0 or 1), and a mathematically model is used to estimate density. The advantage of this method is that a population estimate can be achieved much more quickly than a complete count, although at the cost of accuracy (Binns and Nyrop, 1992; Sileshi, 2007; Mo *et al.*, 2001). Image processing systems can be used to detect and identify insects by analysing morphological characteristics, such as wing vein lengths, angles, and junctions (Yu *et al*, 1992). When dealing with insects caught on sticky traps, these algorithms must be simplified, for example by defining a size and colour for a particularly species of insect, an image processing system can rapidly, and accurately, count the number of this species provided no species which match this definition are present on the trap (Qiao, *et al.*, 2008).

CC Traps, Bug Zappers, and Suction Traps.

Sticky traps are not always ideal, they are unpleasant to handle, can become a saturated, and may catch beneficial insects (Chen *et al.* 2004a; Rodriguez *et al.*, 2012). The CC trap was developed as a response to the aforemention issues with the standard sticky trap design (Chu and Henneberry, 1998). This reusable trap consists of an upside-down clear plastic drinking cup, and a yellow plastic base with a cylindrical, tapering, hole in it. No sticky substances are used to capture the insects, instead a clear plastic disc is placed just above the opening, which prevents the whitefly from escaping. While these traps captured fewer silverleaf

whitefly (Bemisia *argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae)) than yellow sticky traps, the re-usability, ease of handling, and reduced risk of capturing beneficial insects make them a viable alternative, or supplement, to yellow sticky traps in whitefly control (Chu and Henneberry, 1998; Chu *et al.*, 2000).

UV light traps with an electrified grid are a commonly available form of insect trap, and are primarily sold as mosquito control, although this is a dubious claim (Frick and Tallamy, 1996). These traps are generally unsuitable for monitoring insect crop pests, as the electrified grid will damage the insects and make them difficult to identify. There are also concerns that these traps spread unkilled viruses and bacteria (Broce, 1993; Urban and Broce, 2000), although this does not appear to have been researched in regards to crop disease. These traps are also likely to attract and kill beneficial pollinators (Stephen and Rao, 2007; Rao and Ostroverkhova, 2015).

Suction traps are typically use to monitor insect migration, and networks exist throughout Europe and the US (Halbert *et al.*, 1990; Rothamsted Research, 2015), for example Rothamsted Research have maintained a UK network since 1964 (Rothamsted Research, 2015). These trap capture small weak flying insects, including Sciaridae, Aphididae, and Thripidae (Benton *et al.*, 2002; Nielson *et al.*, 2004). These traps are typically used for monitoring aphid migration for dissemination to growers (Woiwod *et al.*, 1984; Halbert *et al.*, 1990; Moreau and Isman, 2011; Rothamsted Research, 2015), rather than pest monitoring within glasshouses.

The Moericke trap, more commonly known as the pan trap (Moericke, 1951), are petri dish shaped traps which are typically mounted on a pole. These traps, as with sticky traps, primarily rely on their visual attraction to the insect, although they can be enhanced with chemical attractants (Iwanaga and Kawamura, 2000). The distinction between these traps, and the sticky trap, is the method of retaining the insect. Moericke traps contain a liquid solution, typically water with detergent added to reduce surface tension (Leong and Thorp, 1999; Döring and Chittka, 2007a). While these traps are commonly used by ecology/conservation researchers (Laubertie *et al.*, 2006; Campbell and Hanula, 2007), they do not appear to be in commgreen bean on use by commercial growers in protected crops. This may be due to the increased handling time associated with insect identification from these traps, as specimens have to be filtered and separated before identification. Pan traps allow for the easier positive identification of specimens as they cause less damage to specimens than sticky trap (Broatch and Vernon, 1997), which is certainly an advantage when expecting a high biodiversity; however, as commercial growers are typically interested

in a small number of pest species, the time saved by using sticky traps likely outweighs this benefit.

Trap Crop

Trap cropping uses a preferred plant host to lure a particular pest away from the commercial crop (Hokkanen, 1991). These plants may be used to monitor pest populations, provide an alternate food source, or provide resources of natural enemies (Zhao *et al.*, 1991; Buitenhuis and Shipp, 2006; Xiao *et al.*, 2012).

Highly attractive trap crops may be used to concentrate pests into a small area where they are easily destroyed. Eggplants are highly effective for luring the whiteflies *Bemisia argentifolii* Bellows & Perring (Hemiptera: Aleyrodidae) and *T. vaporariorum*, and when given the choice between eggplant and poinsettias in a cage, 60% of *B. argentifolii* and 98% of *T. vaporariorum* were observed on eggplant after 3 days (Lee *et al.*, 2009). Luring pests onto specific plants will enable pesticide to be more easily applied; however, this strategy will be ineffective against species which are resistant to insecticides, such as *P. xylostella* (Sarfraz *et al.*, 2005; Hu, *et al.*, 2014; Steinbach *et al.*, 2015). While a grower may choose to contain and destroy a trap crop (i.e. covering a plant with a net and burning it), a more elegant solution may be to use dead end trap crops. These are plants which pests find attractive, but on which they cannot survive. For example *P. xylostella* preferentially oviposit onto *Barbarea vulgaris* R.Br (Brassicales: Brassicaceae), despite their larvae being unable to survive on this plant due to the presence of glucosinolates which stimulate oviposition and amonodesmosidic triterpenoid saponin which acts as a feeding deterrant to the larvae (Shinoda *et al.*, 2002; Badenes- Perez *et al.*, 2004; Lu *et al.*, 2004; Shelton and Nault, 2004; Badenes-Perez *et al.*, 2013).

Banker plants can be used to provide shelter and resources for natural enemies of crop pests. For example the predatory mite *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) can be established on ornamental pepper plants, with each plant maintaining ~1000 *A. swirskii*, resulting in a significant suppression of the populations of *B. tabaci, F. occidentalis,* and *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) on green bean plants (*Phaseolus vulgaris* L.) (Xiao *et al.*, 2012). The provision of these resources will not always improve the effectiveness of natural enemies. Andorno and López (2014) found that the addition of banker plants used in conjunction with the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) provided more effective control of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) within arugula crops, when compared with inoculative releases of

the parasitoid; however, no significant differences were observed within sweet pepper crops. These banker plants were oats infested with *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae), and were intended to provide the parasitoids with a non-pest reservoir for reproduction. By providing the parasitoid with an alternate host which does not feed on the crop, in this case *Rhopalosiphum padi*, it is possible to pre-establish a population of parasitoids before the pest species arrives, allowing for an immediate response by this biological control agent (Andorno and López 2014).

Trap crops are not necessarily biological, and the artificial chrysanthemum flower model trap has proven effective for attracting multiple species of thrips, as well as glasshouse whitefly (*T. vaporariorum*) (Mainali and Lim, 2008a; Mainali and Lim, 2008b; Lim and Mainali, 2009; Lim *et al.*, 2013). When compared against yellow sticky traps in choice tests, the flower model traps captured 4.1 times more *F. occidentalis* and 5.4 times *Frankliniella intonsa* Trybom (Thysanoptera: Thripidae) (Mainali and Lim, 2008b). Flower model traps at a density of 20 traps per $50m^2$ reduced the population of *F. intonsa* on strawberry flower by 82%, and pepper crops by 61% (female, and 49% (male) (Lim *et al.*, 2013). The population of *T. vaporariorum* with $500m^2$ glasshouse was significantly reduced by the installation of 80 flower model traps, and an 85% reduction in sooty mould (caused by mould growing on whitefly honeydew excretions) was observed (Mainali and Lim, 2008a).

Increasing the Number of Insect Captured by Traps with the Addition of an Active Light Source

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

An increase in capture efficiency using LED's has been demonstrated with sticky traps; for example Chu *et al.* (2003) were able to increase the capture of silverleaf whitefly (*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae)) by 100% by equipping plastic cup traps with a lime-green (530 nm) LED. A greater increase in trap capture efficiency (250%) of *F. occidentalis* was found when equipping blue sticky traps with blue LEDs (465 nm) (Chen *et al.*, 2004a), with later work by Chu *et al.* (2005) demonstrating that UV wavelengths (398 nm) are even more effective than blue (465 nm). 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.* (2004a) 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 (530 nm) (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.*, 2004a). This is not an entirely unreasonable approach, as *F. occidentalis* were found to have a high spectral response in the green region (~95-98% at 520 nm) compared with a low response in the blue region (~28% at 450 nm) (Matteson *et al.*, 1992), which is not consistent with their behavioural response to coloured traps.

Nakamoto and Kuba (2004) performed a preference test with the West Indian sweet potato weevil (*Euscepes postfasciatus* Fairmaire (Coleoptera: Curculionidae)) to determine which LED light wavelength to equip their trap with. However, this relied on the simple presentation of four different light wavelengths of varying broadness. Where possible, the determination of a specie's spectral preference would enable more effective LED colour selection.

While an LED attachment may be used to increase the number of individuals captured, the primary benefits are the potential for early detection or detection of pests which would not otherwise have been captured by a sticky trap. Thresholds (i.e. the density of a pest at which control measures provide an economic return) can be measured using sticky traps; however, thresholds based on sticky traps are often unreliable or unavailable (Gillespie and Quiring 1987; Frey, 1993; van Dijken et al., 1994; Cloyd and Sadof, 2003). Furthermore, if reliable sticky trap based thresholds are available, and number of insects captured per sticky trap is increased (e.g. by using an LED attachment), then the threshold should be adjusted to ensure the control measures are used at the same pest density.

Project aims

This project aimed to enhance the trapping efficacy of yellow sticky traps in a range of insect pests by attaching LEDs, enabling more effective timing of pest management by optimising pest monitoring. Examples of similar trap enhancements can be seen in Chen *et al*. (2004a; 2004b), Chu *et al.* (2004), and Muñiz *et al.* (2005). The addition of LEDs may enable more effective mass trapping via yellow sticky traps and minimize the trapping of beneficial insects.

Objectives

1. Design and produce LED attachments for use with sticky traps.
2. Evaluate the efficacy of LED/trap combinations for their use in trapping pests under protected crop conditions with a small group of growers.
3. Evaluate any negative impacts of use of LED/trap combinations in respect to biological control agents and other beneficial insects.
4. Determine the relative spectral preference of *F. occidentalis* and *T. vaporariorum.*

Project Species

Eleven species were initially proposed for this project in agreement with the funding providers, nine pest species, and two beneficial insects (Table 2). However, the majority of the species could not be obtained for lab experiments, due to sourcing issues, the lack of quarantine facilities on site required for containing certain species (*Bemesia tabaci*), or licensing issues (*Aphis gossypii*). It was not possible to predict which species would be present at field sites, although a questionnaire aimed to select sites based on previous pest problems, and many of the proposed species were not captured at any of the field sites. Because of this additional species were included after the fact. These were the diamondback moth (*Plutella xylostella*) and the naturally occurring parasitoid of shore fly *Kleidotoma psiloides.* The final species list for field work and behavioural work are in table 3, and 4 respectively.

Table 2: Proposed species.

Group	Common name	Scientific name
Whiteflies	Glasshouse whitefly	*Trialeurodes vaporarium*
Aphids	Peach-potato aphid	*Myzus persicae*
Thrips	Western flower thrips	*Frankliniella occidentalis*
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*
Parasitoids	N/A	*Encarsia formosa*
	N/A	*Diglyphus isaea*

Table 3: Species captured in sufficient numbers for comparisons between standard yellow sticky traps and LED equipped yellow sticky traps.

Group	Common name	Scientific name
Whiteflies	Glasshouse whitefly	*Trialeurodes vaporariorum*
Thrips	Western flower thrips	*Frankliniella occidentalis*
Flies	Dark-winged fungus gnat	*Bradysia difformis*
Lepidopterans	Diamondback moth	*Plutella xylostella*
Parasitoids	N/A	*Encarsia formosa*
	N/A	*Kleidotoma psiloides*

Table 4: Species list for behaviour experiment.

Group	Common name	Scientific name
Whiteflies	Glasshouse whitefly	*Trialeurodes vaporariorum*
Thrips	Western flower thrips	*Frankliniella occidentalis*

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Chapter 2 Vision and Light

Abstract

This chapter will outline the basic biology of the insect compound eye, the potential for colour vision in insects and how this differs from the human perspective, and the use of visual systems by insects in host-finding. This chapter will also cover the technical information on light, light-emitting diodes (LED), and circuitry required for this project, as well as outlining health concerns which may arise from the use of active light sources.

The vision of the intended target is an essential consideration when designing a trap which relies on a visual component. 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. This is likely a response to the perceived poor visual acuity of the compound eye, which is accredited to diffraction issues from possessing many small lenses. The properties of colour vision and wavelength discrimination should be a consideration when designing a trap which relies on colour, and it should be known that wavelength discrimination is possible regardless of the insect's ability to see in colour, and determining a species spectral sensitivity may provide valuable information when selecting a colour for trapping. Here, the electroretinogram is presented as an option for acquiring this information, and a description of the required equipment and general methodology are discussed. Unfortunately it was not possible to obtain species which had been agreed upon with the funding body for this work, for example due to licensing issues or lack of quarantine facilities. As a result of this no ERG data were obtained and a greater focus was placed on trap comparisons.

The nature of light is complicated by exhibiting the properties of both waves and particles, here light will be discussed as a wave except where the energy of light is measured. At a particular wavelength light possesses a set amount of energy, with more energy per photon being possessed by each photon at longer wavelengths. For the research performed here, light will be produced by LEDs attached to yellow sticky traps, with the aim of increasing the number of pest insects captured. LEDs are semiconductors which produce monochromatic light. When compared with other active light sources they are extremely efficient, durable, and possess an extremely long life-span (half-life of ~12 years), making them an excellent choice sticky trap enhancement.

There are health implications to be considered when introducing an active light source into an environment with workers, particularly in regards to blue and UV light. Care must be

taken to ensure that the output of light in these wavelengths do not exceed thresholds which may damage human retinas, otherwise workers must wear safety glasses. Currently, 5mm LEDs do not emit light in these regions powerful enough for concern, but care should be undertaken to ensure this remains the case when considering future work.

Structure of the Insect Compound Eye, a Brief Overview

There are five known general compound eye structures, three of which will be discussed here. These are the apposition, the refraction superposition, and the neural superposition compound eyes. The remaining two are the reflective superposition and the parabolic superposition (Land, 1992). These are found within lobsters and crabs, and are not relevant to this project. It should be noted that in some species intermediate type eyes are found, for example *Plutella xylostella* possess compound eyes which display characteristics of both superposition and apposition eyes, which have been termed atypical superposition (Wang and Hsu, 1982; Fischer, 2012).

The apposition eye (Fig. 2) is the most common type of compound eye and can be found in a range of groups, for example Hemiptera, Hymenoptera, and Lepidoptera (typically butterflies rather than moths) (Land 1992; Fischer, 2012). Apposition eyes consist of a group of rod shaped cells named the ommatidia, which are adjacent to one another. Each ommatidium has its own cornea at the distal end, which functions as the primary lens. Further focusing is performed in the crystalline cone lying directly beneath it. Both the cornea and crystalline cones are supported by supporting cells. Below the lenses are the retinular cells which contain rhodopsin, a photosensitive pigment formed from retinal and opsin, there are 8 retinular cells in total (R1-8). This cluster of cells is referred to as a retinular, and each have microvilli which point towards the centre of the cluster and combine to form the rhabdome, this is the transduction cascade of the retinular. The rhabdome contains rhodopsin and all the proteins and G proteins which are able to produce an electrical response to light. At the proximal end the retinular cells end with a nerve axon, 7-8 of these exit the ommatidium. This is the same for all ommatidium and as such the combined axons are considered to constitute the optic nerve. The image formed by the apposition eye is an aggregation of the information from these axons (Land, 1992; Land 1997; Ruppert *et al.*, 2004; Hill *et al.*, 2008).

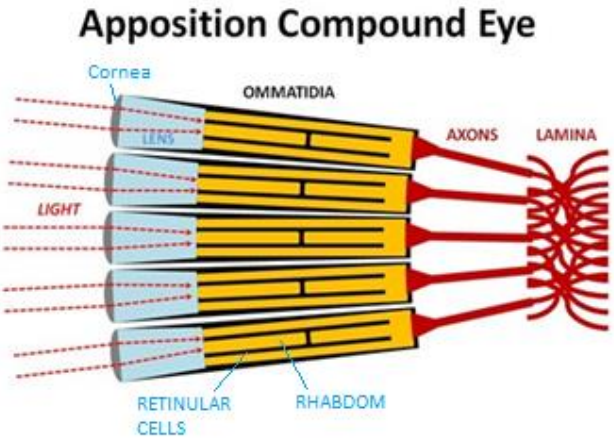

Figure 2. Structure of the apposition compound eye (modified from - Watcher, 2009a).

In the refraction superposition eye (Fig. 3) the image is not aggregated. Rather, the rhabdoms are located further back in the eye, with a clear area between them and the optics of the eye. This allows for the superimposition of multiple images, giving a higher sensitivity to light at the cost of visual acuity (Land, 1992; Land and Nilsson 1992; Land 1997). These are typically found in nocturnal insects such as the firefly *Photuris versicolor* (Fabricius) (Coleoptera: Lampyridae), although they have been found in diurnal species, such as the common Australian moth (*Phalaenoides tristifica* Hübner) (Horridge *et al.*, 1977; Land 1997).

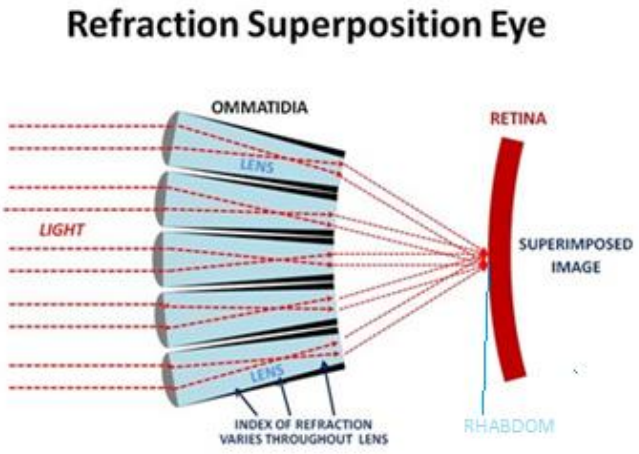

Figure 3. Structure of the refraction superposition compound eye (Modified from - Watcher, 2009b).

The neural superposition eye found in some Dipterans, such as the Bibinidae and the common house fly (*Musca domestica* Linnaeus (Diptera; Muscidae)) (Zeil, 1979; Picaud *et al.*, 1990), is more complex in design (Fig. 4). The rhabdomeres in each ommatidia are optically isolated from one another, and at different optical angles. These angles are parallel across adjacent ommatidia, so six eccentric rhabdomeres (R 1-6) in one ommatidia have a field of view which corresponds to the central rhabdomeres in adjacent ommatidia (Land, 1992) and, although located in different ommatidia, receive light from the same direction. R1-6 have synapses on the lamina where their response is added. This results in the superimposition of the image via neural means. This structure means that the lamina receives images seven times brighter than at a single photoreceptor (Zeil, 1979; van Hateren, 1987; Land, 1992).

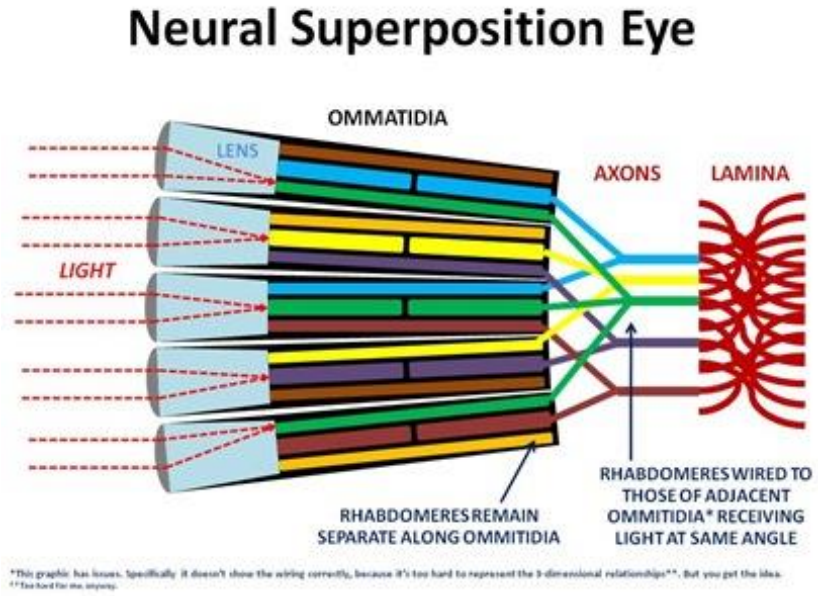

Figure 4. Structure of the neural superposition compound eye. This representation is extremely simplified, and does not fully convey the complex nature of the connections (Watcher, 2009c).

Colour Vision

There are two types of photoreceptor, rods and cones. The cones are then further subdivided. In the human eye cones are divided into three classes termed red, green, and blue, although it would be more accurate to describe these as short, medium, and long wavelength receptors. The rods have a lower response threshold so are more sensitive to light, and are used for low-light vision. The cones, while less sensitive to light, allow for the perception of colour by a comparison between the light wavelengths detected by the cones. The mechanism for

this comparison is not fully understood and although there are two main current mechanisms proposed, the colour opponent mechanism and the complimentary colour theory, the colour opponent mechanism is favoured (Lotto *et al.*, 2010; Pridmore, 2011). The colour opponent mechanism proposes that in the human eye the cone receptors form three opposing pairs: yellow-blue, red-green, and black-white. Activation of one member of a pair inhibits the other, and thus the difference in activation between these opposing cells determines the outcome rather than their absolute level of activation (Hurvich and Jameson 1957; Lotto *et al.*, 2010). For an indept look at the functional roles and features of complementary colours in vision see Pridmore (2011).

Sensitivity to a broad range of wavelengths in no way implies the ability to see in colour. Within the human eye the rods possess a peak sensitivity around 500-510nm (Lotto *et al.*, 2010); however, without additional receptors to compare against the discrimination of colour is not possible, and only the intensity of the light is detected. As such vision at night time is represented in grey scale, and two objects the exact same size and shape, but of different colours will be indistinguishable from one another if they reflect the available light at the same intensity. It is for this reason that the naming of the cone photoreceptors in the human eye would be more accurately named after length than colour, as the wavelength in and of itself does not possess the attributes of a colour; rather, colour is a cognitive property (Skorupski and Chittka, 2009). So it is apparent that the simple detection of a light wavelength does not imply that the subject possesses colour vision. The species of insect being investigated in this project have not all been confirmed to possess colour vision. It is important to note that the ability to distinguish colours is not the same as distinguishing between wavelengths, and the species investigated here display wavelength discrimination regardless of their ability to see colours.

While the healthy human eye is credited with being able to detect wavelength between 380-400nm (the colour violet) and 700-780nm (the colour red), the wavelength detection abilities of insects varies from species to species (Arikawa *et al*., 1987; Briscoe and Chittka, 2001). Commonly three photoreceptors are present in insect eyes; these are typically located within the UVA, blue, and green wavelengths, although some species have red receptors (Qui and Arikawa 2003). This indicates that a colour perceived to be yellow by a human will not be yellow to an insect; for example the flower *Chrysanthemum coronarium* is yellow when viewed by a human, and green when viewed by a bee (FReD, 2011). The implication of this is that the human visual system is not appropriate for selecting colours for non-monochromatic light source traps for insects, and spectral reflectance measurements should

be undertaken if possible (Hall *et al.* 2010). For clarity, trap colours discussed here will be from the human perspective.

Visual cues in Host-Finding

In spite of 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). This idea that vision is not important in host-finding has created a bias towards research regarding chemical cues. A search on ISI Web of Science performed by Reeves in April 2011 found that the search term "host plant location" (without apostrophes) was performed 960 results were returned, further refining this search with the terms "visual" or "chemical" reduced the number of articles to 49 (5.1%) and 138 (14.4%) respectively, demonstrating a bias to chemical based research. The same search performed for this thesis in July 2015, refined to articles between 2012-2015, returned 2155 results, with "visual" being present in 2 (2%) of articles, and "chemical" in 402 (18.65%), demonstrating a slight increase in bias with a larger sample size. It should be noted that Reeves (2011) may have searched papers from as early as 1864, and the increase in bias may be a product of the search presented here not including early articles, when chemistry techniques and equipment availability were less common than in more recent times.

It has been frequently suggested that vision is not an important factor in host-finding in phytophagous insects, often due to the assumption that the insect compound eye suffers from poor visual acuity (Reeves, 2011). This poor acuity is accredited to the issues caused by diffraction when possessing so many small lenses, which has led to comparisons between human and insect eyes suggesting that to match human visual acuity, insects would need eyes 19m in radius (Land, 1997). However, there is clear evidence of insects using vision when selecting a host plant; for example the milfoil weevil, *Euhrychiopsis lecontei* (Dietz) (Coleoptera: Curculionidae), is able to select appropriate hosts in the absence of olfactory cues (Reeves and Lorch, 2009). This use of visual cues to locate a food source is not confined to phytophagous insects, nectar feeders have shown differing preferences between species when a choice is offered between olfactory and visual cues, with the diurnals Lepidopterans *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae) and *Macroglossum stellatarum* (Linnaeus) (Lepidoptera: Sphingidae) preferentially selecting the visual cue, while the nocturnal Lepidopteran *Deilephila elpenor* (Linnaeus) (Lepidoptera: Sphingidae) selected olfactory cues (Balkenius *et al,*. 2006; Goyret *et al*., 2007). These preferences have

been shown to be flexible, and may change as an individual gains foraging experience (Kelber, 1996; Balkenius and Kelber, 2006; Goryet *et al*., 2007). Predatory insects also use visual cues to find host plants, for example the host-specific beetle *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) took significantly longer periods of time to find their host-plant when in darkness, despite there being no efforts to mask chemical cues (Mausel *et al.*, 2011). It is worth bearing in mind that olfactory cues may be used for distance, while visual cues are close range (Reeves, 2011).

Colour is typically assumed to be the most important visual stimulus for phytophagous insects, and numerous phytophagous species appear to possess trichromatic vision (Matteson, *et al.*, 1992; Kirchner *et al.*, 2005; Döring and Chittka, 2007b), and may even possess colour vision, although this is difficult to define (Skorupski and Chittka, 2009). Further evidence of the importance of colour in host-finding can be found in autumn leaf colouration, which has been suggested to be a signalling mechanism to warn herbivores they are either chemically defended, or low in nutrients (Hamilton and Brown, 2001; Döring *et al.*, 2009). This is still debated, and alternative mechanisms have been proposed, such as autumn leaf colouration being a non-adaptive consequence of senescence or a protective sun screen (Wilkinson *et al.*, 2002). The use of coloured traps to capture phytophagous insects also provides compelling evidence towards the importance of colour in host-finding, with numerous phytophagous insects demonstrating a preference for a particular colour of trap (Vernon and Gillespie, 1990; Bernays and Chapman, 1994; Yaku *et al.*, 2007; Moreau and Isman, 2011).

Colour should not be considered the only factor and the ability to differentiate between plant species has been demonstrated in the cabbage root fly, *Delia radicum* L., which is able to differentiate between hosts using colour (Prokopy and Owens, 1983). Furthermore, despite the observation that the host-finding in the glasshouse whitefly (*T. vaporariorum*) is not influenced by leaf structure and shape (van Lenteren and Noldus, 1990), an increase in the capture of tobacco whitefly (*B. tabaci*) has been demonstrated by both triangular and circular shaped yellow sticky traps against a black background (Kim and Lim, 2011). The size of the yellow sticky trap area is a factor in attracting insects, typically with a larger surface area capturing more insects (Carrizo, 2008; Kim and Lim, 2011); however, when adjusted for capture effectiveness per sample area, this is not always the case. Kim and Lim (2011) found traps with two 13cm diameter yellow circles on a black background captured 1.8 times more *Bemisia tabaci* than standard yellow sticky traps when adjusted for whiteflies per sampled

area (50.2cm^2). No significant differences were found between standard yellow sticky traps and those with two 18cm^2 yellow circles on a black background.

In addition to size and shape, the increase in number of pests captured may be the result of the contrast between the attractive area (yellow sticky trap) and the black background influencing the insects landing response by affecting their optomotor response (Smith, 1976).

A reduction in the densities of *T. vaporariorum, B. tabaci, Aphis gossypii, Myzus persicae* (Sulzer) (Hemiptera: Aphididae) has been observed by using UV absorbing films or nets (Chyzik *et al.,* 2003: Mutwiwa *et al.,* 2005; Kumar and Poehling, 2006; Gulidov and Poehling, 2013). The mechanism for this behaviour is uncertain, but may be due to UV being a stimulus for flight initiation, orientation, and host-finding (Kumar and Poehling, 2006; Kigathi and Poehling, 2012).

Electroretinogram: Determining the Spectral Sensitivity of an Insect

Spectral sensitivity, the efficiency at which light is detected by the photoreceptors, can be determined using an electroretinogram (ERG) (Kirchner *et al.,* 2005). 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 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.

When determining spectral sensitivity using an ERG, the quantity and quality of the light available to the subject must be controlled, and the amount of light (photonflux) at the position a subject's eye will be located during experimentation must be known (Kirchner *et al.,* 2005). The wavelength of light is most easily regulated by using a light source with a broad spectral output, such as a xenon arc lamp, and filtering this light to a narrow

wavelength using bandpass filters. The subject's eye does not necessarily contain an equal number of the different classes of photoreceptor and may, for example, possess more red than blue receptors. In order to account for this difference, the amount of light can be adjusted to a range of different log intensities by using neutral density filters (Pers. comms Thomas Döring). In addition to this problem, the different sensitivities to wavelengths can create a masking effect. For example if the subject has a high sensitivity to green, and a low sensitivity to UV, then the UV can be masked (Fig. 5) (Kirchner *et al.*, 2005). Because of this the subjects must be tested under different conditions of light adaptation, for example under dark adapted, white light adapted, and yellow light adapted conditions. By adapting the subject to white light the sensitivity the wavelengths within the visible spectrum are reduced, and the response to UV is seen much more strongly (Fig. 6) (Kirchner *et al.*, 2005). To detect the presence of a blue receptor the sensitivity to green light should be reduced using yellow light; this is because a reduction by use of green light adaptation can result in a reduced sensitivity to blue light. The subject should be contained within a light proof Faraday cage to prevent both stray light and the influence of outside electrical sources. Using this set up a subject is exposed to short flashes of light across a range of narrow wavelengths, and the spectral sensitivity of the tested eye type can be determined (Fig. 7) (Kirchner *et al.*, 2005).

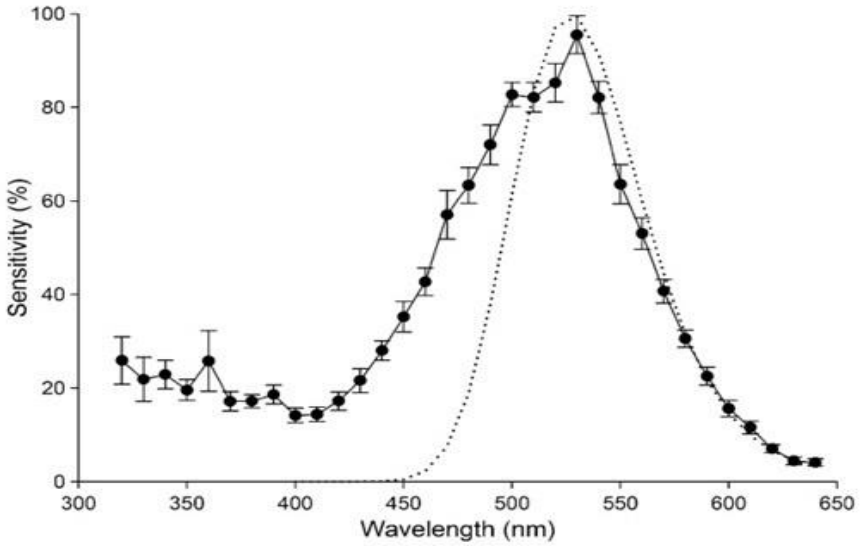

Figure 5. The spectral sensitivity of alate *Myzus persicae* under conditions of dark adaptation. Demonstrating the masking effect of the much higher sensitivity to light within the green range of the spectrum (dotted line is a model of a green receptor, λ_{max} = 527 nm)(Kirchner *et al*., 2005).

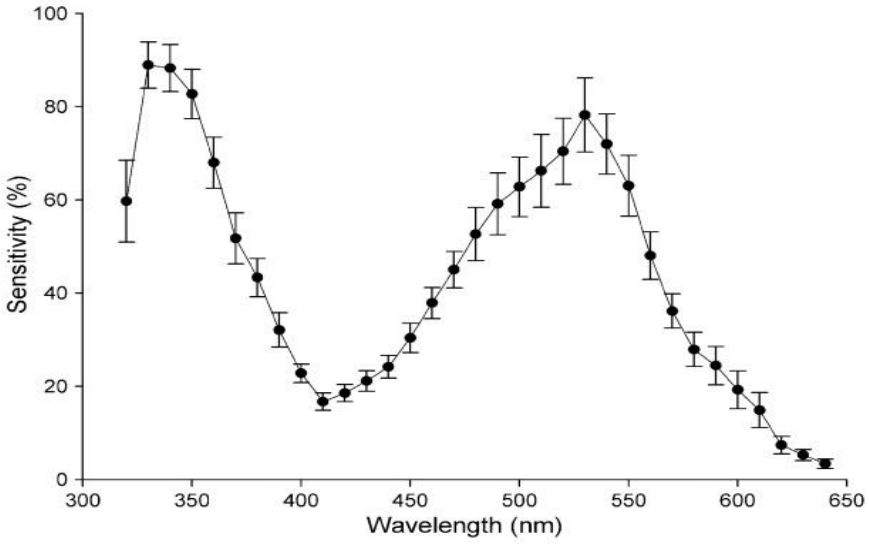

Figure 6. The spectral sensitivity of alate *Myzus persicae* under conditions of dark adaptation. Demonstrating the increased visibility of the response to UV in the visual output of the ERG (Kirchner *et al*., 2005).

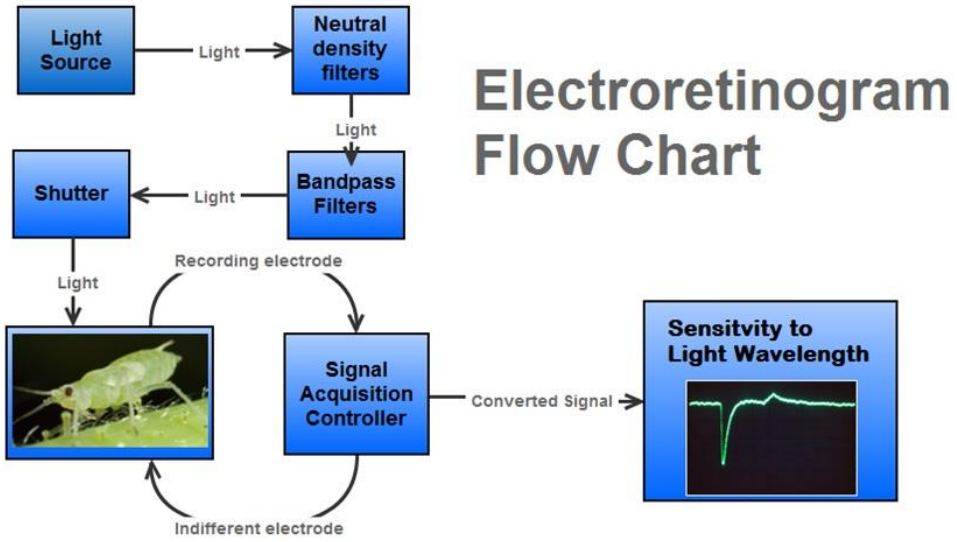

Figure 7. The subject's eye is pierced by a recording electrode; an indifferent electrode is placed into the body. An output is then obtained by exposing the eye to short flashes of light across a range of narrow wavelengths. By measuring the magnitude of the response to these wavelengths, the spectral sensitivity of the insect can be determined (Diagram produced by author; Aphid photo: Delvaux 2011).

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 ERG was initially a key component of this research project, and the equipment to perform this procedure was assembled (Fig. 8) and test data were gathered. Unfortunately it was not possible to obtain species which had been agreed upon with the funding body for this work, for example due to licensing issues or lack of quarantine facilities. As a result of this no ERG data were obtained and a greater focus was placed on trap comparisons.

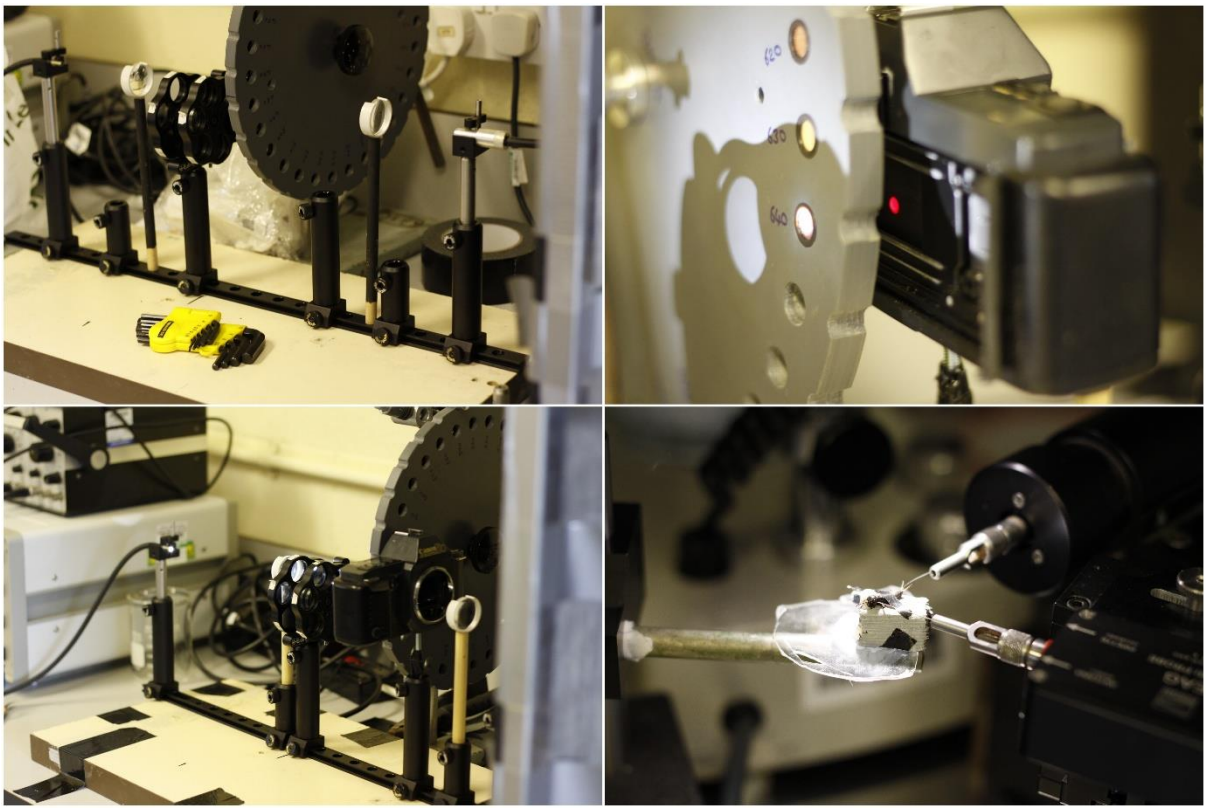

Figure 8. Electroretinogram equipment.

Introduction to Light, Refraction and Dispersion

The nature of light is complicated by exhibiting the properties of both waves and particles. For convenience sake light will generally be treated as part of the electromagnetic spectrum within this thesis, except when measuring the brightness of light, where particles are more appropriate. As part of the electromagnetic spectrum light can be described as a wave, which has a wavelength (λ), a frequency, and an electrical and magnetic field, both of which are described by a vector. Light can be graphically represented using a sine wave (Fig. 9), with the amplitude representing the magnitude of the electrical vector, and the distance between the wave crests, or troughs, the wavelength. If the velocity which the waves vibrate is increased the distance between the wave crests shortens, giving a shorter wavelength and a higher frequency. This relationship is described by the following formula: $V = \lambda.F$, where V is velocity (m/s), λ is wavelength (m), and F is frequency (Hz) (Tilley, 2000). The implications of this are that the shorter the wavelength, the higher the frequency, and thus the

more energy contained by each photon (i.e. blue light possesses more energy than red). The magnetic vector will not be relevant to this project.

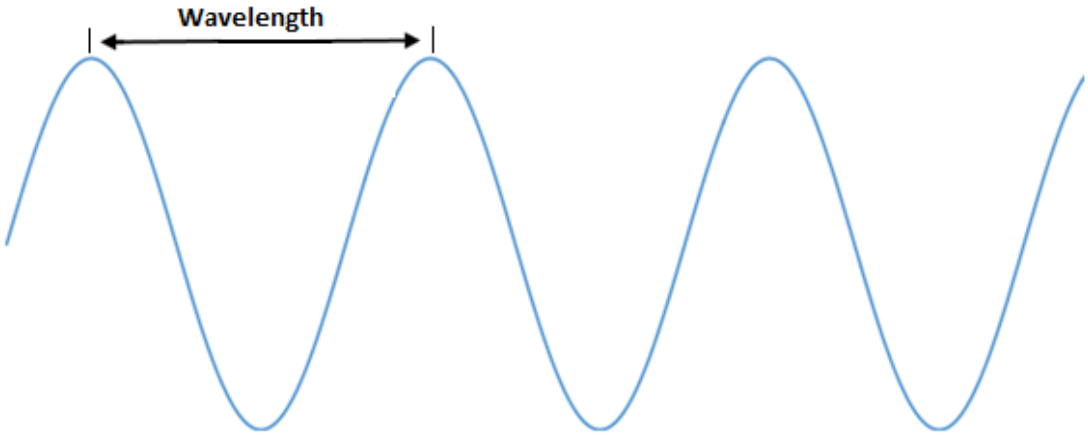

Figure 9. Sine wave representing light as a wave. The wavelength is the distance between the peaks in the wave and the amplitude is the maximum distance from the wave's undisturbed position (Diagram produced by author).

When light changes medium the direction of the wave is changed, this is termed refraction. The angle of refraction is determined by a number of factors, for example the velocity of light within that material, or the material density. Of relevance to this project is the effect termed dispersion, this describes a relationship between the refraction of light and wavelength. The index of refraction will increase as the wavelength decreases, so blue light will refract at a greater angle than green, or red (Tilley, 2000). This is relevant to the behaviour experiment described in chapter 7, where there will be a small unavoidable inequality between the numbers of photons at equal distances from the light sources with the exception of the centre.

Light-emitting Diodes: Advantages and Disadvantages

An LED is a semiconductor which produces light. It is composed of a silicon semiconductor chip possessing a positive side (anode) and negative side (cathode) the gap between these two sides is named the p-n junction (Fig. 10). As with all semiconductors the voltage will flow in one direction, from the p-side to the n-side, and it is not ordinarily possible for a reverse flow of voltage. Due to this LEDs must be powered using direct current (DC), as in

alternating current (AC) the flow of electrons will periodically reverse direction and the LED will not be powered for this period. Fortunately batteries use DC, and mains power is easily converted from AC to DC using a converter, for example a laptop charger possesses an AC-DC converter.

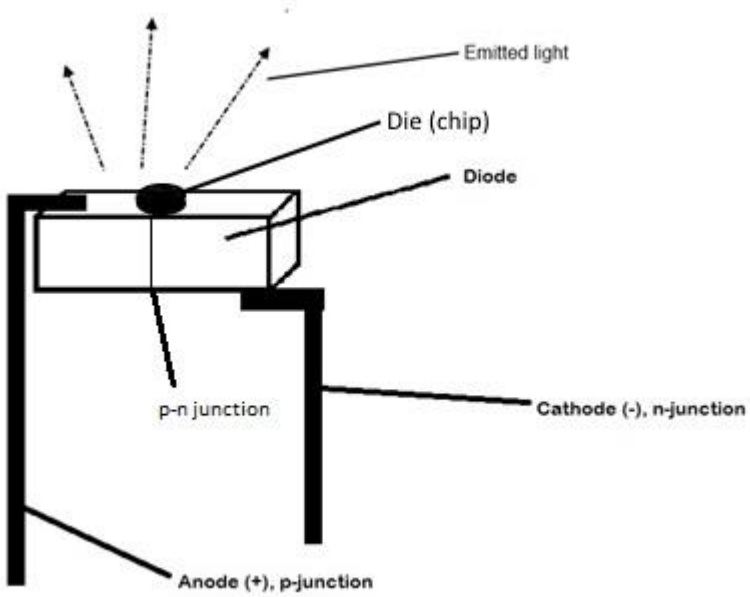

Figure 10. A simplified diagram showing the construction of an LED. The emitted light can be focused, or dispersed, using an epoxy casing (Diagram produced by author).

When a forward voltage is applied to the LED the electrons in the n-junction and the holes in the p-junction will be pushed towards the p-n junction. This lowers the barrier potential and it becomes thin enough that electrons can tunnel across the barrier of the p-n junction; allowing the electrons to enter the p-type silicon and move from hole to hole. When a barrier cross occurs and the electron meets a hole it will fall into a lower energy state and, due to the first law of thermodynamics, energy is released. In this case the energy is released in the form of photons, and thus light is produced. The wavelength of light produced depends on the band-gap energy of the materials which make up the p-n junction. For example gallium(III) phosphide (GaP) and Indium gallium nitride (InGanN) can be used to produce green and blue light respectively (Held, 2009).

It is apparent that the way LEDs function limits them to monochromatic light output. This means that colours produced by combinations of wavelengths, such as pink, purple, or white cannot be produced using a single die (i.e. the chip which produces the light). These can be produced by either a combination of dies, for example white can be produced using a mix of

red, green, and blue dies. Alternatively, a phosphor coating can be placed over the die, which emits light when illuminated by the die; the combination of these light sources produces the desired colour, for example a blue LED with a red phosphor coating produces the appearance of purple light (Schubert, 2003; Held, 2009).

The narrow wavelength produced by LEDs is a great advantage for attracting insects for two primary reasons, 1. The absence of other light wavelengths prevents a reduction in attraction from a photonegative response to unwanted wavelengths. 2. Power is not wasted in producing unwanted wavelengths. It should be noted that it is possible that certain wavelength combinations result in a greater attraction than monochrome light sources, if this is the case LEDs can be combined while still maintaining these two advantages.

In terms of power consumption a standard 5mm LED uses 10-30mA (Avago, 2016), and is also much more efficient than other light sources; for example tungsten light bulbs have a luminous efficacy of ~2-5% (Energy saving trust, 2016; Keefe, 2007), with the remainder being output as heat. While there is great variance in LED luminous efficacy it would not be unusual for it to be over 15%, and a luminous efficacy of over 100% (~230%) was recently demonstrated using a non-standard LED which made use of environmental heat to increase the electrical efficiency, although this was performed at very low power level and efficiency should be expected to decrease as power level is increased (Santhanam *et al.*, 2012). A result of this combination of low power consumption and high luminous efficacy is that current LEDs produce far less heat than other light sources, as a higher percentage of a smaller amount of power is used to produce light. In the case of the LED used by Santhanam *et al.* (2012) heat is absorbed. The advantage of this in a crop growing environment is that LEDs can be placed closer to a plant than currently used light sources, which enables a much more compact growing environment as well as intercrop (within crop) lighting (van Ieperen and Trouwborst, 2007).

Although LEDs consume very little power, they have a forward voltage which ranges from 1.5V to 3.4V (Avago 2016). As a general rule the longer the wavelength the lower the forward voltage required, so a UV LED may have a forward voltage of 3.4V compared with 1.8V for a red LED. The forward voltage is the minimum voltage required to light up the LED, this creates difficulties when having to power LEDs without access to mains power, as high voltage batteries typically suffer from low capacity. The capacity of an alkaline 9v battery is ~300-500mAh (Rightbattery, 2016), which would power a 20mA LED continuously for a period of 25 hours (500mAh/20ma=25hrs). Conversely an alkaline D cell battery has a capacity of around 12,000-20,000mAh and a voltage output of 1.5V (Energizer,

2016) (Note: some capacities are estimates as manufacturers do not typically publish full battery specifications). Because of the relatively high forward voltage requirements of LED, in order to power an LED using D cell batteries, multiple batteries must be arranged in series to combine their voltages. A minimum of three D cell batteries are required to power a single green (540 nm, 3.2V forward voltage) LED. In situations where more than one LED must be powered by a single source, it is possible to wire the LEDs so that they all benefit from the full voltage of the power source (Fig. 11); this applies to any number of LEDs, so very large numbers of LEDs can be powered from a single battery pack, although each LED will draw an additional 10-30mA and the power source will expire sooner. If a rechargeable power source is desired, it is preferable to use AA batteries instead of D cell, as although these have a much lower capacity (~500-1000mAh) they suffer far less from voltage drop, i.e. the reduction in voltage as the battery power depletes (Energizer, 2016; Rightbattery, 2016). As the voltage decreases, the light output of the LED will also decrease. When the voltage drops below the forward voltage of a particular LED, the LED will switch off. Because of this battery life estimates (mAh/mA) will always overestimate the length of time an LED will be powered to some extent, and a live test on a setup must be performed.

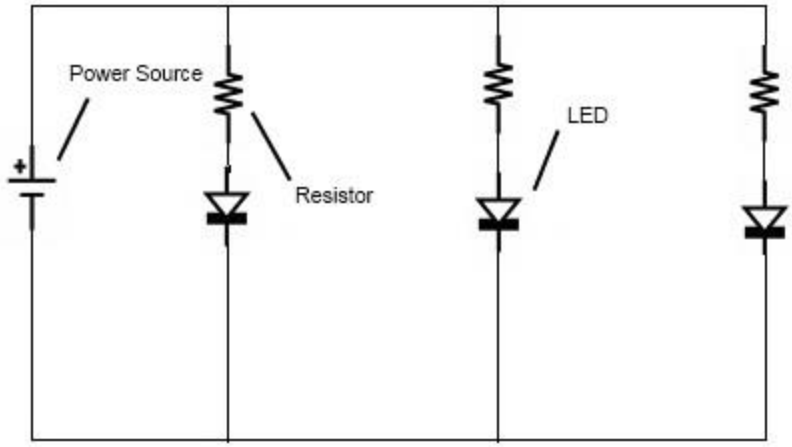

Figure 11. Circuit diagram demonstrating how to wire multiple LEDs to a power source which only produces enough voltage to drive a single LED. Here each LED is directly wired to the power source, so receives the full voltage.

LEDs are solid state, which is to say they are built of solid materials and have no moving parts. This gives them a high degree of durability. They also possess a very long half-life of around 11 years, so will theoretically lose only half of their output after this time period. A

further advantage is the small size of the LED, which enables LEDs to be included into existing trapping systems with ease.

In terms of cost, 5mm LEDs differ in price based on wavelength and light output. LEDs within the visible spectrum are generally priced between 7p and 50p per unit. UV LEDs are more expensive, with 400 nm LEDs costing around £1.20 per unit (Avago, 2016). This cost increases dramatically further into the UV spectrum.

Health Implication of Artificial Light Sources

When using light to attract crop pests it is often the case that hazard to the human eye are not considered, for example Mutwiwa and Tantau (2005) experimented with the use of a UV lamp to attract the greenhouse whitefly (*Trialeurodes vaporariorum*), and made no mention of concerns of the irreversible damage that may be caused by exposure to UV light. This is concerning, considering that the damaging effects of UV light are widely known (Pfiefer *et al.*, 2005; Chalam *et al.*, 2011).

Blue light is also known to cause damage to the eyes, with the photooxidative damage blue light causes being associated with the causation of age-related macular degeneration (Barker *et al.*, 2011; Kernt *et al.*, 2012). In some respects this is of greater concern, as much less blue light is filtered by the lens when compared with UV, particularly in younger individuals. The lens yellows with age, filtering a large portion of blue light (Fig. 12).

Sources of blue light can be categorised into four different risk groups as defined by the European standard EN 62471 (Table 5). These exposure limits were determined by experiments involving monkeys and rabbits. The subjects were exposure to light until a white lesion was observed on the retina, the level of exposure to cause these lesions were then multiplied by a safety factor of ten (Behar-Cohen, 2011).

Behar-Cohen (2011) determined that a blue LED with an output of 0.07W would belong to group 1, and thus represent a low risk. As the blue LEDs used in this thesis do not exceed 0.01W these will likely be classified as group 0 and present very little risk. However, the potential for damage from these light sources should be considered as the potential for LED brightness increases with advancing technologies.

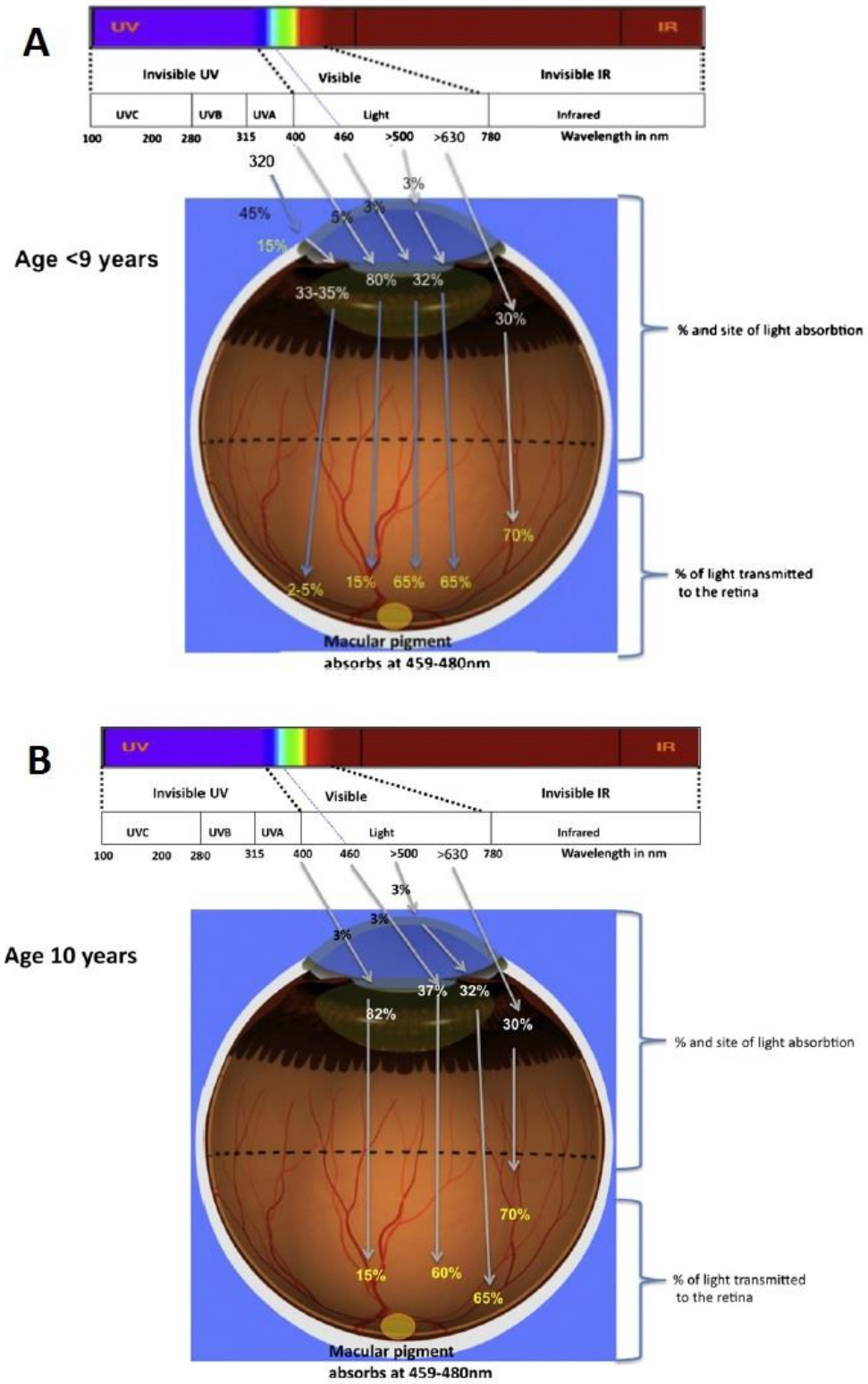

Figure 12. Percentage of UVA and visible light absorbed by the human eye, and transmitted to the retina within an eye. A: Aged <9 years. B: 60-70 years (Behar-Cohen, 2011).

Table 5. Risk groups which sources of blue light under by EN 62471. Risk groups are categorised by the level of hazard in the light emitted from a device (Behar-Cohen, 2011).

Maximum admissible exposure time (t)	Risk group
t ≥ 10,000s	Group 0
11s ≤ t 10,000s	Group 1 (low risk)
0.25s ≤ t < 100s	Group 2 (moderate risk)
T< 0.25s	Group 3 (high risk)

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Chapter 3 Development of LED attachments

Abstract

Coloured sticky traps are commonly used in commercial horticulture to monitor for the presence of insect pests. These traps primarily rely on their visual attraction to the pest and can be enhanced with the addition of an artificial light source. Light-emitting diodes (LEDs) are small, solid state, monochromatic light producing devices, and are currently the most suitable available light source for the purposes of trap enhancement.

A cheap, and easily produced, LED attachment was designed for clipping LEDs to sticky traps. This chapter describes the LED attachment design and the considerations for this. Alternate designs are briefly discussed.

Introduction

Coloured sticky traps are a commonly used insect trap, and rely primarily on their visual attraction to pests (Vernon and Gillespie, 1990). The addition of an active light source can increase the number of insects captured by these traps, for example equipping yellow sticky traps with green (530 nm) LEDs increased the number of *Bradysia coprophila* by 136.6% and *Trialeurodes vaporariorum* by 31% when compared with standard yellow sticky traps (Chen *et al.*, 2004).

LEDs are small, solid state, monochromatic light producing devices. They are ubiquitous, and are found in many household devices, such as TV screen, microwave displays, and power indicators on a range of appliances. They possess a high degree of durability, are more efficient than other available light sources, possess an extremely long lifespan (half-life ~11 years), and are cheap (~7-50p per unit within the visible light spectrum) (Avago, 2016). These properties make them an ideal active light source for crop pest trap enhancement. For a more detailed overview of the properties of LEDs see chapter 2.

LED Attachment

Basic Design

It was essential that LED attachments be cheap, durable, easy to produce, and easily integrated into an existing monitoring system in commercial growing facilities. Broadly speaking, LED attachments consist of a light source, a power source, a means of attachment, and a central unit which is used to hold the components together. Here, LEDs were soldered to 0.2mm equipment wire which was connected to a power source (mains or battery), and used a dual pronged curling clip as the attachment method. The central unit was a terminal block (Fig. 14).

LED attachments produced by Chu *et al.* (2004) used perforated circuit board as the central unit, the main advantage of which when compared against a terminal block is cost (~7p versus ~20p per attachment device) (CPC 2016). However, perforated circuit board must be sawed into segments before use, which is a time consuming task if a power saw and appropriate facilities are unavailable. Furthermore, there is no easy way to attach the curling clip and care must be taken to avoid crossing circuits when soldering the LED to the circuit board, further increasing production time. The channels within terminal blocks are electrically isolated from one another, and come equipped with screws to hold wires in place, enabling devices to be assembled quickly, more easily, and result in more durable units than

those made using perforated circuit board. Here, 5mm LEDs were used as these are commercially available in a broad range of wavelengths (Avago, 2016).

A potential disadvantage of this design is that it is not possible to point the LED downwards towards the crop, although it is not known if this would increase the effectiveness of the trap enhancement. Possible solution to this would be to either use perforated circuit board as the central unit of the attachment, or to remove the central unit entirely and attach the LED directly to the clip, while ensuring that the cathode and anode are electrically isolated from one another using insolating sheathing (e.g. heat shrink tubing). Alternatively, a reflector could be fixed to the attachment which re-directs the light downwards. These alteration would incur a cost in terms of expense and construction time (if using perforated circuit board or reflectors), or the stability and durability of the attachment (if using perforated circuit board or no central unit).

A further concern with this design is the distance of the LED from the trap surface. When the attachment is clipped to a sticky traps the light producing surface of the LED sit ~2cm away from the surface of the trap. Although the mechanism for trap enhancement is unknown, in circumstances where it is due to an increase in attraction or the insect's flight being arrested, then it may be advantageous for the light producing surface of the LED to be position more closely to the surface of the trap. This can be achieved by either removing the central unit (as suggested in the above paragraph) or by piercing the sticky trap and clipping the attachment on with the LED going through the hole (i.e. turn the LED inwards towards the trap rather than outwards).

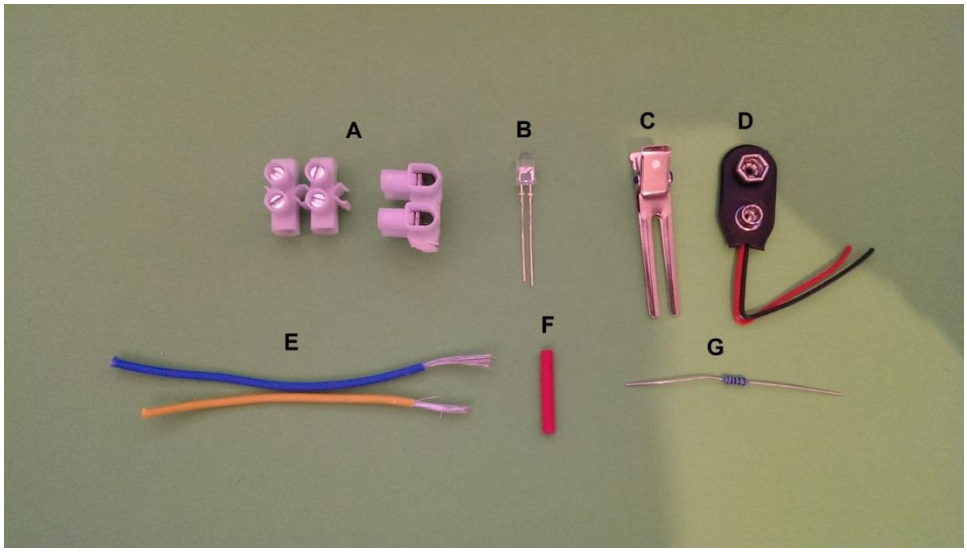

Figure 14. LED attachment components. A. Terminal block. B. LED. C. Curling clip. D. Battery clip. E. 0.02mm equipment wire. F. Heat shrink tubing. G. Resistor.

Power Supply

Providing power to the LED attachments in a growing environment is challenging. Power can be provided via an AC/DC mains adaptor; however, due to the dispersed nature of the sticky traps this will not be viable in all growing facilities as wires will become tangled, or present a tripping hazard. Due to this, battery power was the preferred method for this research. A standard 5mm LED uses 10-30mA and a forward voltage between 1.5V and 3.4V, a battery pack of 4 D cells will power a single LED for roughly 600-1000 hours, depending on LED and battery specifications. Unfortunately, battery power is expensive, costing around £3 per month for each trap compared with ~0.07p using mains power (based on 15.2p/kWh) (UKPower 2015).

Solar power is a viable alternative with a 5w solar panel costing ~£12 (Sunstore, 2016), and an LED can be powered directly via a solar panel, or preferably via a rechargeable battery connected to a solar panel. Solar powered garden nightlights are an extremely cheap source for solar panels (£1 per unit) (Homebase, 2016), and the LED provided with the nightlights can be switched easily. These devices use the solar panel to charge a battery, then use this power to turn on the LED when the voltage on the solar panel drops below a certain threshold, so will only produce light during periods of darkness. Unfortunately, it is difficult, if not impossible, to modify these devices to function during the daytime (i.e. power the LED while over a certain voltage threshold), making them unsuitable for diurnal pests or facilities which operate crop lighting during the night time. The availability of power via solar cannot be guaranteed, making this an unsuitable power source for research purposes. For commercial applications LED attachments would have to be tested on a case by case basis in each facility.

Water Resistance

LED attachments and battery packs are susceptible to corrosion, and steps must be undertaken to prevent this in facilities were overhead irrigation or misting are used. As LED attachments were homemade with limited manufacturing facilities, it was not possible to produce water proof enclosures; instead, they were wrapped in cling film which was then taped, which provided ample protection for the study period. Battery packs were suspended within plastic containers, which contained a silica satchel to reduce humidity.

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Chapter 4 Insects Pests and Biological Control Agents

Abstract

Four species of pest insect, and two species of beneficial were captured in sufficient numbers for analysis in the experiments outlined in chapter 5. Here, the pest species are introduced (*Bradysia difformis, Frankliniella occidentalis, Trialeurodes vaporariorum, Plutella xylostella*) and the damage they cause, monitoring strategies, and management practices are described. The two beneficial species, *Encarsia formosa* and *Kleidotoma psiloides*, are introduced and their use is also described.

Introduction

The term protected cropping broadly refers to crops grown either in glasshouses, under cover (e.g. plastic sheeting), or to mushroom cultures, which are grown in dark room (EGTOP, 20013). Glasshouses allow the grower a much higher degree of environmental conditions than outdoors, for example temperature, humity, light, water supply, and atmospheric composition are all alterable to some extent (EGTOP, 20013). The European Union Expert Group for Technical Advice on Organic Production define glasshouses as: "*all permanent structures, with or without heating, covered by glass or plastic or other material that lets daylight through, in which crops, transplants or ornamentals are cultivated, are considered as 'greenhouses'*" (EGTOP, 20013). It should be noted that the terms 'greenhouse' and 'glasshouse' are interchangeable, and the term glasshouse does not necessarily imply the covering material is glass. The field work components of this project took place in glasshouses growing poinsettia or herbs.

Poinsettia (*Euphorbia pulcherrima* (Willd. ex Klotzsch)) are ornamental plants which flower between December and January, making them a popular decorative flower around Christmas time (Benson *et al.*, 2001). Pests include whitefly, thrips, fungus gnats, and aphids (Jeon *et al.*, 2007*;* Cuthbertson *et al.*, 2011; Cloyd, 2015). These pests potentially have a large economic impact on the grower, for example *B. tabaci* are a notified pest in the UK and there is a policy of containment and eradication if an infestation is found at a nursery. If the infestation is not dealt with within a prescribed time, the crop is destroyed (Cuthbertson, 2005; Cuthbertson *et al.*, 2011). Herbs are defined as the leaves (dried) of aromatic plants, which are used to impart flavour and/or odour to food (International Standard Office, 2015). A broad range of herbs are grown in the UK, one of the study site used for this thesis grew a variety of herbs such as basil, chives, and thyme, and the pest species of interest was dark-winged fungus gnat (*Bradysia difformis*), as these were a known pest at the site. Here three pest species, and two biological control agents, will be discussed, these are *B. difformis*, glasshouse whitefly (*Trialeurodes vaporariorum*), and western flower thrips (*Frankliniella occidentalis*). The biological control agents are the commercially available parasitic wasp of whitefly *Encarsia formosa* and the naturally occurring parasitoid of shorefly *Kleidotoma psiloides* Westwood (Figitidae: Eucoilinae).

This chapter will provide information on species which were captured in sufficient numbers for an analysis to be performed are presented. *Plutella xylostella* are a special case, in that they are not a pest of the protected crops studied here; however, this species were captured in

sufficiently different numbers by the trap types at one study site to be of interest to this work. There will be no discussion of action thresholds, as no relevant thresholds exist.

Introduction to Project Species

Dark-winged fungus gnat (*Bradysia difformis*)

Physical description and life cycle

Bradysia difformis are small black flies, with long legs and beaded antennae (Fig. 15). Adults are between 1-5mm in length, and males are usually smaller than females. They are weak flyers and are typically found just above the soil surface. Their lifecycle is in 4 stages and lasts between 20-28 days, and generations are continuous and overlapping (Fig. 16) (Nielson 1997; Menzel *et al.*, 2006). Females are able to mate within a few hours of emerging, and lay small (~0.1-0.25mm) yellowish white eggs on the ground close to plant roots. A single female can lay 50-1000 eggs during their lifetime (Nielson 1997; Malais and Ravenberg, 2003). Larvae (Fig. 16) emerge after 4 days, and progress through four morphologically identical instar stages, and a pupal stage, before reaching adulthood (Malais and Ravenberg, 2003).

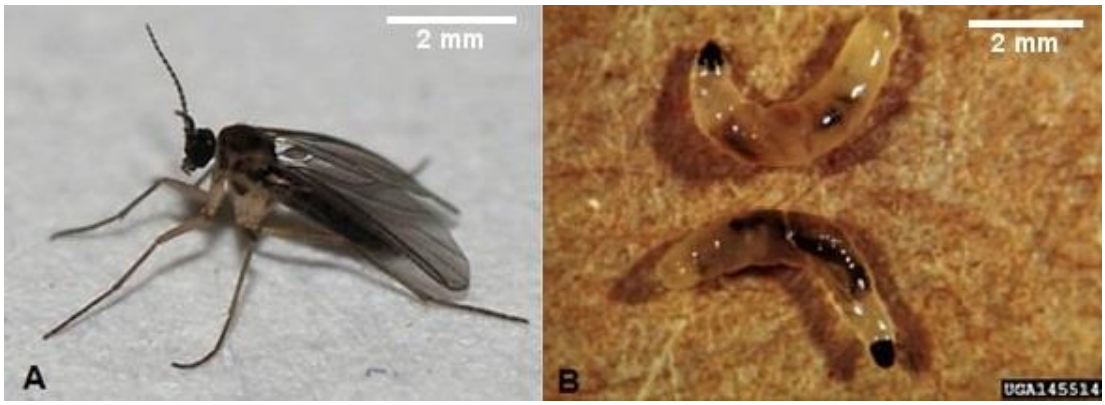

Figure 15. A: *Bradysia difformis* adult. B: *Bradysia difformis* larvae (Whitney Cranshaw, 2005; Alvesgaspar, 2007).

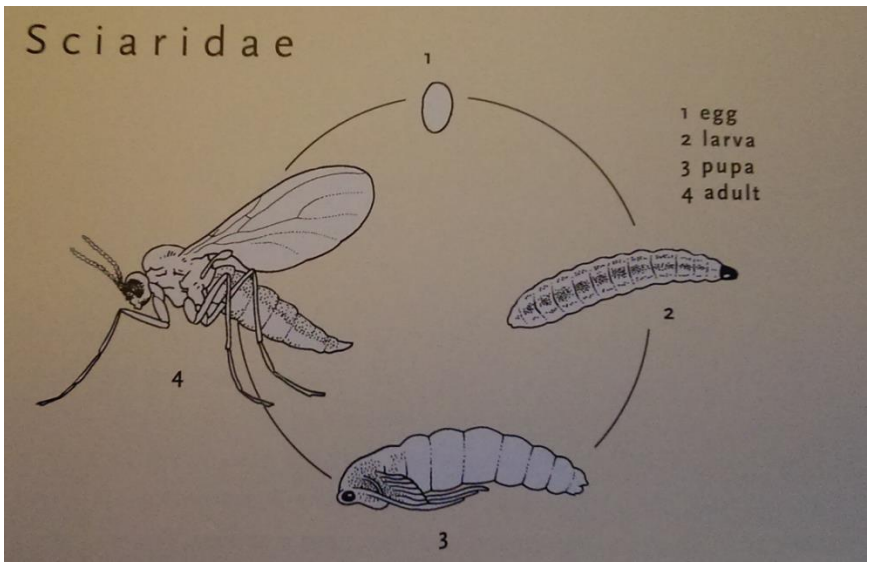

Figure 16. *Bradysia difformis* life cycle (not to scale) (Malais and Ravensberg, 2003).

Damage

Sciaridae are broadly distributed, and have been recorded across Europe, USA, Brazil, Asia, and South Africa (White *et al.,* 2000; Menzel *et al.,* 2003; Hurley *et al.,* 2007; Santos *et al.* 2012; Shin *et al.,* 2012*;* Han *et al.,* 2015). *Bradysia difformis* infest a broad range of crops including glasshouse ornamentals, orchids, and forestry nurseries (White *et al.,* 2000; Chen *et al.,* 2004b; Hurley *et al.,* 2010; Han *et al.,* 2015), despite this they are poorly researched in comparison to related species, particularly *Bradysia coprophila* Lintner (Diptera: Sciaridae) (Harris *et al.,* 1995; Chen *et al.,* 2004b). Where *B. difformis* data is unavailable, studies of related Sciaridae species will be used. Although Sciaridae are a common pest in mushroom crops, the mushroom sciarid fly (*Lycoriella ingenua* (Dufour) (Diptera: Sciaridae)) is the dominant Sciaridae mushroom pest in the UK and *B. difformis* are rarely found (White *et al.,* 2000). Sciaridae primarily cause direct damage through larval feeding, and the adults and larvae of are known to transmit fungal pathogens (Freeman, 1983; Kalb and Millar, 1986; Gardner *et al.,* 1990). Data for the economic impact of *B. difformis* in the UK are not available.

Sciaridae larvae feed on plant tissues, fungi, animal excrement, and decaying organic matter (Anas and Reeleder, 1988; Gillespie and Menzies, 1993; Han *et al.,* 2015). Direct damage by larval root feeding hinders water and nutrient absorption, leading to plant discolouration, and eventually death (Leath and Newton 1969; Jagdale *et al.,* 2007). This is of particular concern in facilities where the plants have minimal space for root systems, such as those grown on

benches (Harris *et al.,* 1995). The loss of vigour caused by this damage may make the plants more susceptible to infection by plant pathogens (Leath and Newton 1969; Kennedy 1974; Springer, 1995).

Sciaridae larvae act as a vector for a number fungi, for example *Pythium* spp. and *Coniothyrium minitans* Campb (Pleosporales: Leptosphaeriaceae) (Gardiner *et al.,* 1990; Whipps and Budge, 1993). These fungi are ingested by the larvae while feeding or moving through the soil, and are then spread in faeces, larval cadavers, or adult cadavers via transstadial transmission. *Pythium* spp. causes root rot in a broad range of crops, for example poinsettia, bell peppers, tomato, and cucumber (Owen-Going, *et al.,* 2012; Miyaki *et al.,* 2014). This is particularly the case in hydroponic systems (Sutton *et al.,* 2006; Owen-Going, *et al,.* 2012; Miyaki *et al.,* 2014). While no UK specific data are available on yield loss in protected crops, in Kenya *Pythium aphanidermatum* (Edson) Fitzp. has been reported to reduce tomato crop yields by 30% (Muriungi et al., 2014).

In contrast to this *C. minitans* is a biological control agent of the fungus *Sclerotinia sclerotiorum* de Bary (Helotiales: Sclerotiniaceae). *C. minitans* achieves this control by secreting antifungal metabolties and a range of enzymes which degrade the cell walls of *S. sclerotiorum* (Hu *et al.,* 2009; Zeng *et al.,* 2011). As *C. minitans* can be spread by infected Sciaridae, a Sciaridae population infected with *C. minitans* may enable more effective control of *S. sclerotiorum* (Whipps and Budge, 1993; Zeng *et al.*, 2011).

Adult Sciaridae are vectors to pathogenic fungus such as *Verticillium albo-atrum* Reinke & Berthold (hypocreales: Incertae sedis) and *Fusarium oxysporum* f.sp. *radices-lycopersici* Jarvis & Shoemaker (Hypocreales: Nectriaceae) (Gillespie and Menzies, 1993; Scarlett *et al.,* 2013).

Monitoring

Monitoring of adult Sciaridae is typically performed using yellow sticky traps. Yellow sticky traps were found to be inaccurate at measuring the population changes of *B. coprophila* (Harris *et al.,* 1995). There are currently no thresholds to determine control implementation in *Bradysia* spp., and the inaccuracy of yellow sticky traps when used to measure population size means they do not provide sufficient information for determining when the apply control measures or to assess their effectiveness (Cloyd, 2008; Harris *et al.,* 1995). It has been suggested that placing the traps close to the soil would be more effective than the usual placement above the canopy (Harris *et al.,* 1995). Yellow sticky traps were found to be more

effective than blue for capturing the Sciaridae *Ctenosciara hyalipennis* (Meigen) (Diptera: Sciaridae), although both trap colours were effective (Górska-Drabi *et al.,* 2011). Larvae can be monitored by taking soil core samples, placing these within a cage, and awaiting adult emergence (Grewal *et al.,* 1993). Alternatively larvae can be floated out of the core samples using a magnesium sulphate solution (Calvert, 1987).

The addition of a green (530 nm) LED to yellow sticky traps increased their effectiveness by 136.6% for capturing *B. coprophila* (Chen *et al.,* 2004b). An attraction to light in the blue/UV region of the spectrum has been previously demonstrated in a related species *Bradysia paupera* Tuomikoski (Diptera: Sciaridae) (Ishitani *et al.,* 1997).

Management

A broad range of management options exist for the control of Sciaridae, including insecticides, insect growth regulators, biopesticides, and biological control agents (Ludwig and Oetting, 2002; Jagdale *et al.,* 2007; Shamshad *et al.,* 2008).

Organophosphate and pyrethroid chemicals are commonly used in Scaridae management strategies, and can be used as spray to target adult flies, or mixed into the soil substrate to target larvae (Shamshad *et al.,* 2008: Shamshad, 2012). The prevalence of these pesticides has resulted in instances of insecticide resistance developing, particularly in the United Kingdom and Canada (Brewer and Keil, 1989; White and Gribben, 1989; Smith and White, 1996). Pyrethoid resistance has been demonstrated to be stable across multiple generations, with *Lycoriella mali* (Fitch) (Diptera: Sciaridae) retaining a 42-fold resistance at LD_{50} over 13 generations (~10 months) without insecticide exposure (Brewer, 1990). The use of chemical pesticides may influence crop production, for example a 30% reduction in yield in mushroom crops has been demonstrated (Cantelo, 1981).

Insect growth regulators are a form of insecticide which may be used in response to the development of resistence to conventional insecticides, or where environmental pollution is a concern (Shamshad, 2012). A range of insect growth hormones have been demonstrated to be effective alternatives to conventional pesticides for example effective control of *Lycoriella ingenua* (Dufour) (Diptera: Sciaridae) was achieved using six different insect growth hormones (diflubenzuron, flufenoxuron, lufenuron, methoprene, novaluron, pyriproxyfen, teflubenzuron, and triflumuron) without a significant loss of *Agaricus bisporus* (Lange) (Agaricales: Agaricaceae) yield (Erler *et al.,* 2011). White (1986) observed an increase in mushroom crop yield when using the commercial rate of diaflubenzuron 30µg g^{-1}

to manage *Lycoriella auripila* (Winnertz) (Diptera: Sciaridae), although a reduction in yield was observed if these rates were increased to 190 and 1080µg g^{-1}.

Entomopathogenic nematodes are an effective, environmentally friendly, alternative to conventional pesticides for Sciaridae management. Predatory nematodes of the families Steinernematidae and Heterorhabditidae are available, with *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) being the most commonly used in Europe (Gouge and Hague, 1994; Jagdale *et al.,* 2004). *S. feltiae* are sprayed onto the soil, where they infect their host larvae via the mouth, anus or spiracles, then enter the haemocoel and release bacteria (*Xenorhabdus* sp.) which causes septicaemia, and eventual death, in the host (Poinar and Thomas 1966). *S. feltiae* has proven effective against both *Bradysia* spp. and *Lycoriella* spp., for example Nickle and Cantelo (1991) demonstrated a 72-81% reduction in *L. mali* when applying 620 nematodes cm^{-2}, and Gouge *et al.* (1995) demonstrated a 92% decrease in adult emergence of *B. paupera* when applying 780, 000 nematodes/m^2.

If applied early in the infestation the predatory mite *Stratiolaelaps scimitus* (formerly *Hypoaspis miles*) (Berlese) (Mesostigmata: Laelapidae) has a comparable success rate to *Steinernema feltiae*, and an application of 700 mites m^{-2} reduced the emergence of *Lycoriella solani* (Winnertz) (Diptera: Sciaridae) by 87% (Jess and Bingham, 2004). Similarly, an application of 55 *S. scimitus* per pot in pot grown cyclamen and poinsettia, greatly reduced the emergence of adult *Bradysia* spp. with no later emergence (Chambers *et al.*, 1993).

Western flower thrips (*Frankliniella occidentalis*)

Physical description and life cycle

Western flower thrips, *Frankliniella occidentalis*, are small, light yellow to dark brown coloured, winged insects of the order Thysanoptera (Fig. 17). An *F. occidentalis* population consists of both males and females; females are slightly larger (1.3 – 1.4mm) than males (1mm) and possess a darker coloured body. Both sexes possess the fringed wings, from which the order is named. The life cycle of *F. occidentalis* is in 6 stages (Fig. 18), with feeding occurring in stages two, three, and six. Stages four and five, while capable of movement, do not feed. The feeding stages can be found amongst the petals, and the underside of the leaves, on infected plants. Reproduction can be sexual or asexual. Fertilised females produce females and males in a 2:1 ratio, while unfertilised females produce only male progeny. This results in a higher proportion of females in an established population (Malais and Ravensberg, 2003; Higgins and Myers, 1992).

Figure 17. *Frankliniella occidentalis* (Sparks and Riley, 2015).

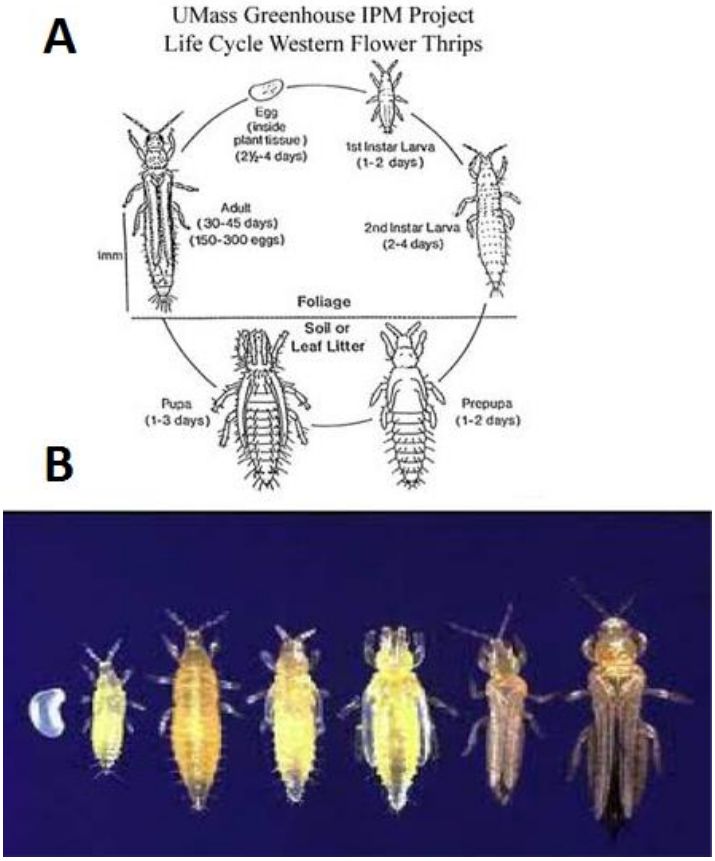

Figure 18. The life-cycle of *Frankliniella occidentalis* (not to scale) (A: The Center for Agriculture, Food, and the Environment, 2012; B: Himmelein, 2011).

Damage

Originating in North America, *F. Occidentalis* has now been spread around the world, most likely due to international trade in ornamental crops. It is considered to be a serious pest species across a wide range of crops, with host-plants within ornamental crops, fruits, and vegetables. *F. occidentalis* causes direct damage to host plants by feeding or ovipositing in developing fruit, as well as by the transmission of topoviruses (Chisholm and Lewis, 1984; Moritz *et al.*, 2004).

F. occidentalis feeds by piercing the plant cells using their mandible, and ingesting the cell contents via their maxillary stylets (Chisholm and Lewis, 1984). The tissue surrounding the point of feeding dies, which creates silver patches on the leaves or petals (Fig. 19). These patches will eventually turn brown. In fruit the cosmetic damage may reduce the commercial grade of the crop, for example short periods of high density *F. occidentalis* have been shown to lower the food grade of sweet pepper and greenhouse cucumber from grade #1 to grade #2 (Shipp *et al.*, 1998; Hao *et al.*, 2002). Although no significant loss of yield was seen in the sweet peppers, glasshouse cucumber yields decreased by ~2.5kg per plant between low and high densities of *F. occidentalis* (Shipp *et al.*, 1998). In addition to this, the tendency of *F. occidentalis* to feed on developing tissues can lead to deformities in the leaves and fruit, and may prevent flower buds from opening. These deformities may make a portion of the crop unmarketable, such as the pig-tail deformity in cucumber crops, which is characterised by the cucumber growing in a curl (Hardgrave, 1993).

Tomato spotted wilt virus (TSWV), of the genus *Tospovirus*, is a globally distributed plant virus which can infect at least 1000 different species of plant (Adkins, 2000; Parella *et al.*, 2003). TSWV causes severe damage to plant hosts in both the leaves, and fruit (Fig. 20). Due to these factors, TSWV is ranked in the top 10 economically important plant viruses, and causes over one billion U.S. dollars in crop damage worldwide annually (Adkins, 2000; Parella *et al.*, 2003). UK specific economic data are not available. TSWV can be transported by at least 8 species of thrips (Mound, 1996); *F. occidentalis* is the most efficient of these, and is considered to be the most important vector of TSWV (Peters, 1998).

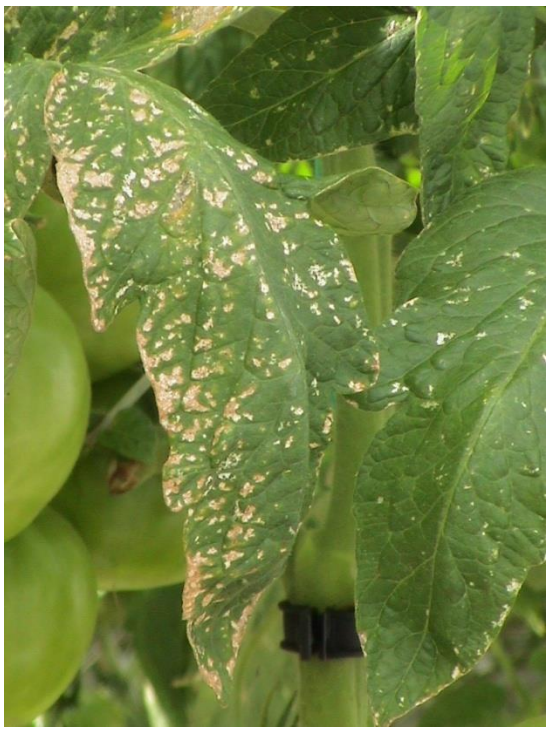

Figure 19. Thrips damage (Pagliarulo and Giacomelli, 2005)

Figure 20. *Tomato spotted wilt* damage in leaves and a tomato (Sherwood *et al.*, 2009).

Monitoring

Monitoring of *F. occidentalis* can be performed using sticky traps.In comparisons between yellow and blue sticky traps, blue has consistently been found superior to yellow for the trapping of *F. occidentalis.* For example Broughton and Harrison (2012) found that blue traps were around twice as effective as yellow traps, and a comparison between twenty different coloured traps by Brødsgaard (2009) found a shade of blue to be the most effective. Hoddle *et al.* (2002) found white to be a more effective colour than blue; however, it should

be noted that the white traps used here reflected strongly across the blue area of the spectrum, with the white trap reflecting ~88% of 480 nm and the blue trap ~60%. Similar results in comparisons between blue and yellow sticky traps have been observed in both field (Chen *et al.*, 2004a) and glasshouse conditions (Gillespie and Vernon, 1990). Matteson and Terry (1992) provided further evidence of *F. occidentalis*' attraction to blue by demonstrating cotton reflecting highly in the blue spectrum attracted more *F. occidentalis* than a range of other colours.

A relationship between the number of *F. occidentalis* captured by sticky traps and their population size has not been established (Shipp *et al.*, 2000), and although an action threshold of 20 *F. occidentalis* adult per blue sticky trap per week was established in a cut carnation (*Dianthus caryophyllus* L.) crop (Cloyd and Sadof, 2003), there are a multitude of factors which may confound this. For example the attractiveness of the crop, *F. occidentalis* population, sticky trap placement, or the use of chemical lures on the sticky traps (Cloyd, 2009; Broughton and Harrison, 2012); furthermore, the susceptibility of the crop to viruses vectored by *F. occidentalis* has been shown to vary thresholds from 10 to 40 adults per sticky trap per week (Frey, 1993; van Dijken *et al.*, 1994). With this in mind growers may be more comfortable setting their own action thresholds gained through experience with their own crops and growing facilities (Cloyd, 2009).

Chu *et al.* (2005) compared a range of wavelengths by releasing *F. occidentalis* at 83cm, and 165cm, from a filtered light source. Of the wavelengths tested UV (369 nm) and UV (398 nm) were found to be vastly more attractive than the other wavelengths, including blue (460 nm). Field experiments compared blue sticky traps against those which were attached with either a UV (398 nm) LED or a Blue (465 nm) LED in three sites. The blue (465 nm) LED significantly increased the number of *F. occidentalis* captured across all weeks of the experiments at all sites. The UV (398 nm) LED equipped traps were less effective, and although significant results were observed, these were sporadic. It should be noted that the blue (465 nm) LED was likely to have a much higher light output (mW) than the UV (398 nm) LED, and as such a direct comparison between these should be viewed with caution. Unfortunately Chu *et al.* (2005) did not provide the output of either LED.

There has also been an attempt at a novel trap design, where a standard yellow sticky trap was compared against a model of a chrysanthemum flower (Mainali and Lim, 2008b). The model trap was found to be very effective, and captured 2.6 times the number of *F. occidentalis* in choice tests. Unfortunately no comparison against blue sticky traps was

made, and given the apparent low effectiveness of yellow sticky traps, when compared with blue, for capturing *F. occidentalis*, the effectiveness of this model needs further assessment.

Sticky traps can be enhanced using chemical attractants, for example the addition of the male aggregation semiochemical Thripline$_{ams}^{®}$ increased the capture of *F. occidentalis* by three times on both yellow and blue sticky traps (Broughton and Harrison, 2012). This chemical is particularly attractive to female *F. occidentalis* (Broughton and Harrison, 2015), suggesting this chemical would be beneficial to mass trapping programmes in established populations, where females typically outnumber males (Higgins and Myers, 1992).

Management

Management of *F. occidentalis* may be achieved using insecticides; however, numerous instances of insecticide resistance have been reported and *F. occidentalis* are sheltered from insecticides by living amongst flower petals (Jensen 2000; Bielza, 2008; Allsopp, 2010; Shan *et al.*, 2012). The prevalence of insecticide resistance in *F. occidentalis* necessitates the inclusion of alternative management methods such as entomopathogenic nematodes, predatory mites, parasitic fungi, mass trapping, and low oxygen treatments (Maniania *et al.*, 2002; Ebssa *et al.*, 2006; Messelink *et al.*, 2006; Bielza, 2008; Weintraub *et al.*, 2011, Liu 2012; Sampson and Kirk, 2013).

Few chemical options are available for *F. occidentalis* management. The organophosphate phoxim was the most effective in a comparison between 36 chemicals, although this suffers from a short half-life (Shan *et al.*, 2002); however, this chemical is now banned in the EU (Directive 2007/166/16/EN). Chemical mixtures have also proven effective, although a combination of spinosad and bifenazate appeared to be antagonistic (Willmott *et al.*, 2013). Care must be taken to avoid the development of resistance when managing *F. occidentalis* using chemical pesticides, and the three-spray strategy has been recommended to minimise the development of insecticide resistance (Herron and Cook, 2002). This strategy involves three sprays of an insecticide 3-6 weeks apart, followed by a repeat of this cycle with an insecticide from a different chemical group if required.

Predatory mites are available in the UK, and have been shown to be highly effective for controlling *F. occidentalis* (Kutuk, *et al.*, 2011; Weintraub *et al.*, 2011). For example, in laboratory experiments Kutuk found that a single release of 50 *Amblyseius swirskii* adults per m^2 were able to maintain populations of less than 2 *F. occidentalis* per flower in pepper plants. The successful combination of predatory mites and entomapathogenic nematodes has

also been reported, with the application of 10 adult *Neoseiulus cucumeris* (Oudemans) (Phytoseiidae: Acari) (formerly *Amblyseius cucumeris)* and the application of 200 infective juvenile *Heterorhabditis bacteriophora* Ponar (Rhabditida: Heterorhabditidae) cm^{-2}, resulting in 83% reduction in an adult *F. occidentalis* population (Ebssa, 2006).

Entomopathogenic fungi are an effective means of *F. occidentalis* control, and may aid in preventing the build-up of insecticides resistance by reducing selection pressure (Maniania *et al.*, 2002). Maniania *et al.* (2002) reported a 72% reduction in larval and adult stages when *Metarhizium anisopliae* (Metsch) (Hypocreales: Clavicipitaceae) was applied in a soil drench, similarly Vestergaard *et al.* (1995) demonstrated a 94% mortality rate at 7 days post-inoculation in adult thrips infected with *M. anisopliae*, and 20-70% in those infected with *Verticillium lecanii* (Zimmerman) (Hypocreales: Clavicipitaceae). While *M. anisopliae* is not available to buy in the UK, it has been approved for use in Europe (Bio-Pesticides DataBase 2016).

Glasshouse Whitefly (*Trialeurodes vaporariorum*)

Physical description and life cycle

Trialeurodes vaporariorum are small, beige bodied flies with white wings of the Hemiptera order (Fig. 21). *T. vaporariorum* populations consist of both males and females, with females being slightly larger (1.1mm) than males (0.7mm). The life cycle of *T. vaporariorum* has six stages; the egg, larvae (four instars), and the adult. Eggs attach to the underside of leaves by hooks. First stage larvae are mobile, enabling them to disperse. Second and third stage larvae are immobile and transparent. On progression to the fourth stage larva, development of genitalia and the re-growth of legs occur, after which the adult form emerges (Fig. 22) (Malais and Ravensberg, 2003).

Figure 21. *Trialeurodes vaporariorum* adult (Dem, 2010).

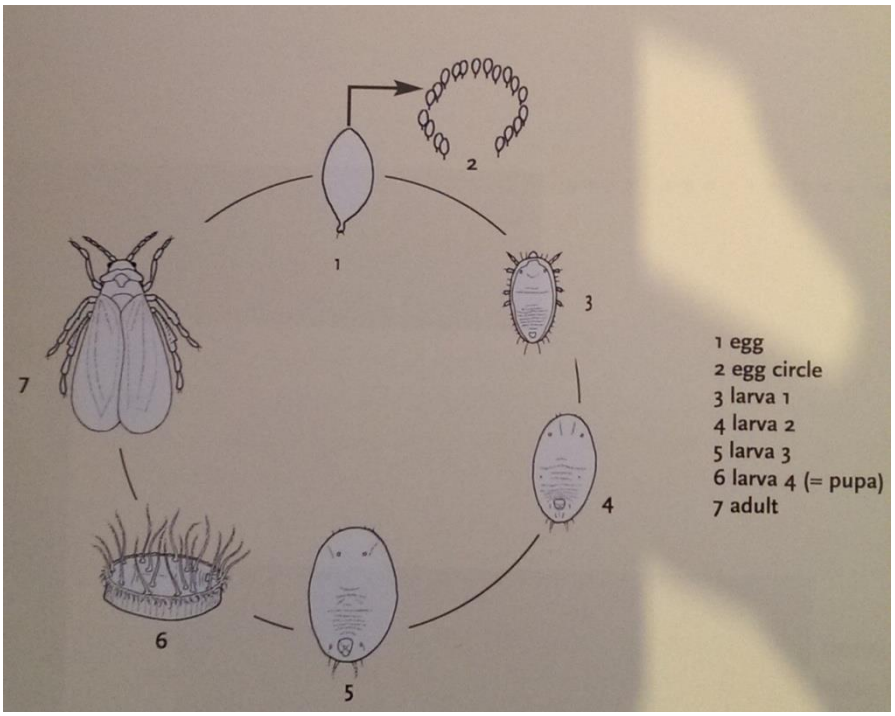

Figure 22. *Trialeurodes vaporariorum* life cycle (not to scale) (Malais and Ravensberg, 2003).

Damage

Thought to have originated in the tropical or subtropical Americas, *T. vaporariorum* is now a serious pest worldwide (van Lenteren and Noldus, 1990; Byrne and Bellows, 1991; Bi *et al.*, 2002; Chio and Kim, 2004), causing damage to a broad range of crops including tomato, strawberry, peppers, and raspberry (Bi *et al.*, 2002). The estimated damage of *T.*

vaporariorum in British Columbia in Canada is $3-4 million per year (Moreau and Isman, 2010). *Trialeurodes vaporariorum* specific UK economic loss data are not available, but insecticide cost in protected cropping for invasive insect species is estimated at £40,408 in ornamentals, and £50,694 in edibles. A portion of this cost is attributed to *T. vaporariorum;* though the specifics are not available (Williams, *et al.* 2010). *Trialeurodes vaporariorum* causes damage directly and indirectly. Direct damage is caused by feeding damage (van Lenteren and Noldus, 1990; Byrne and Bellows, 1991). Indirect damage is caused by the excretion of honeydew, which sticks to the leaves providing a surface for mould to grow, or the spread of disease (van Lenteren and Noldus, 1990; Morales and Jones, 2004).

T. vaporariorum feeds on plant sap and, in large numbers, can retard plant growth and cause aesthetic damage. It has been suggested that the effects of feeding by *T. vaporariorum* in Hawaii can cause a loss of 5% of a tomato crop over a period of 70 cumulative days of *T. vaporariorum* infection (Johnson *et al.,* 1992). The economic damage caused by the growth of sooty mould on the excreted honeydew was estimated to have less of an economic impact, resulting in a 5% loss of 300 cumulative days of *T. vaporariorum* infection (Johnson *et al.,* 1992). A 20-25% loss of strawberry yield was reported in California (California Strawberry Commission, 2003) with subsequent experiments estimating a loss of ~80g/plant 20 weeks after planting on an untreated field with an average whitefly population (McKee *et al.,* 2007).

T. vaporariorum can also act as a vector for a number of plant viruses. For example, *Tomato chlorosis virus* induces interveinal yellowing and necrosis in a range of important crop and ornamental species, e.g. tomato (*Lycopersicon esculentum* Mill (Solanales: Solanaceae)) and petunia (*Petunia hybrid* (Hook) (Solanales: Solanaceae)) (Duffus *et al.,* 1996; Wisler *et al.,* 1997). Additional viruses spread by *T. vaporariorum* include *golden mosaic virus* and *beet pseudo yellows virus* (Morales and Jones, 2004; Boubourakas *et al.,* 2006). There are no current experimentally determined reports on economic or yield loss on these viruses, although concern has been expressed for their potential impact (e.g. Zhao *et al.*, 2013).

Monitoring

Yellow sticky traps are an effective monitoring tool for *T. vaporariorum* within glasshouses (Gillespie and Quiring, 1987; Gillespie and Quiring, 1992; Heinz *et al.,* 1992; Moreau and Isman, 2010), and are effective at restricting *T. vaporariorum* population growth in early stages of infection (Dowell, 1990). *Trialeurodes vaporariorum's* attraction to yellow has

been attributed to their laying eggs on the underside of young leaves, which are more yellow (from the human perspective) than mature leaves (Ekbom and Rumei, 1990). While this may be the case, the super-normal foliage stimulus suggested by Prokopy and Owens (1983), should also be considered, 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 is supported by *T. vaporariorum's* preference for a green (526 nm) LED when compared with a range of wavelengths, including yellow (570-580 nm), to which *T. vaporariorum* showed very little attraction (Jahan *et al.*, 2013).

The reliability of yellow sticky traps for estimating the population density of *T. vaporariorum* is uncertain, and while Kim *et al.* (1999) found the yellow trap catches correlate with density up 1 trap per 50 m^2, Gillespie and Quiring (1987) found a correlation at just 7 m^2. This would suggest an action threshold developed around sticky traps would be unreliable, as such thresholds are not used when controlling this pest. As biological control agents are generally favoured over chemical control measures for *T. vaporariorum* in Europe (van Lenteren, *et al.*, 1996; Perdikis and Lykouessis, 2000) (see page 68 for more details on *T. vaporariorum* management), growers may follow preventative release protocols of biological control agents and monitor, progressing to curative if necessary, then reducing or stopping the release of biological control agents once a certain success threshold is released. For example, *Encarsia formosa* parasitism levels have been correlated with the number of *E. formosa* captured by yellow sticky traps (Webb and Smith, 1980; Vande Veire and Vacante, 1984).

Attempts to enhance the effectiveness of yellow sticky traps for capturing *T. vaporariorum* have met with some success. Chu *et al.* (2004) found that attaching a green LED (530 nm) increased the number of *T. vaporariorum* captured, with LED equipped traps capturing 31% more than standard yellow sticky traps. Stukenberg *et al.* (2015) found green (517 nm), UV (368 nm), and the combination of the two to increase the capture of *T. vaporariorum*, although this was in small (1.6x1.1x.19m) gauze cages, and rates may have been positively influenced by the initial close proximity of the light source, as has been demonstrated in other species (Chu *et al.*, 2005). It has also been demonstrated that in conditions where UV is the only light source available, yellow sticky traps closest to the light source captured more *T. vaporariorum* (Mutwiwa and Tantau, 2005). It should be noted that as UV was the only light source available, this does not demonstrate an enhancement of yellow sticky traps using UV light. In the absence of additional wavelengths, the sticky traps used in the study are

effectively UV traps; furthermore, this experiment demonstrates only that *T. vaporariorum* will preferentially congregate on surfaces reflecting more strongly in the UV spectrum when no other wavelengths are present, this does not provide evidence that UV light is effective for attracting *T. vaporariorum* in glasshouse conditions.

Natural oils have been successfully used to increase the effectiveness of yellow sticky traps for capturing *T. vaporariorum*, with sandalwood oil, basil oil, and grapefruit oil increasing the number of *T. vaporariorum* captured by 487.64%, 483.20%, and 333.09% respectively (Górsk, 2004).

Management

Trialeurodes vaporariorum are typically managed using a combination of pesticides and biological control agents (van Lenteren *et al.,* 1996), although there has been suggestion of mass trapping using the novel CC trap in co-ordination with the parasitoid *Encarsia formosa* may enable pesticide free management in the related species tobacco whitefly (*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)), and *Bemisia argentifolii* (Chu *et al.,* 2000; Chu *et al.*, 2003).

Relatively few chemical compounds are available for the control of *T. vaporariorum*, and a history of insecticide resistance developing in *T. vaporariorum* and *B. tabaci* necessitate careful use of insecticides in whitefly control, preferable as a supplement to biological control in instances of high whitefly population (Gorman *et al.,* 2002; Quesada-Moraga *et al.*, 2005; Gorman *et al.*, 2007; Erdogan *et al.*, 2008; Yuan *et al.,* 2012).

Encarsia formosa and *Macrolophus* spp. are the most commonly used biological control agents of *T. vaporariorum* within Europe (van Lenteren, *et al.,* 1996; Perdikis and Lykouessis, 2000), with *E. formosa* being considered more effective for management of *T. vaporariorum* than chemical insecticides, without the risk of resistance developing (van Lenteren, *et al.,* 1996). The presence of other pest species can influence the effectiveness of biological control agents via apparent competition, an indirect interaction between two prey species which share a common predator, where the predator's population increases in response to the abundant food source, leading to an increase in predation pressure (Messelink *et al.,* 2008). While ordinarily ineffective for controlling *T. vaporariorum,* the predatory mite *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) is an extremely effective control measure when thrips are present, eliminating almost all *T. vaporariorum* over a 10 week period in a glasshouse growing cucumber (Messelink *et al.*,

2008). This is believed to be due to higher juvenile development and survival rates in *A. swirskii* with a mixed diet, and the presence of thrips allows the *A. swirskii* population to grow to a size where effective control of .*T vaporariorum* is achieved (Messelink *et al.,* 2008). This interaction emonstrates the need to consider interactions, direct and indirect, between pests and biological control agents when developing a pest management system (Messelink *et al.,* 2008).

Diamondback moth (*Plutella xylostella*)

Physical description and life cycle

Diamondback moths (*Plutella xylostella*), are small greyish-brown moths (Fig. 23). *Plutella xylostella* have a typical Leipodopteran life cycle consisting of an egg, caterpillar (four instars), pupae, and adult stages (Fig. 24). Eggs are ~1mm in length and are typically laid along the veins on the top and underside of leaves, at a ratio of 3:2 respectively. The caterpillars go through four instars, reaching ~12mm in length before pupating after 10-28 days depending on the temperature (Hsu and Wang, 1971; Bhalla and Dubey, 1986; Sarnthoy *et al.,* 1989). Pupae are ~6mm in length and are attached to leaves via a mesh cocoon, with pupal period varying between 4-15 days depending on temperature (Lu *et al.,* 1984; Hoy, 1988). The adult moth is ~8mm long with a wingspan of ~13mm. The adult lifespan is around 16 days, with adult females laying around 160 eggs during this period (~10 per day) (Talekar, 1993).

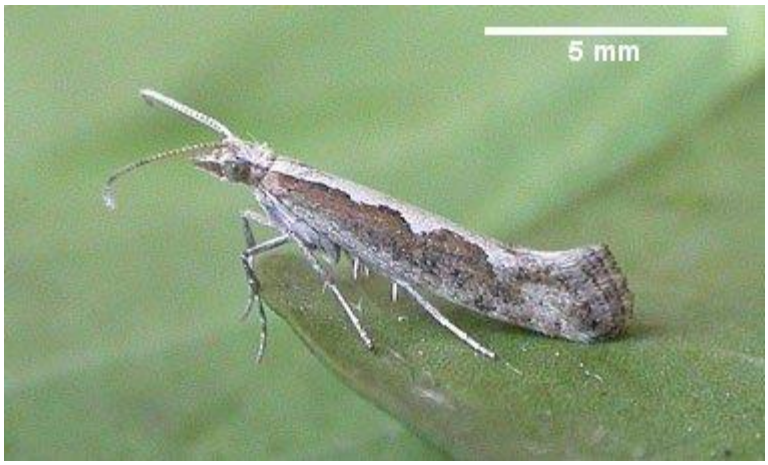

Figure 23. *Plutella xylostella* adult (Kitchener, 2015)

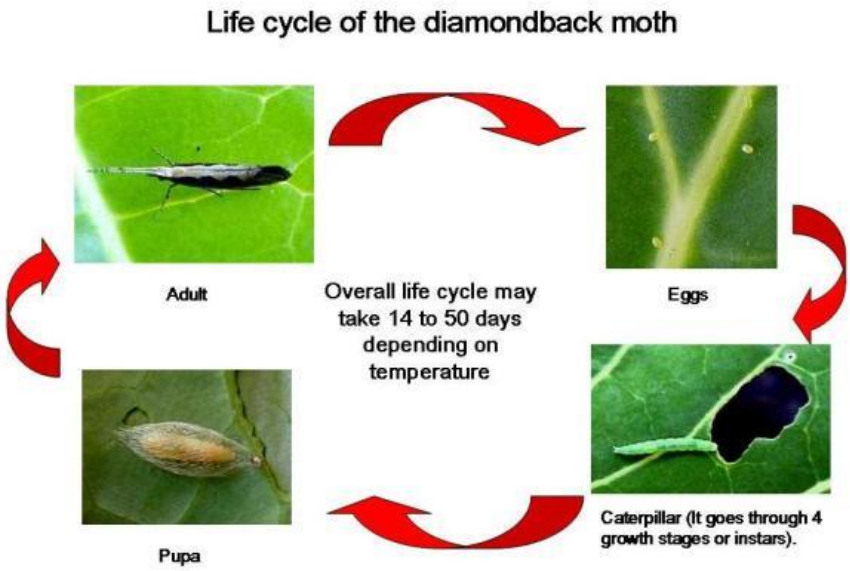

Figure 24. *Plutella xylostella* life cycle (not to scale) (Varela, 2015)

Damage

While there is no UK specific data is available *P. xylostella* is the most destructive insect pest of cruciferous plants worldwide (e.g. cauliflower, cabbage, and broccoli), capable of causing complete crop destruction when insecticides are not applied (Macharia *et al.*, 2005). In 1993 it was reported that *P. xylostella* required an estimated U.S. $1billion worldwide in management costs annually, and a more a recent report estimates this has risen to U.S. $4-5 billion (Javier, 1992; Talekar and Shelton, 1993; Zulucki *et al.*, 2012). The cost of damage to crops can be severe, with a severe infestation in California in 1997 resulting in an estimated loss >U.S. $6 million (Shelton *et al.*, 2000). In Canada an estimated CN $50 million was used to spray 1.25 million ha of canola crops during a *P. xylostella* outbreak in Canada (Dosdall *et al.*, 2004). In Australia, the estimated damage and control costs of *P. xylostella* on 136,000 ha of cruciferous plants are $A 8 million and $A 12 million respectively. Furthermore, it has been suggested that if crops in Texas were left untreated, 100% of cabbage and 20% of broccoli would be unmarketable, translating to losses of U.S. $40-70 million for cabbage and U.S. $400,000 for broccoli (Shelton, 2004). This assertion is supported by losses of 99% in 1992 and 80% in 1994 of yield in summer cabbages in China when no *P. xylostella* insecticides were applied, when compared with insecticide treated-plots (Zhao *et al.*, 1996).

Plutella xylostella are capable of migrating long distances, enabling them to spread to newly planted crops, an ability their natural enemies appear to lack (Bretherton**,** 1982; Talekar and Shelton, 1993; Xing *et al.,* 2013). The result of this is that effective natural enemies are not present in many locations inhabited by *P. xylostella*; furthermore, they possess a phenotypic plasticity, which is defined as 'the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions' (Pigliucci *et al.* 2006). This plasticity facilitates the rapid adaptation to pesticides (Mohan and Gujar, 2003) and they were the first species of crop pest to develop a resistance to DDT (Asakawa, 1975).

Damage is caused by caterpillar feeding, with first instar caterpillars burrowing into the leaf and feeding on the inside (Fig. 25). The remaining instars are surface feeders (Fig. 26). Adult moths feed on water droplets and dew, and do not typically cause damage to crops (Fleischer, 2003).

Figure 25. Feeding damage caused by first instar *P. xylostella* caterpillar (Rowell, 2004)

Figure 26. Feeding damage caused by 2nd-4th instar *P. xylostella* caterpillar (Sparks and Riley, 2015)

Monitoring

Monitoring is typically performed using sex pheromone traps to capture adult males (Chishold *et al*., 1983), for example a pest monitoring network in the Canadian Prairie provinces makes use of pheromone based traps (Miluch *et al.*, 2013). The effectiveness of these traps as an estimator of population density of larval stages is unclear, and relationships between moth catch and larval density are typically infrequent (Walker *et al.* 2003; Campos, *et al.*, 2006; Miluch *et al.*, 2013). While sex ratios in established *P. xylostella* populations are close to 1:1, in migrating populations the sex ratio is not known, and as the number of female moths would typically be the limiting factor in reproduction a high prevalence of male moths would not necessarily imply a high density of larvae (Miluch *et al.*, 2013). Additionally migrating males may be less responsive to sex pheromones, leading to an underestimation of population in migrating populations (Miluch *et al.*, 2013).

In comparisons between sticky trap colours Silvapragasm and Saito (1986) found yellow to be the more effective colour when compared with blue, red, and clear. As with pheromone traps a higher proportion of males were captured despite there being a larger number of females in the study group (128 males: 172 females), suggesting either lower activity levels (Harcourt, 1963), or a lesser influence of the visual stimuli on their behavior than exhibited by males. Hallett (1986) found that yellow sticky traps placed within or at the crop canopy captured significantly more *P. xylostella* than those placed above the crops (mean (±SE)

1.8±0.8 and 0.7±0.4 respectively). Horizontally placed traps captured more (3.4±0.9) than the traditional vertical trap placement (north-facing: 0.3±0.2; south –facing: 0.0±0.0). These results suggest that the traditional placement of yellow sticky traps renders them ineffective as a monitoring tool for *P. xylostella*.

Light traps have been used with some success (Harcourt and Cass, 1958), and more recently the spectral preference of *P. xylostella* to a selection of LEDs was investigated (Cho and Lee, 2012). Adult *P. xylostella* were introduced to a linear chamber with light being projected into one side, giving a "dark" side and a "light" side. Individuals were exposed to the light source for a 15 minute period, after which their position in the chamber was recorded. Of the LEDs tested green (520 nm) and UV (365 nm) were found to be most effective.

Management

Management of *P. xylostella* is usually achieved using chemical pesticides; however, high levels of chemical resistance have prompted the development of strategies which make use of biological control agents (Talekar and Shelton, 1993; Sarfraz *et al.,* 2005). As *P. xylostella* are a migratory species, capable of migration over long distances, developed chemical resistance may spread between populations in different countries, and insecticide resistance may be unpredictable (Talekar and Shelton, 1993; Chapman et al., 2002; Shortall and Foster, 2016).

In 1953 *P. xylostella* became the first crop pest insect to be reported as resistant to DDT in Java, Indonesia (Ankersmit, 1953). Since then *P. xylostella* has developed resistance to almost all insecticides (Sarfraz *et al.,* 2005; Hu, *et al.,* 2014; Steinbach *et al.,* 2015). *P. xylostella* is able to retain insecticide resistance over multiple generations with no contact with the chemical, and has demonstrated stable resistance ratios to diamides chemicals after almost 4 years (Steinbach *et al.,* 2015).

Over 135 parasitoids are known to target *P. xylostella*, at various stages in their life cycle (Delvare, 2004). For example, *Diadegma insulare* (Creddon) (Hymenoptera: Ichneumondidae), a larval endoparasitoid, can parasitise 70%-90% on *P. xylostella* larvae on average and causes a 35-80% reduction in food consumption in parasitised larvae (Mukenfuss *et al.,* 1992; Mitchell *et al.,* 1997; Monnerat *et al*., 2002). Although *D. insulare* can be reared in a *P. xylostella* infected glasshouse (Xu *et al.,* 2001), it has not been possible to rear them in captivity, making them unsuitable for commercial application (Sieglad*d et al.,* 1998, Johanowicz and Mitchell 2000). Despite the apparent effectiveness of these

parasitoids and predators, management of *P. xylostella* using these methods has proven ineffective (Sarfraz *et al.,* 2005). This is likely because *P. xylostella* possess greater resistance to the insecticides used in their management, and are able to better survive the chemical treatments used in their management, when compared against their biological control agents. For example, while laboratory bioassays have demonstrated that *D. insularei* is able to increase its resistance to some pyrethroids, this build up is limited and develops more slowly than *P. xylostella* (Xu *et al.,* 2001; Shelton, 2004).

Viruses, bacteria, nematodes, microsporidia, and fungi are also available for *P. xylostella* management, some of which have demonstrated promising effects. For example, the microsporidum *Vairimorpha* sp was able to cause 100% mortality in larvae at $1.5x10^3$ spores per larva (Harque, *et al.,* 1999) and an insecticide spray applied at a rate of 3×10^6 conidia mL^{-1} containing the fungi *Beauveria bassiana* causes 100% mortality after 3-7 days (Furlong, 2004).

Encarsia formosa

Physical description and life cycle

Encarsia formosa (Fig. 27) is a commercially available whitefly parasitoid. These small wasps have a life cycle consisting of six stages consisting of the egg, larvae (four instars), pupal instar, and adult. The egg is ~0.08mm long and ~0.03mm wide. Female wasps deposit their eggs into the larval stages of whitefly, preferentially selecting the third or fourth stage. The parasite will develop inside the whitefly, and parasitised whitefly will turn black during the pupal stage. Adult *E. formosa* will emerge from the pupal stage of the whitefly, and are ~0.6mm in length. Adult females can deposit ~5 eggs per day, and a total of ~59.2 over their lifetime (Hoddle, *et al.,* 1998; Malais and Ravensberg, 2003) (Fig. 28).

Figure 27. *Encarsia formosa* adult (Cappaert, 2014).

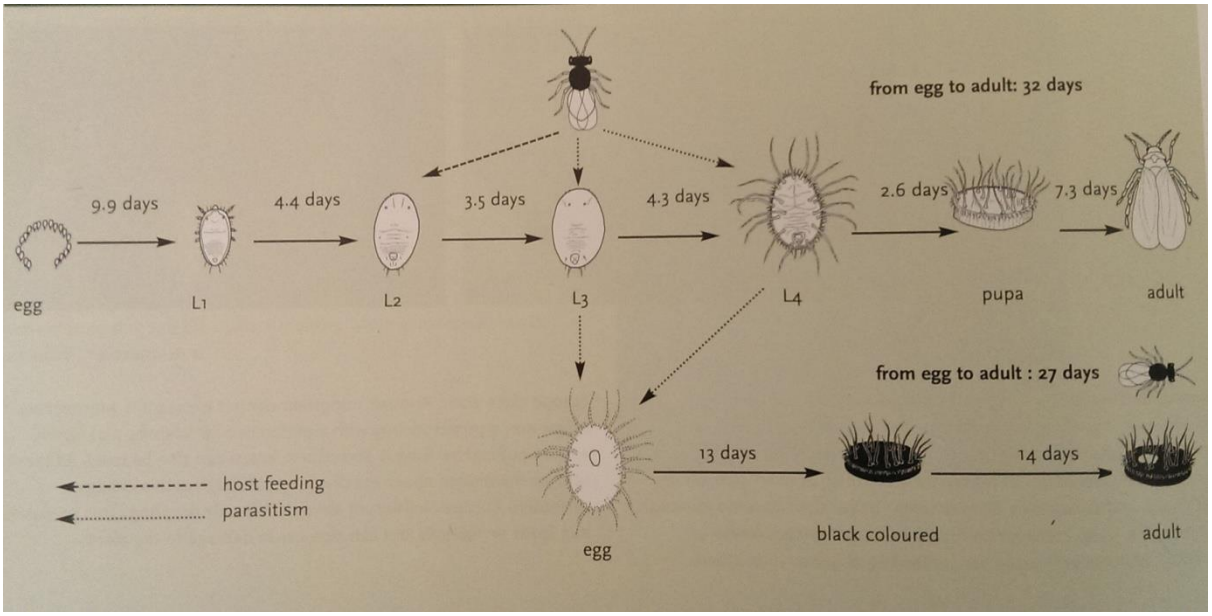

Figure 28. *Encarsia formosa* lifecycle (not to scale) (Malais and Ravensberg, 2003).

Use of *Encarsia formosa* as a biological control agent

Encarsia formosa is an effective biological control agent of a range of whitefly species, including glasshouse whitefly (*T. vaporariorum*), tobacco whitefly (*B. tabaci*), and silverleaf whitefly (*B. argentifolii*) (Hoddle *et al.*, 1997a; Hoddle *et al.*, 1997b; Malais and Ravensberg, 2003). Growers consider *E. formosa* to be more effective for the control of whitefly than chemical pesticides, and it has been estimated that in Western Europe *E. formosa* is used on around 4000 ha (van Lenteren, *et al.*, 1996). An advantage to using *E. formosa* in protected cropping when compared with chemical control methods, is the ability

to maintain a population by releasing *E. formosa* at regular intervals, enabling an immediate response by this biological control agent to the presence of a pest (Hoddle and Van Driesche, 1996; Hoddle, 1997).

Populations consist almost entirely of females, and reproduction is achieved via parthenogenesis. *Encarsia formosa* are active between ~12°C-38°C, and will operate optimally at around 25°C (Malais and Ravensberg, 2003). *Encarsia formosa* appear to locate their host by randomly searching for whitefly signs (e.g. life whitefly, dead hosts) (van Lenteren *et al.*, 1996). Although there is some evidence of attraction to the honeydew excreted by the whitefly (Hussey *et al.*, 1976), a more recent study has concluded that they are unable to detect infected plants from a distance (van Roermund and van Lenteren, 1994). Once a whitefly larvae is detected the *E. formosa* female will either oviposit or feed on the larvae. If the larva has been previously parasitized *E. formosa* is highly unlikely to parasitise it (Malais and Ravensberg, 2003). *Encarsia formosa* use has been demonstrated to be compatible with yellow sticky traps (Dowell, 1990).

There are occasions where *E. formosa* will be ineffective at controlling whitefly. The more common reasons for this are; 1. The intrinsic rate of increase of whitefly is too high and *E. formosa* are unable to parasitise the population quickly enough. This may occur where the host plants provides excellent resources for the whitefly, and although it may be possible to overcome this by releasing a larger number of *E. formosa*, this is unlikely to be cost effective; 2. *Encarsia formosa* mobility are restricted (e.g. hairs on cucumber leaves slow down the walking speed of *E. formosa*), reducing the number of whitefly they are able to parasitise per day; 3. Chemical pesticides may harm *E. formosa* (van Vianen and van Lenteren, 1986; van Lenteren *et al.*, 1996).

Cost is a consideration in any management strategy, and a cost-benefit analysis by Stevens *et al.* (2000) reported the cost of an *E. formosa* management strategy to be >300% greater than using chemical pesticides. This was based on the assumption that *E. formosa* effectiveness was comparable to the chemical pesticide treatments (Stevens *et al.*, 2000). It should be noted that cost is not the only consideration, and as there are relatively few chemical pesticides available for whitefly management, a system relying solely on pesticides is discouraged (Gorman *et al.*, 2002; Quesada-Moraga *et al.*, 2005; Gorman *et al.*, 2007; Erdogan *et al.*, 2008; Yuan *et al.*, 2012).

Kleidotoma psiloides

Kleidotoma psiloides Westwood (Figitidae: Eucoilinae) is a naturally occurring parasitic wasp of shore flies (family: Ephydridae) (Fig. 29). Very little is known about this parasitoid, and it is not currently commercially available. Tilley *et al.* (2011) suggested this species may be of minor importance for the control of shore fly when compared against the parasitoid *Aphaereta debilitate* Morlay (Hymenoptera: Braconidae)*;* however, this was purely based on the relative populations at each of their eight sites, a factor which can be influenced by many variables.

There is anecdotal evidence suggesting that *K. psiloides* is exhibits a strong positive behavioural response (i.e. A change in the behaviour of the insect (e.g. arrested flight) which results in the insects moving closer to the visual stimulus) to the colour yellow; for example a grower (Pers comm grower*) in England found that large numbers of *K. psiloides* would fly near, and land on, their children's yellow plastic bucket and spade at the seaside. This suggests that, at a site with a high population of *K. psiloides*, yellow sticky traps may be detrimental to their use as a control for shore fly.

*Grower name withheld for privacy.

Figure 29. *Kleidotoma psiloides* (Stho002, 2012).

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Chapter 5 Comparison between LED Equipped and Standard Yellow Sticky Traps

Abstract

Sticky traps are common used in commercial horticulture to monitor insect pest populations. Typically coloured yellow or blue, these traps rely primarily on their visual attraction to pests. These traps can be enhanced with the addition of light-emitting diodes (LEDs), for example in research performed by others, the addition of a green (530 nm) LED to yellow sticky traps increased the number of *Bradysia coprophila, Bemisia tabaci* , and *Frankliniella occidentalis* compared with standard yellow sticky traps.

Comparisons between standard yellow sticky traps and those equipped with green (540 nm) or blue (480 nm) LEDs were carried out at four commercial growing facilities. Green (540 nm), and blue (480 nm) LED equipped traps captured significantly more dark-winged fungus gnats (*Bradysia difformis* Frey (Sciaridae: Diptera)) and diamondback moths (*Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae)) than those without.

The addition of LEDs to yellow sticky traps enhanced their capture efficiency for some key pests in commercial protected crop growing environments, and has the potential to enable pest detection at an early stage, consequently optimising the timing of pest management options.

Introduction

Sticky traps are the most common used insect trap, and rely primarily on their visual attraction to pests (Vernon and Gillespie, 1990), and are commonly commercially available in yellow or blue. 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), although this does not fully account for this preference for yellow (see chapter 1, Sticky Traps). Rectangular shaped sticky traps are the most commonly used shape, although other shapes have been found effective these are rarely used in practice (see chapter 1, Sticky Traps).

From discussion with UK growers, it is atypical for commercial growers to use blue traps, and none of the partner, or potential partner, sites used blue sticky traps. This is because blue sticky traps capture a much narrower range of pests then yellow (Byrne *et al*., 1986; Vernon and Gillespie, 1990; Moreau and Isman, 2011), while being more attractive to some beneficial insects (Broughton and Harrison, 2012) (see chapters 1, Sticky Traps, and chapter 2).

The capture efficiency of yellow sticky traps can be improved with the addition of an active light source. For example Chen *et al.* (2004) increased the capture of dark-winged fungus gnat, sweet potato whitefly, and western flower thrips by equipping a yellow sticky traps with a green LED (no wavelength given). Similarly, Nakamoto and Kuba (2004) increase the capture of *Euscepes postfasciatus* using a green (536 nm) LED. The unintended increase in the capture of beneficial insects is a possibility which must be considered, and one which may have a negative impact pest management (Chen *et al.*, 2004b) (see chapter 1, Sticky Traps).

Chen *et al.* (2004b) compared standard yellow sticky traps against those equipped with green LEDs, for capturing a broad range of species in a greenhouse growing poinsettia. The difference in capture between the two trap types was much larger for *Bradysia coprophila,* and rove beetles (Staphylinidae), between June and August. This may be an indication of seasonal behaviour (Tauber and Tauber, 1981), with these species being more active, perhaps for reproduction, during these months. This may also be due to the combination of the green LED and brighter sunlight during the summer months reflecting from the traps, passing some critical point which alters the behaviour of these species. An alternative

explanation may be that the large number of *B. coprophila* captured by both trap types covered the attractive surface area of the yellow sticky traps. This would reduce the attractiveness of the traps; however, the green LED equipped traps would retain the qualities of the green LED which produce a positive behavioural response, and a larger difference in capture would result. As the research for this thesis covered multiple species where this difference was not observed, including *Bemesia tabaci* which has a known attraction to green LEDs (Chu *et al.*, 2003; Chu *et al.*, 2004), this does not seem likely.

Materials and Methods

Experimental Design and LED Attachment Specifications

Yellow sticky traps (10x25cm) equipped with LED attachments were compared against those without at six sites (Table 4, Fig. 30). The sticky traps used at Sites 1, 2 and 3 were Oecos branded. Oecos yellow sticky traps had glue on both sides, which was covered by waxy paper. This paper is removed when the trap is in use. Sites 5 and 6 were Horiver branded and supplied by Koppert, these had glue on both sides and no wax paper covering.

LED attachments consisted of LEDs soldered to 0.2mm equipment wire, which were held in place in a terminal block by screws. The stripped wire was covered by heat shrink tubing to provide water resistance. These were powered by either four D cell batteries or via a 9V ac/dc mains adaptor depending on the site. An appropriate resistor was selected according to Ohm's law (Formula 1, see page 96). The attachments were clipped into the traps using curling clips which were fixed into the terminal block using screws. In sites which operate overhead irrigation, or misting, battery packs were suspended within plastic containers (Fig. 31), a silica satchel was included to reduce humidity. Attachments were permanently switched on, and the batteries were changed every seven weeks. Three different types of LED were used, and the power output of the LEDs was determined by Professor John Allen (St Andrews University) (Table 5).

LED attachment placement was standardised to be in the mid-point of the trap (vertically), although some slippage over time is expected due to the weight of the attachment. Growers were shown this positioning by the researcher. Where one side of the trap was used at a time, when the trap side was changed, the trap was turned so the LED attachment remained on the sticky side of the trap facing in the same cardinal direction.

Traps were integrated into the existing pest monitoring procedures of the study site, with the intention of limiting the additional work on the part of the grower, with the exception of trap density, which was increased for these experiments. The aims of this were to make taking part in this study as convenient as possible for the growing, with the hope they were be more inclined to take part in future research. It was also thought that the growers were more likely to follow existing procedures accurately, rather than take on a new procedure specifically for traps being used in this research. With this in mind blue sticky traps were not used, as the growers expressed a preference for yellow sticky traps. While this is a disadvantage for capturing thrips (Gillespie and Vernon, 1990; Brødsgaard 2009; Broughton and Harrison 2012), it provided the opportunity to determine whether equipping yellow sticky traps with blue (480 nm) LEDs may increase the number of *Trialeurodes vaporariorum* captured.

Initially the experimental design was randomised; however, this caused some confusion with the growers, and it was decided that a simpler layout would reduce the chance of mistakes being made. With this in mind a paired treatment layout was used, and traps were paired together (i.e. each pair contained one LED equipped trap, and one standard trap along side one another) (Appendix 2). While this layout A paired treatment design also ensured good coverage of the crop, and avoided clusters of either standard or LED equipped traps. Randomisation reduces bias in the experimental design and increase the reliability of statistical inferences, and the disadvantage to a paired treatment design is the potential introduction of bias (Dythan, 2011; Quinn and Keough, 2011). Paired treatments have previously been used by others in LED equipped sticky trap comparisons (Chen *et al.*, 2004b; Nakamoto and Kuba, 2004). Replications were chosen based on the room available in each glasshouse, and the number of traps each grower was amenable to having placed.

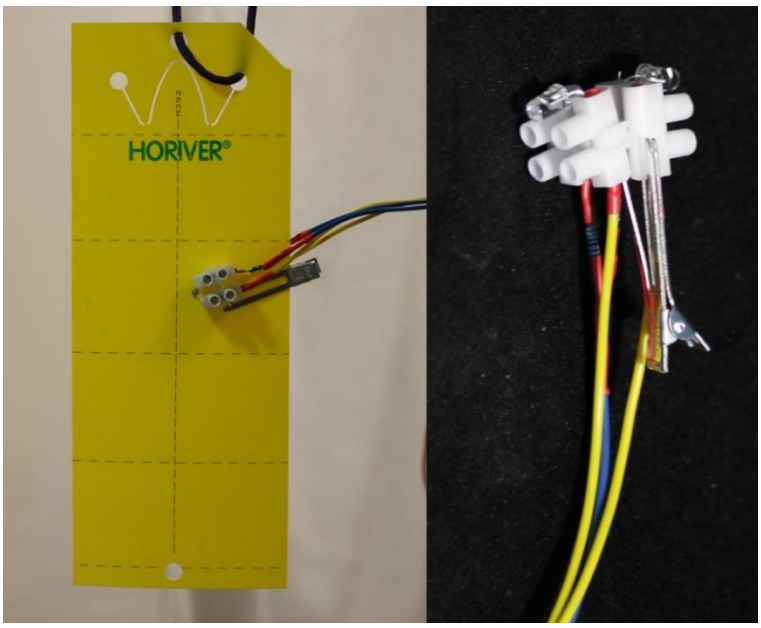

Figure 30. LED attachment.

Table 4. Locations of study sites and crops

Site Number	*Crop*	*Location*
Site 1	Poinsettia	Stratford-upon-Avon
Site 2	Variety of herbs, e.g. basil, thyme	Littlehampton
Site 3	Poinsettia	West Sussex
Site 4	Mint	Dundee
Site 5	A variety of flowering plants which were changed frequently	Edinburgh
Site 6	A variety of Scottish wild flowers	Edinburgh (SRUC)

Power source voltage = 3 V

LED voltage drop = −2 V

Desired current = 25 mA

R = V÷I

V = 3 V−2V

V = 1

1 V÷25 mA = 0.04

0.04×1000 = 40OΩ

A 40Ω resistor would be appropriate for this set up.

Formula 1. Example formula demonstrating the appropriate selection of a resistor for driving an LED with −2 V forward voltage with a current of 25 mA, via a 3 V power source. V = power source voltage minus the forward voltage of the LED, I = desired current to drive the LED, R = resistance, Ω ohms.

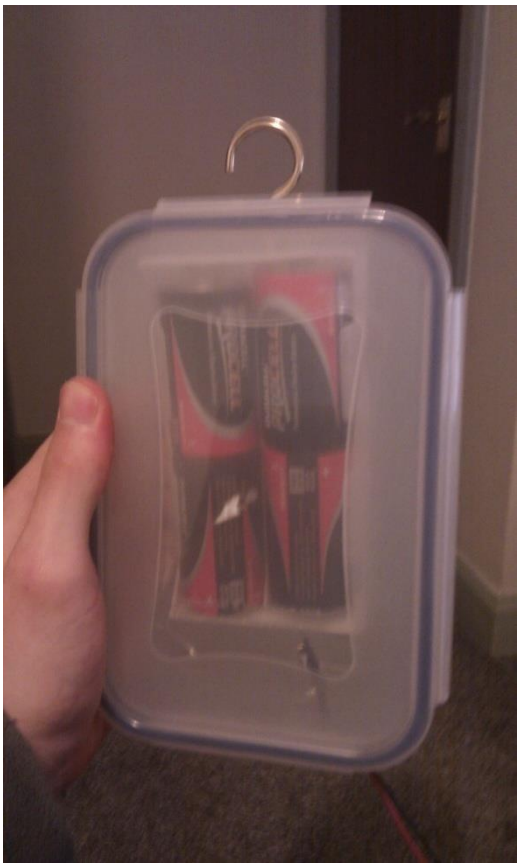

Figure 31. Water resistant battery pack container. A hole is cut in the bottom to allow the LED attachment to clip to the battery pack.

Table 5. Properties of LEDs. *Power output is not known for this LED, light output is given in candela.

Colour	*Wavelength*	*Angle*	*Power*	*Manufacturer*
Green	520nm	30°	13cd*	Avago Technologies
Green	540nm	30°	6.1mW	Multicomp
Blue	480nm	30°	10.4mW	CREE

Study Sites

Study sites 1-4 were selected from amongst a small group of commercial growers who, via HDC contacts, had expressed an interest in the project. These growers, and others, attended a presentation where they were given overview of the project, the project aims and objectives, and past research was discussed. A short questionnaire, aimed to determine the suitability of the site, was send to the growers (Appendix 1). All attachments and batteries were posted to the growers. Used yellow sticky traps were posted to SRUC for insect identification on a regular basis throughout the project. Growers were updated on the project via regular e-mails and HDC conferences.

Study sites 5 and 6 were located in Edinburgh. Site 5 was a local (Edinburgh) site known to have a whitefly infestation through business contacts of Andy Evans. This site was contacted directly, and agreed to take part in the project after a visit. Equipment was delivered and retrieved personally. Site 6 was based at SRUC. Despite requests sites 1, 2, 3, and 5 did not provide lists of biological control agents used at their sites. The request was initially made in the questionnaire (Appendix 1), and followed up towards the middle and end of the project.

Specimen Identification

Specimen were identified to species level using light microscopy with the aid of a dichotomous key, and by looking for known identifying features in common species of a particular pest (Table 6). Due to the difficulty of reliably sexing specimens captured by sticky traps, this was not attempted. *Kleidotoma psiloides* were identified with the assistance of the grower, who had previously identified this species (pers comm grower). Specimen with damaged key identifying features were not identified.

Table 6. Sources for species identification.

Species	Sources
Bradysia difformis	Menzel *et al*, 2003; Malais and Ravensberg, 2003
Frankliniella occidentalis	Parrella, 1995; New South Wales; Malais and Ravensberg, 2003; Department of Primary Industries, 2016
Trialeurodes vaporariorum	Martin *et al*, 2000, Malais and Ravensberg, 2003
Plutella xylostella	Moriuti, 1986; Bhalla and Dubey, 1986
Encarsia formosa	Malais and Ravensberg, 2003
Kleidotoma psiloides	pers comm grower

Site 1: Experimental Design

Yellow sticky traps equipped with a single green (540 nm) or blue (480 nm) LED powered by battery packs were compared against those without (Table 7). One half (side) of the trap was exposed for a week, this was then re-covered with the wax paper and the other half (side) was exposed. Each half (side) will be discussed as a separate batch. The crops were poinsettia, and were grown on benches in a 46.5×44m glasshouse. Random numbers for trap positions were generated using an internet tool (Random, 2016).

There were further batches in study year 1 (2012), but due to corrosion of the battery packs data from later dates were unreliable and will not be included here. During year 2 battery packs were enclosed in water resistant plastic containers.

Table 7. Experimental design, LED specifications, and dates for comparison between green (540 nm) or blue (480 nm) LEDs and standard yellow sticky traps at site 1.

Study dates	*Batch dates*	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
09/08/12-23/08/12	09/08/12-16/08/12 16/08/12-23/08/12	21 standard and 21 LED equipped	540 nm (green) Avago Technologies, 5mm, 30° angle, power output 6.1mW	Randomised design (see appendix 2). Positions were not re-randomised when traps were changed.	See appendix 2
12/09/13-02/10/13	12/09/13-19/09/13 19/09/13-26/09/13 26/09/13-02/10/13	10 standard and 10 LED equipped	480 nm (blue) CREE, 5mm, 30° angle, power output 10.4mW	Traps were arranged in two rows of paired replicates (see appendix 2 for layout pattern)	See appendix 2

Site 2: Experimental Design

Yellow sticky traps equipped with a single green LED powered by battery packs were compared against those without (Table 8). One half (side) of the trap was exposed for 7 days time, this was then re-covered and the other half (side) was exposed. Traps were changed fortnightly. Each half (side) will be discussed as a separate batch (Table 9). Crops were a wide variety of herbs which were cycled e.g. basil, chive, and thyme, and were grown on benches in a 130×45m glasshouse. Traps were placed above the same type of crop.

Table 8. Experimental design, LED specifications, and dates for comparison between green (540 nm) LEDs and standard yellow sticky traps at site 1.

Study dates	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
01/10/12-26/11/12	17 standard and 17 LED equipped	540 nm (green) Avago Technologies, 5mm, 30° angle, power output 6.1mW	Traps were arranged in two rows of paired replicates (see appendix 2 for layout pattern)	~2m

Table 9. Batch numbers and dates for comparison between traps equipped with green (540 nm) LEDs and standard yellow sticky traps at site 2.

Batch number	1	2	3	4	5	6	7	8
Batch date	01/10/ 12	08/10/ 12	15/10/ 12	22/10/ 12	29/10/ 12	05/11/ 12	12/11/ 12	19/11/ 12

Site 3: Experimental Design

Yellow sticky traps equipped with a single green (540 nm) or blue (480 nm) LED powered by battery packs were compared against those without (Table 10). One half (side) of the trap was exposed for a set time, this was then re-covered and the other half (side) was exposed. Each half (side) will be discussed as a separate batch (Table 11). The crops were poinsettia, and were grown in pots on top of capillary matting covered by perforated plastic sheet. The glasshouse was 58×70m. This site scales down their operations as crops are sold, so the number of traps decreases over time (Table 12). The exact method for downscaling was not provided by the grower, but the traps remained in pairs with crops beneath them for the duration they were in use. Batch 5 were misplaced by the grower, so these data were not collected.

Table 10. Experimental design, LED specifications, and dates for comparison between green (540 nm) or blue (480 nm) LEDs and standard yellow sticky traps at site 1.

Study dates	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
11/10/12-04/12/12	21 standard and 21 LED equipped	540 nm (green) Avago Technologies, 5mm, 30° angle, power output 6.1mW	Traps were arranged in two rows of paired replicates (see appendix 2 for layout pattern)	~1m
02/09/13-15/08/13	10 standard and 10 LED equipped	480 nm (blue) CREE, 5mm, 30° angle, power output 10.4mW	Traps were arranged in two rows of paired replicates (see appendix 2 for layout pattern)	~1m

Table 11. Batch numbers, dates, and number of replicates for comparison between traps equipped with green (540 nm) LEDs and standard yellow sticky traps at site 3.

Batch number	Batch 1	Batch 2	Batch 3
Batch date	11/10/12 - 08/11/12	08/11/12 – 22/11/12	22/11/12 – 04/12/12
Time (days)	28	14	12
Replicates	12	10	8

Table 12. Batch numbers, dates and number of replicates for comparison between traps equipped with blue (480 nm) LEDs and standard yellow sticky traps at site 3.

Batch number	1	2	3	4	5	6	7
Batch date	02/09/13-09/09/13	09/09/13-16/09/13	13/09/13-20/09/13	20/09/13-05/10/13	Unknown	01/11/13-08/11/13	08/11/13-15/08/13
Time (days)	7	7	7	15	N/A	7	7
Replicates	8	6	6	7	N/A	7	7

Site 4: Experimental Design

Traps returned from this site did not show sufficient numbers of pests for data collection to be worthwhile. The grower did not suffer from any flying pest problems during the study period. The traps primarily captured Syrphidae.

Site 5: Experimental Design

LED attachments at this site were constructed to allow the LED to be changed without replacing the entire device. LEDs were not soldered to the wire; rather, the LED anode and cathode were held in place against the wire solely using the terminal block screw.

Yellow sticky traps equipped with a single green (520 nm) or blue (480 nm) LED powered by battery packs were compared against those without (Table 13). Note that 520 nm LEDs were used at this site, rather than the standard 540 nm, this wavelength was selected based on results from the behaviour study, and the purpose of using this wavelength was to gather data for this wavelength. One half (side) of the trap was exposed for a set time, this was then re-covered and the other half (side) was exposed. Each half (side) will be discussed as a separate batch (Table 13). The plants were a collection of Scottish wild flowers grown for a student display, and were frequently changed. The glasshouse was 20×13m.

Table 13. Experimental design, LED specifications, and dates for comparison between green (520 nm) or blue (480 nm) LEDs and standard yellow sticky traps at site 5.

Study dates	*Batch dates*	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
21/08/13-02/10/13	21/08/13-04/09/13 04/09/13-18/09/13 18/09/13-02/10/13	6 standard and 6 LED equipped	520 nm (green) Multicomp, 5mm, 30° angle, luminous intensity 13cd	Traps were arranged in two rows of paired replicates (see appendix 2 for layout pattern)	~1m
02/10/13-14/11/13	02/10/13-16/10/13 16/10/13-31/10/13 31/10/13-14/11/13	6 standard and 6 LED equipped	480 nm (blue) CREE, 5mm, 30° angle, power output 10.4mW	Traps were arrange in two rows of paired replicates (see appendix 2 for layout pattern)	~1m

Site 6: Experimental Design

Yellow sticky traps each equipped with a single green (540 nm) LEDs powered by a 9V ac/dc mains adaptor were compared against those without (Table 14). As Koppert traps were used here, both sides of the trap were uncovered. The crops were a frequently changed assortment of Scottish flowering plants grown in pots on top of benches. The glasshouse was 30×12m.

Table 14. Experimental design, LED specifications, and dates for comparison between green (520 nm) LEDs and standard yellow sticky traps at site 6.

Study dates	*Batch dates*	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
10/04/13	10/04/13-27/04/13	6 standard and 6 LED equipped	520 nm (green) Avago Technologies, 5mm, 30° angle, power output 6.1mW	Traps were arranged in two pairs of rows (see appendix 2)	~1m

General Statistical Methods for LED and Standard Yellow Sticky Trap Comparisons

To test for significant differences between the capture efficiency of the standard and LED equipped yellow sticky traps, either a One-Way ANOVA or a Mann-Whitney U test (the nonparametric equivalent of a One-Way ANOVA/t-test) were used (Dythan, 2011; Quinn and Keough, 2011). When comparing just two sets of data using a One-Way ANOVA the output is exactly the same as with a t-test, and the decision to use a One-Way ANOVA was personal preference as well as due to the intention of performing three-way comparisons between trap types. The One-Way ANOVA was preferred over the Mann-Whitney U as this test operates under more stringent assumptions, enabling a more powerful analysis confidence (Dytham, 2011).

The assumptions of an ANOVA are that the data are normally distributed, the population variance are equal, and the observations are independent. Normality and homogeneity were tested Shapiro-Wilk and Levene's tests respectively. While the Kolmogorov-Smirnov test is more commonly recommended as a test of normality (Dythan, 2011; Quinn and Keough, 2011), the Shapiro-Wilk is more effective for small sample sizes (Shapiro and Wilk, 1965; Shapiro *et al.*, 1968).

In instances of non-normal data a log10 transformation was performed, with 0s being changed to 1s, to attempt to normalise the data. Normality was then retested, and if these data were then normally distributed a One-Way ANOVA was used the test for differences between the populations, otherwise a Mann-Whitney U test was performed on the original data. The Mann-Whitney U test does not make assumptions of normality or homogeneity of data, and operates by converting the data into ranks before performing the analysis, making this test more suitable for data with extreme values (Dytham, 2011). Where Log10 data are used for analyses, actual data are presented in graphical form.

Traps were analysed in 'batches', with a batch being all traps which were used over the same time period. Comparisons of the overall data will be made, with the batches being combined, i.e. all standard yellow sticky traps compared with all LED equipped stick traps. The time period over which each batch was in use is termed as a 'study period'.

All tests were conducted with 95% confidence (Dytham, 2011). In instances where data are normal, averages are expressed as mean (±SE). Non-normal averages are expressed as median, quartile 1 and quartile 3 (Q1, Q3). All percentage differences are expressed as the difference between the mean, or median, number of insects captured (Formula 2).

$V_1 = 10$

$V_2 = 5$

$V_1 - V_2 \div ((V_1 + V_2) \div 2) \times 100$

$10 - 5 \div ((10 + 5)/2) \times 100$

$(5 \div 15) \times 100$

$= 33.33\%$

Formula 2. Example formula demonstrating percentage difference calculation.

Normally distributed data are displayed on column charts and show mean (±SE). Non-normal data are displayed as boxplots, which show median (Q1, Q3), and the smallest and largest sample value. When outliers are present SPSS modifies the whiskers showing the smallest and largest sample value to not include extreme values. Outliers (data points greater than 1.5 times the interquartile range) are shown as numbered empty circles, and extreme outliers (data points greater than 3 times the interquartile range) as asterix.

The decision to display data in this way were made for the following reasons. Boxplots provide a succinct summary of a data set, typically presenting the median, first and third quartiles, and the highest and lower values (here these are adjusted for those values below 1.5 times the interquartile range) (Sheskin, 2000; Yates *et al.*, 2002). Boxplots give an indication of the skewness of a distribution, for example in a right skewed distribution the third quartile will usually be farther away from the median than the first quartile (Yates *et al.*, 2002). Furthermore, boxplots display outliers, enabling the reader to have a better grasp of the overall data set (Sheskin, 2000; Yates *et al.*, 2002). It would be inappropriate, and potentially misleading, to present the mean when the data set is of a non-normal distribution, as the mean is likely to be skewed by outliers (Sheskin, 2000), with this in mind the median is presented for all non-normal data in this thesis.

The ANOVA is a comparison of means (Sheskin, 2000; Yates et al., 2002; Crawley, 2007), so it is reasonable to present these means when displaying a graphical representation of this comparison. Deviating from the boxplot conventions described above (e.g. by presenting mean data on a boxplot) could cause confusion for the reader, and the program used here to

generate boxplots (SPSS) does not provide an option to produce boxplots which display means rather than median. Bar charts displaying means with errors bars are a commonly used in the graphical representation of mean data, and although these are termed 'Dynamite plots' by some, with strong arguments presented for the presentation of all data points (Bolker, 2011; Koyama, 2011), bar charts with error bars remain the prominent graphical display of mean data in papers comparing sticky trap types (Hoddle *et al.*, 2002; Chen *et al.*, 2004b; Moreau and Isman, 2011; Broughton and Harrison, 2012). There are instances in this thesis were data log transformed and analysed using an ANOVA, where this is the case median data are presented in boxplots rather than the transformed data, this was for consistency between boxplots.

Decision to Select Statistical Methods

The statistical methods employed here are based on the normal distribution, or where normality could not be established, a non-parametric alternative. This is supported by a large number of papers within the fields of research discussed here which follow the same procedure (Table 15). A small number of the papers reviewed log or square root transformed non-normal data in an attempt to establish normality (Knight *et al.*, 2002; Facchinelli *et al.*, 2008; Yaku *et al.*, 2008; Steenken and Halaweh, 2011; Gharekhani *et al.*, 2014), the remaining papers did not comment on the distribution of their data. The prevalence of these methods, particularly in the case of LED trap comparisons, as well as the authors own statistics education formed the decision to use the methods found in this thesis.

Although tests based on the normal distribution (and non-parametric alternative) are commonly used (Table 15), it has been recommended that insect count data should not be analysed using methods based on the normal distribution (Sileshi, 2006). The primary reasons given for this are that insect count data often overdispersed (i.e. the variance is greater than the mean) and have a high proportion of zeros, with the remaining data having a skewed distribution (Sileshi, 2006; Sileshi, 2008). These reasons are not properly accounted for in statistical models based on the normal distribution, which can lead to biased estimations of ecological effects (Sileshi, 2006).

Generalized linear models are an extension of linear models (e.g. ANOVA, ANCOVA, linear regression), which allow more distributions than the normal distribution to be used (O'Hara and Kotze, 2010). The Poisson and negative binomial distribution have been suggested as alternatives to the normal distribution when analysing count data (Sileshi, 2006;

Sileshi, 2008; O'Hara and Kotze, 2010). A model based on the Poisson distribution assumes that the mean and variance are equal, so if the data are overdispersed this may lead to P values which are too small or narrow confidence intervals (Sileshi, 2008). These issues may be overcome by using a Poisson model which has been correction for overdispersal, often termed a quasi-Poisson model (Sileshi, 2008; O'Hara and Kotze, 2010). The decision to select from these models must be taken on a case by case basis, as although O'Hara and Kotze (2010) reported almost identical estimates in simulations, Ver Hoef and Boveng (2007) and Sileshi (2008) reported differing results gained from these models.

It is apparent that the quasi-Poisson or negative binomial would be more suitable for the analysis of the data collected for this thesis, and any published papers or future work will seek to use more appropriate analyses.

Table 15: Papers which compare insect count data using statistical methods based on the normal distribution, or a non-parametric alternative.

Comparison type	**References**
Trap types and/or monitoring methods	Knight *et al.*, 2002; Tong-Xian and Chu; Hall, Hentz, and Ciomperlik, 2007; Facchinelli *et al*., 2008; Aliakbarpour and Rawi, 2011; Górska-Drabik *et al.*, 2011; Premalatha and Rajangam 2011
Sticky trap configurations	Puckett *et al*., 2013
Chemical enhanced sticky traps	Górsk, 2004; Premalatha and Rajangam, 2011; Broughton and Harrison 2012
Trap colour	Chu *et al.*, 2006; Demirel and Yildirim, 2008; Yaku *et al*., 2008; Gharekhani *et al.*, 2014; Thongjua *et al.*, 2015
Host plant preference	Steenken and Halaweh, 2011
Light enhanced traps	Chu *et al.*, 2000; Nombela *et al.*, 2003; Chu *et al.* 2003; Chu *et al.* 2004b; Nakamoto and Kuba, 2004; Chen *et al.*, 2004a; Chen *et al.*, 2004b; Chu *et al.*, 2005; Castresana and Puhl, 2015

Site 1: Statistical Methods

Table 16. Species captured at site 1.

Species	*Common name*	*Relevance to crop growing*
Bradysia difformis	Dark-winged fungus gnat	Pest species
Frankliniella occidentalis	Western flower thrips	Pest species
Trialeurodes vaporariorum	Glasshouse whitefly	Pest species

Table 17. Statistical analyses used for study year 1 (09/08/12 – 23/08/12) data at site 1 comparing green (540 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Bradysia difformis	09/08/12-16/08/12	1	One-Way ANOVA	Yes
	16/08/12-23/08/12	2	One-Way ANOVA	Yes
	09/08/12-23/08/12	Combined data	One-Way ANOVA	Yes
Frankliniella occidentalis	09/08/12-16/08/12	1	Mann-Whitney U	No
	16/08/12-23/08/12	2	Mann-Whitney U	No
	09/08/12-23/08/12	Combined data	Mann-Whitney U	No

Table 18. Statistical analyses used for study year 2 (12/09/13 – 02/10/13) data at site 1 comparing blue (480 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Trialeurodes vaporariorum	12/09/13 – 19/09/13	1	One-Way ANOVA	Yes
	19/09/13 – 26/09/13	2	One-Way ANOVA	Yes
	26/09/13 – 12/09/13	3	One-Way ANOVA	No
	12/09/13 – 03/10/13	Combined data	One-Way ANOVA	Yes

Site 2: Statistical Methods

Table 19. Species captured at site 2.

Species	*Common name*	*Relevance to crop growing*
Bradysia difformis	Dark-winged fungus gnat	Pest species

Table 20. Statistical analyses used for study year 1 (01/10/12 – 26/11/12) data at site 2 comparing green (540 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Bradysia difformis	01/10/12	1	One-Way ANOVA	No
	08/10/12	2	One-Way ANOVA	No
	15/10/12	3	One-Way ANOVA	No
	22/10/12	4	One-Way ANOVA	No
	29/10/12	5		
	05/11/12	6	One-Way ANOVA	Yes
	12/11/12	7	One-Way ANOVA	No
	19/11/12	8		
	01/10/12 – 26/11/12	Combined data	N/A (See page 110)	N/A

Site 3: Statistical Methods

Table 21. Species captured at site 3.

Species	*Common name*	*Relevance to crop growing*
Bradysia difformis	Dark-winged fungus gnat	Pest species
Frankliniella occidentalis	Western flower thrips	Pest species
Plutella xylostella	Diamondback moth	Pest species

Table 22. Statistical analyses used for study year 1 (11/10/12 – 04/12/12) data at site 3 comparing green (540 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Bradysia difformis	11/10/12	1	One-Way ANOVA	No
	08/11/12	2	One-Way ANOVA	Yes
	22/11/12	3	One-Way ANOVA	No
	11/10/12 – 04/12/12	Combined data	Mann-Whitney U	No
Plutella xylostella	11/10/12	1	N/A (sample size too small to be reliably analysed – Data were included as *P. xylostella* are not typically captured on yellow sticky traps in their standard placements (Hallet, 1986)).	
	08/11/12	2	N/A	N/A
	22/11/12	3	N/A	N/A
	11/10/12 – 04/12/12	Combined data	Trap numbers and the time period each batch was used for differed at this site. Statistical advice was sought on how these data may be analysed, but no solution was provided.	

Table 23. Statistical analyses used for study year 2 (02/09/13 – 08/11/13) data at site 3 comparing blue (480 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Bradysia difformis	02/09/13 – 09/09/13	1	One-Way ANOVA	No
	09/09/13 – 16/09/13	2	One-Way ANOVA	No
	13/09/13 – 20/09/13	3	One-Way ANOVA	No
	20/09/13 – 05/10/13	4	One-Way ANOVA	Yes
	N/A	5	N/A (traps were lost by grower)	
	08/11/13 – 15/08/13	6	One-Way ANOVA	Yes
	02/09/13 – 15/08/13	Combined data	A comparison of the combined data could not be performed, as this site varied in the length of time each batch of traps were used, with batch 4 being used for 15 days and the remaining batches used for 7 days.	
Frankliniella occidentalis	02/09/13 – 09/09/13	1	One-Way ANOVA	No
	09/09/13 – 16/09/13	2	One-Way ANOVA	No
	13/09/13 – 20/09/13	3	One-Way ANOVA	No
	20/09/13 – 05/10/13	4	One-Way ANOVA	No
	N/A	5	N/A (traps were lost by grower)	
	08/11/13 – 15/08/13	6	N/A (No *F. occidentalis* were captured)	
	02/09/13 – 15/08/13	Combined data	A comparison of the combined data could not be performed, as this site varied in the length of time each batch of traps were used, with batch 4 being used for 15 days and the remaining batches used for 7 days.	
Plutella xylostella	02/09/13 – 09/09/13	1	Mann-Whitney U	No
	09/09/13 – 16/09/13	2	Mann-Whitney U	No
	13/09/13 – 20/09/13	3	Mann-Whitney U	No
	02/09/13 – 15/08/13	Combined data	Mann-Whitney U	No

Site 5: Statistical Methods

Table 24. Species captured at site 5.

Species	***Common name***	***Relevance to crop growing***
Frankliniella occidentalis	Western flower thrips	Pest species
Trialeurodes vaporariorum	Glasshouse whitefly	Pest species

Table 25. Statistical analyses used for study year 2 (21/08/13 – 02/10/13) data at site 5 comparing green (540 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Frankliniella occidentalis	21/08/13 – 04/09/13	1	One-Way ANOVA	Yes
	04/09/13 – 18/09/13	2	One-Way ANOVA	No
	18/09/13 – 02/10/13	3	One-Way ANOVA	No
	21/08/13 – 02/10/13	Combined data	One-Way ANOVA	Yes
Trialeurodes vaporariorum	21/08/13 – 04/09/13	1	One-Way ANOVA	No
	04/09/13 – 18/09/13	2	One-Way ANOVA	No
	18/09/13 – 02/10/13	3	One-Way ANOVA	No
	21/08/13 – 02/10/13	Combined data	One-Way ANOVA	Yes

Table 26. Statistical analyses used for study year 2 (02/10/13 – 14/11/13) data at site 5 comparing blue (480 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Frankliniella occidentalis	02/10/13 - 16/10/13	1	One-Way ANOVA	No
	16/10/13 - 31/10/13	2	One-Way ANOVA	Yes
	31/10/13 - 14/11/13	3	One-Way ANOVA	No
	02/10/13 – 14/11/13	Combined data	Mann-Whitney U	No
Trialeurodes vaporariorum	02/10/13 - 16/10/13	1	One-Way ANOVA	No
	16/10/13 - 31/10/13	2	One-Way ANOVA	Yes
	31/10/13 - 14/11/13	3	One-Way ANOVA	No
	02/10/13 – 14/11/13	Combined data	Mann-Whitney U	No

Site 6: Statistical Methods

Table 27. Species captured at site 6.

Species	*Common name*	*Relevance to crop growing*
Trialeurodes vaporariorum	Glasshouse whitefly	Pest species

Table 28. Statistical analyses used for study year 2 (10/04/13 – 27/04/13) data at site 6 comparing green (540 nm) LED equipped yellow sticky traps against standard yellow sticky traps.

Species	*Batch dates*	*Batch number*	*Statistical analysis*	*Log10 transformation*
Trialeurodes vaporariorum	10/04/13 – 27/04/13	1	Mann-Whitney U	No

Results

Bradysia difformis

Site 1

Bradysia difformis: Green (540 nm) LEDs (09/08/12 – 23/08/12)

LED traps captured significantly more *B. difformis* in batches 1 (P=0.049) and 2 (P=0.001) (Table 29), with LED traps captured 16.7% more than standard traps in batch 1 (Fig. 32), and 56.4% more in batch 2 (Fig. 33). LED traps captured significantly more *B. difformis* across the study period (P=0.004) (Table 29), with LED traps capturing 29% more than standard traps (Fig. 34).

Table 29. No. of *B. difformis* caught on green (540 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 1. *Significant at P < 0.05.

Batch number	***Dates***	***F statistic***	***P value***	***LED traps. Median, Q1, Q3.***	***Standard traps. Median, Q1, Q3.***
Batch 1	09/08/12 – 16/08/12	$F_{1,40} = 4.138$	0.049*	39 (31, 57)	33 (25, 41)
Batch 2	16/08/12 – 23/08/12	$F_{1,40} = 12.045$	0.001*	25 (15, 32)	14 (11, 17)
Entire study period	09/08/12 – 23/08/12	$F_{1,81} = 8.938$	0.004*	31.5 (24.25, 39)	23.5 (14.25, 33)

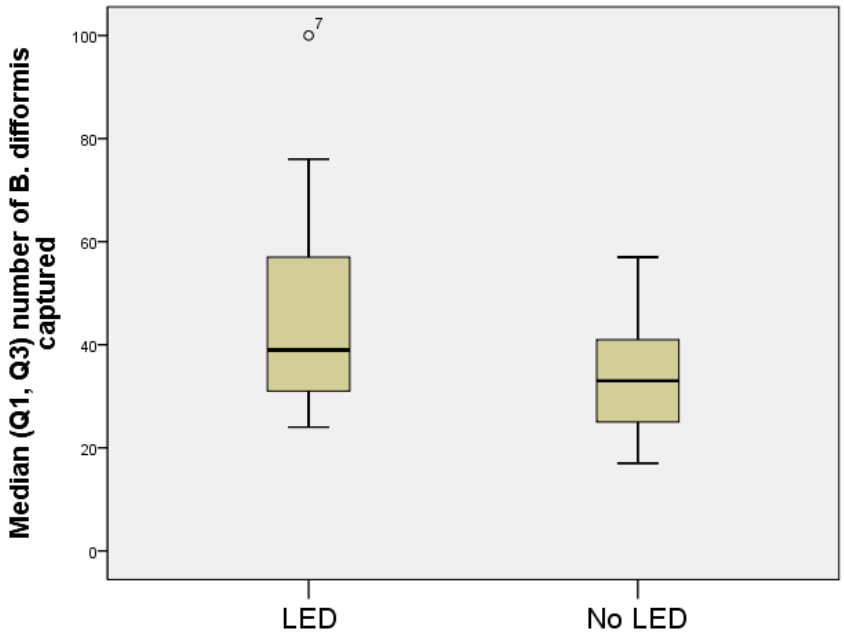

Figure 32. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *B. difformis* captured on green (540 nm) LED and standard yellow sticky traps in Batch 1 at site 1 (09/08/12 – 16/08/12).

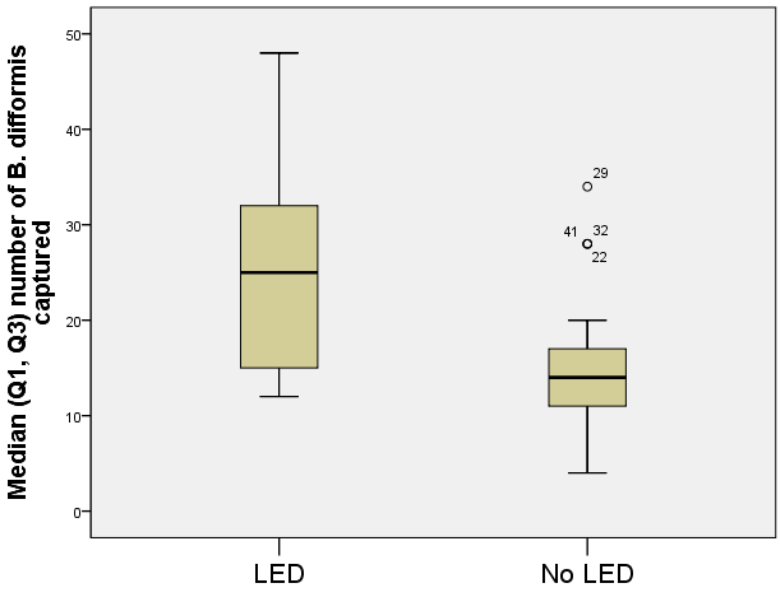

Figure 33. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of B. *difformis* captured on green (540 nm) LED and standard yellow sticky traps in Batch 2 at site 1 (16/08/12 – 23/08/12).

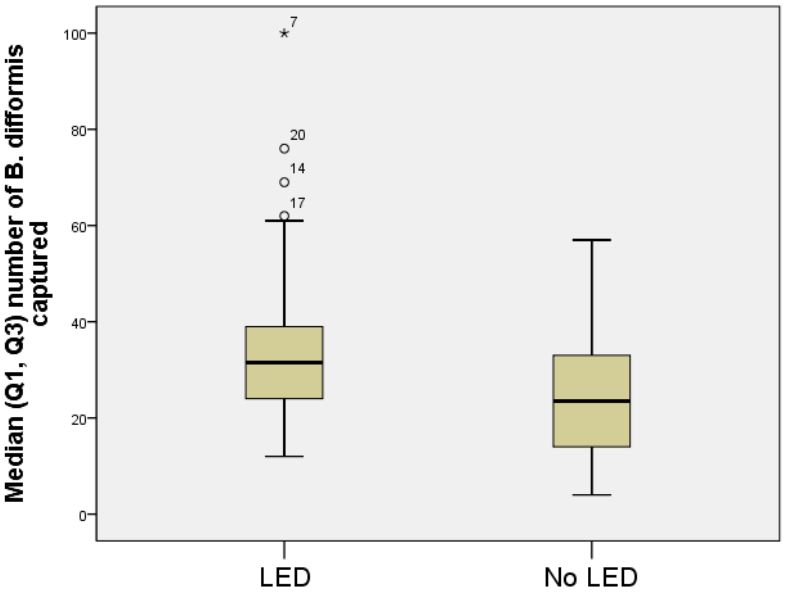

Figure 34. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *B. difformis* captured on green (540 nm) LED and standard yellow sticky traps across whole study period at site 1 (09/08/12– 23/08/12).

Site 2

Bradysia difformis: Green (540 nm) LEDs (01/10/12 – 26/11/12)

No significant differents were found in batches 1, 2, 4, and 5. LED traps captured significantly more *B. difformis* in batches 3, 7, and 8. LED traps captured 37.3% more *B. difformis* than standard yellow sticky traps in batch 3, 47.5% in batch 7, and 46.6% in batch 8 (Table 30) (Fig. 35).

LED traps captured significantly more *B. difformis* than standard traps across the entire study period (P=0.002), with LED traps capturing 37.8% more than standard yellow sticky traps (Table 30) (Fig. 36).

Table 30. No. of *B. difformis* captured on green (540 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 2. Traps were changed weekly. *Significant at P < 0.05, **Median (Q1, Q3).

Batch number	***Dates***	***F statistic/ Mann-Whitney U***	***P value***	***LED traps. Mean (±SE)***	***Standard traps. Mean (±SE)***
Batch 1	01/10/12	$F_{1,32}$ = 0.076	0.785	206.35 (±18.61)	198.12 (±23.41)
Batch 2	08/10/12	$F_{1,32}$ = 0.807	0.376	163.41(±18)	140.12 (±18.652)
Batch 3	15/10/12	$F_{1,32}$ = 5.282	0.028*	122.24 (±13.31)	83.76 (±10.15)
Batch 4	22/10/12	$F_{1,32}$ = 0.148	0.703	78.12 (±9.81)	84.12 (±12.14)
Batch 5	29/10/12	$F_{1,32}$ = 0.639	0.403	71.94 (±9.6)	61.47 (±8.91)
Batch 6	05/11/12	$F_{1,32}$ = 3.117	0.087	67 (46, 124)**	48 (33, 64)**
Batch 7	12/11/12	$F_{1,32}$ = 5.942	0.021*	61.12 (±8.1)	37.65 (±5.21)
Batch 8	19/11/12	$F_{1,32}$ = 4.616	0.039*	62.53 (±6.4)	38.88 (±8.95)
Entire study period	01/10/12 – 26/11/12	U = 7233, Z= -3.106	0.002*	86.5 (52, 149.5)**	59 (35, 129)**

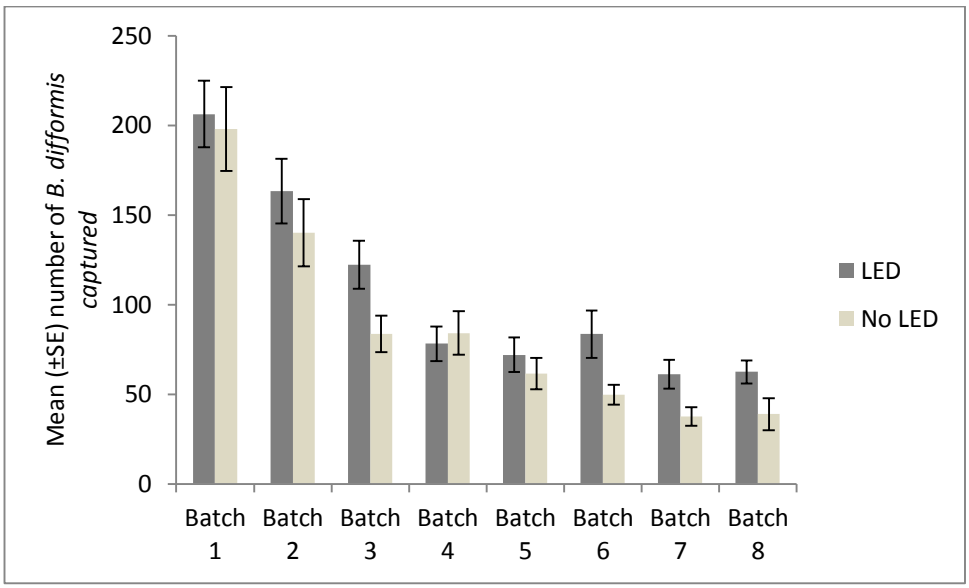

Figure 35. Mean (±SE) number of *B. difformis* captured on green (540 nm) LED and standard yellow sticky traps at site 2.

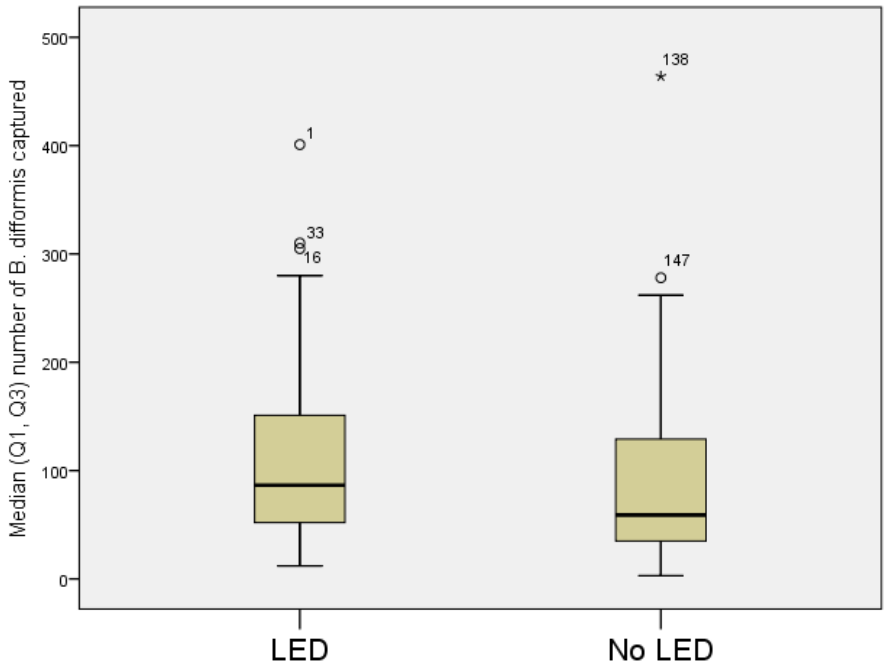

Figure 36. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *B. difformis* captured on green (540 nm) LED and standard yellow sticky traps across study period at site 2 (01/10/12 – 26/11/12).

Site 3

Bradysia difformis: Green (540 nm) LEDs (11/10/12 – 04/12/12)

Significantly more *B. difformis* were captured by LED traps in batch 1 (P<0.001), with LED traps capturing 129.2% more than standard sticky traps. No significant differences were found in batches 2 (P=0.169) or 3 (P=0.184) (Table 31) (Fig. 37). No significant difference was found over the complete study period (P=0.281) (Table 31) (Fig. 38).

Table 31. No. of *B. difformis* captured on green (540 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3. *Significant at P < 0.05. **Median (Q1, Q3).

Batch number	***Dates***	***F statistic/ Mann-Whitney U***	***P value***	***LED traps. Mean (±SE)***	***Standard traps. Mean (±SE)***
Batch 1	11/10/12 – 08/11/12	$F_{1,22}$ = 66.08	<0.001*	790.67 (±70.07)	170 (±30.29)
Batch 2	08/11/12- 22/11/12	$F_{1,18}$ = 2.050	0.169	8.5 (4, 11)**	4.5 (3.25, 6)**
Batch 3		$F_{1,13}$ = 1.970	0.184	4.5 (±0.88)	6.86 (±0.78)
Entire study period	11/10/12 – 04/12/12	U =364, Z= -1.078	0.281	11 (4.25, 653.75)**	9 (5, 130)**

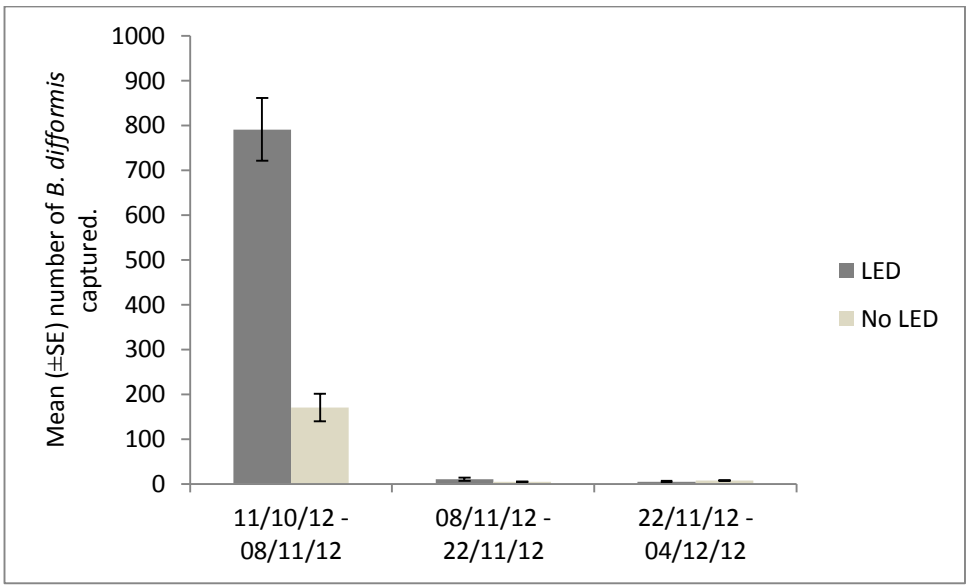

Figure 37. Mean (±SE) number of *B. difformis* captured on green (540 nm) LED and standard yellow sticky traps at site 3.

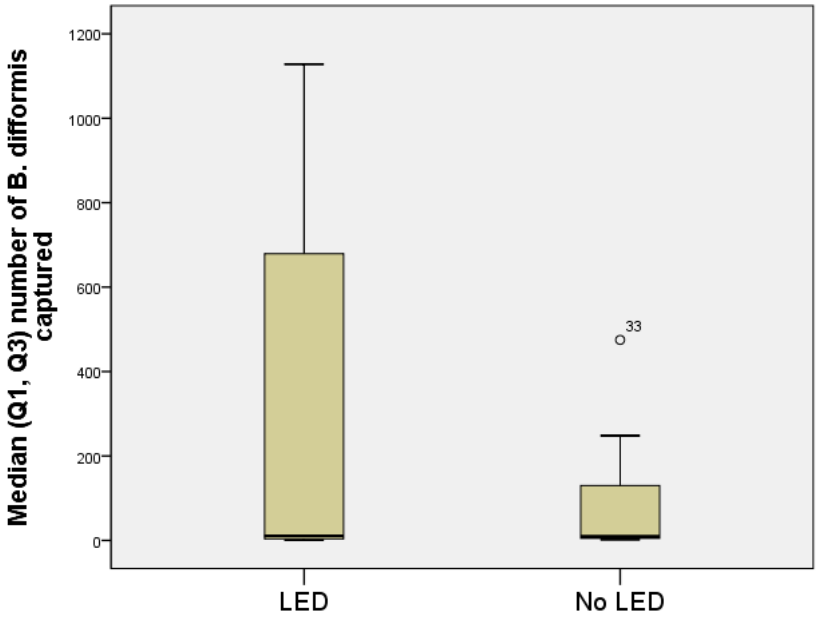

Figure 38. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of B. difformis captured on green (540 nm) LED and standard yellow sticky traps across study period at site 3 (11/10/12 – 04/12/12).

Bradysia difformis: Blue (480 nm) LEDs (02/09/13 – 08/11/13)

LED traps captured significantly more *B. difformis* in batches 4 and 6. In batch 4 LED traps captured a median (Q1, Q3) of 28 (24.5, 66) and standard traps captured 16 (11.5, 26.5), a 75% difference. In batch 6 LED traps captured 4 (3, 10.5) and standard traps captured 1 (0, 1) (Table 32) (Fig. 39).

Table 32. No. of *B. difformis* captured on blue (480nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3. *Significant at P<0.05. **Median (Q1, Q3).

Batch number	***Dates***	***F statistic***	***P value***	***Standard traps. Mean (±SE)***	***LED traps. Mean (±SE)***
Batch 1	02/09/13 – 09/09/13	$F_{1,14} = 1.591$	0.228	45.75 (±6.43)	64.25 (±13.18)
Batch 2	09/09/13 – 16/09/13	$F_{1,10} = 0.160$	0.698	19 (±3.79)	21 (±3.27)
Batch 3	13/09/13 – 20/09/13	$F_{1,10} = 0.007$	0.935	34 (±6.52)	33.17 (±7.58)
Batch 4	20/09/13 – 05/10/13	$F_{1,12} = 0.473$	0.038*	16 (11.5, 26.5)**	28 (24.5, 66)**
Batch 6	08/11/13 – 15/08/13	$F_{1,12} = 17.681$	0.001*	1 (0, 1)**	4 (3, 10.5)**

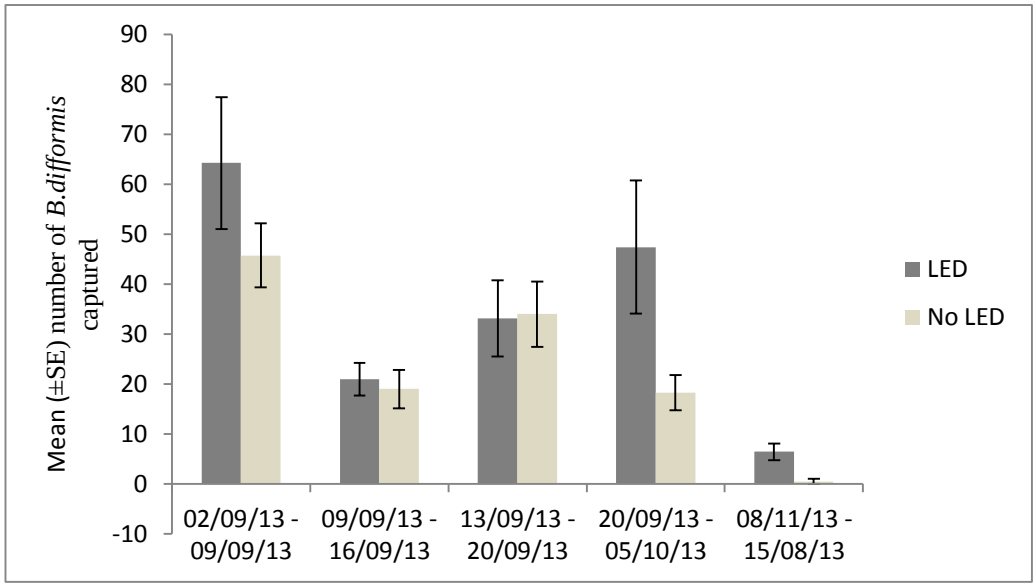

Figure 39. Mean (±SE) number of B. difformis captured on blue (480 nm) LED and standard yellow sticky traps at site 3.

Frankliniella occidentalis

Site 1

Frankliniella occidentalis: Green (540 nm) LEDs (09/08/12 – 23/08/12)

No significant differences were found between LED traps and standard yellow sticky traps in batches 1 (P=0.650) (Fig. 40) or 2 (P=0.504) (Fig. 41). No significant difference was found across the study period (P=0.423) (Fig. 42) (Table 33).

Table 33. No. of *F. occidentalis* captured on green (540 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3. *Significant at P<0.05.

Batch number	***Dates***	***Mann Whitney U***	***P value***	***Standard traps. Median (Q1, Q3)***	***LED traps. Median (Q1, Q3)***
Batch 1	09/08/12 – 16/08/12	U = 202.5, Z= -0.454	0.650	12 (9, 17)	13 (8, 16)
Batch 2	16/08/12 – 23/08/12	U = 194, Z= -0.668	0.504	20 (12, 27)	18 (10, 25)
Entire Study Period	09/08/12 – 23/08/12	U = 792.5, Z= -0.802	0.423	15 (9.5, 21)	14 (10, 19)

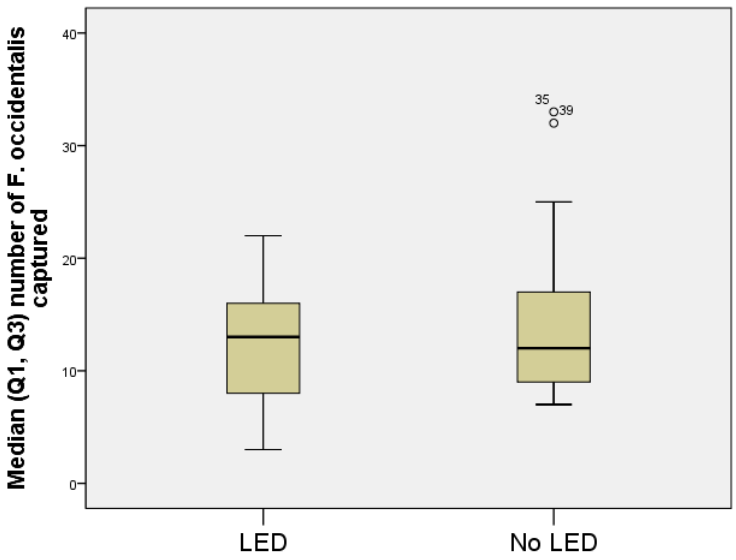

Figure 40. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *F. occidentalis* captured on green (540 nm) LED and standard yellow sticky traps in batch 1 at site 1 (09/08/12 – 16/08/12).

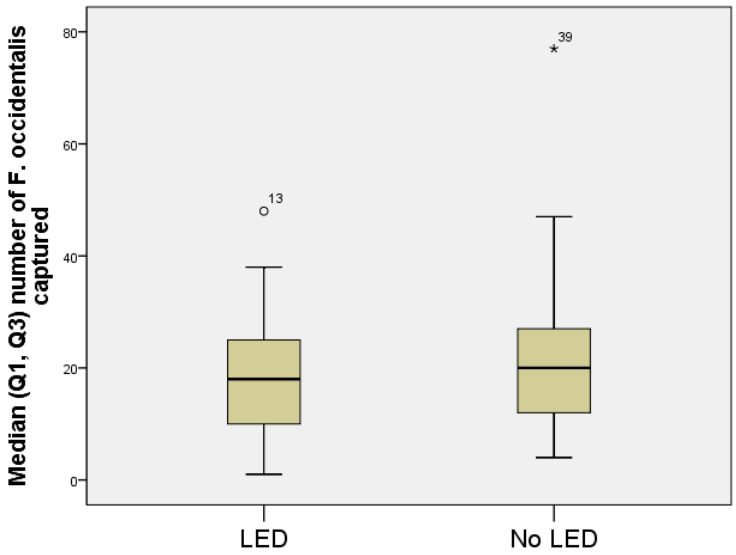

Figure 41. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *F. occidentalis* captured on green (540 nm) LED and standard yellow sticky traps in batch 2 at site 1 (16/08/12 – 23/08/12).

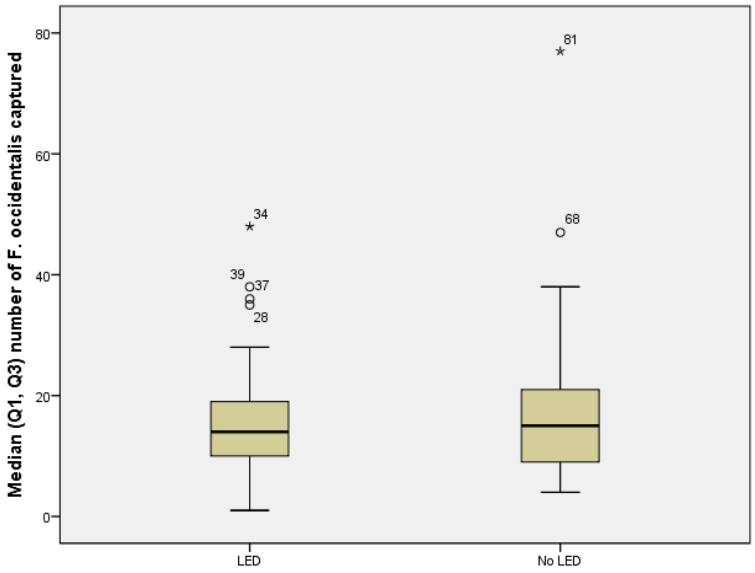

Figure 42. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *F. occidentalis* captured on green (540 nm) LED and standard yellow sticky traps across study period at site 1 (09/08/12– 23/08/12).

Site 3

Frankliniella occidentalis: Blue (480 nm) LEDs (02/09/13 – 05/10/13)

No significant differences were found between LED traps and standard yellow sticky traps for batches one to four, though a pattern of reducing the catch was observed (Fig. 43) (Table 34).

Table 34. No. of *F. occidentalis* captured on blue (480 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3. *Significant at P<0.05.

Batch number	***Dates***	***F statistic***	***P value***	***Standard traps. Mean (±SE)***	***LED traps. Mean (±SE)***
Batch 1	02/09/13 – 09/09/13	$F_{1,14} = 0.688$	0.421	14.5 (±1.97)	12.13 (±2.08)
Batch 2	09/09/13 – 16/09/13	$F_{1,14} = 2.855$	0.122	8 (±2.45)	3.67 (±0.89)
Batch 3	16/09/13 – 20/09/13	$F_{1,14} = 3.926$	0.076	5.17 (±1.01)	2.5 (±0.89)
Batch 4	20/09/13 – 05/10/13	$F_{1,14} = 4.267$	0.061	18.57 (±4.12)	9 (±2.12)

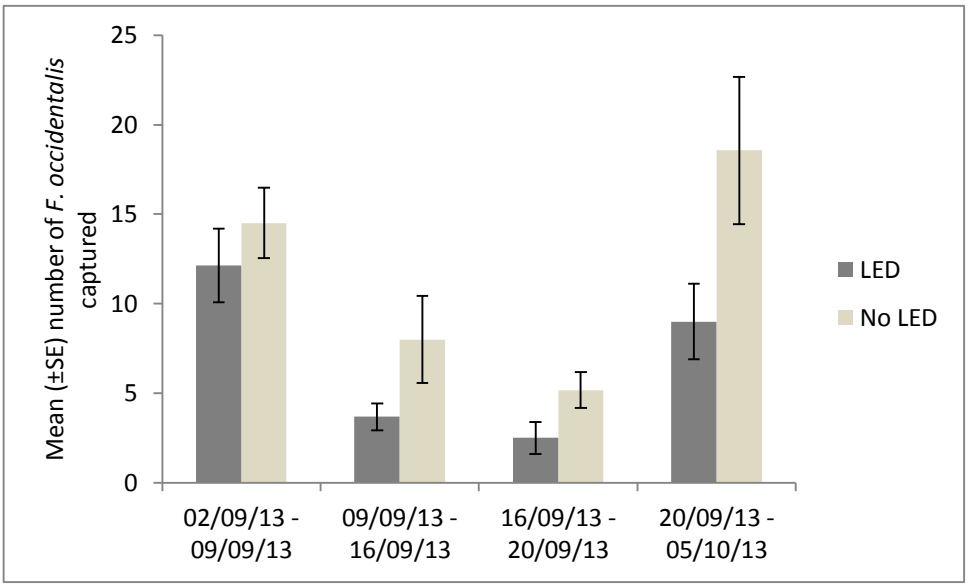

Figure 43. Mean (±SE) number of *F. occidentalis* captured on blue (480 nm) LED and standard yellow sticky traps at site 3.

Site 5

Frankliniella occidentalis: Green (520 nm) LEDs (21/08/13 – 02/10/13)

No significant differences were found between LED traps and standard yellow sticky traps in any of the individual batches (Fig. 45). No significant difference was found between the trap types across the entire trapping period (P=0.697) (Fig. 46) (Table 35).

Table 35. No. of *F. occidentalis* captured on green (520 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3.

Batch number	***Dates***	***F statistic***	***P value***	***Standard traps. Mean (±SE)***	***LED traps. Mean (±SE)***
Batch 1	21/08/13 – 04/09/13	$F_{1,10} = 1.769$	0.213	27.67 (±3.37)	42.83 (±10.89)
Batch 2	04/09/13 – 18/09/13	$F_{1,10} = 0.623$	0.448	12.00 (±1.44)	9.17 (±3.29)
Batch 3	18/09/13 – 02/10/13	$F_{1,10} = 0.007$	0.937	36.83 (±8.71)	38.00 (±11.55)
Entire Study Period	21/08/13 – 02/10/13	$F_{1,34} = 0.154$	0.697	25.5 (±3.86)	30 (±6.23)

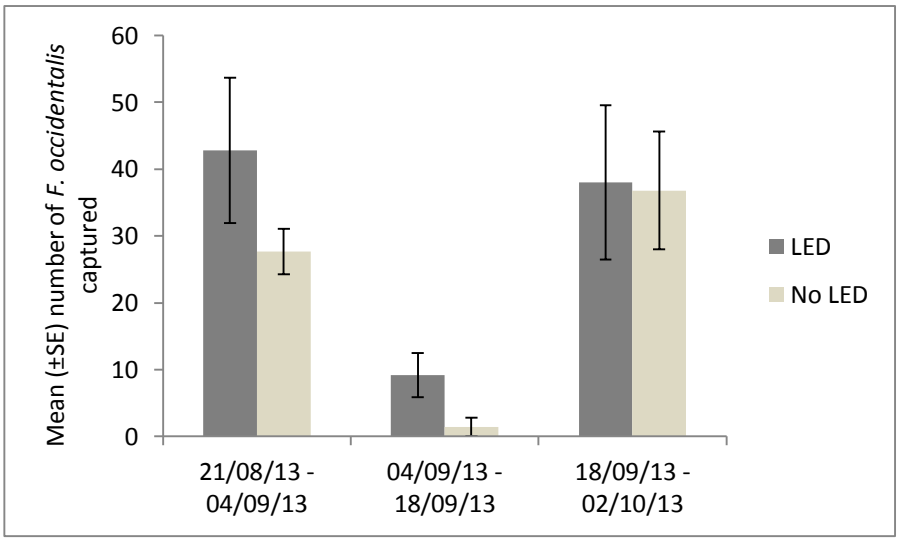

Figure 45. Mean (±SE) number of *F. occidentalis* captured on green (520 nm) LED and standard yellow sticky traps at site 5.

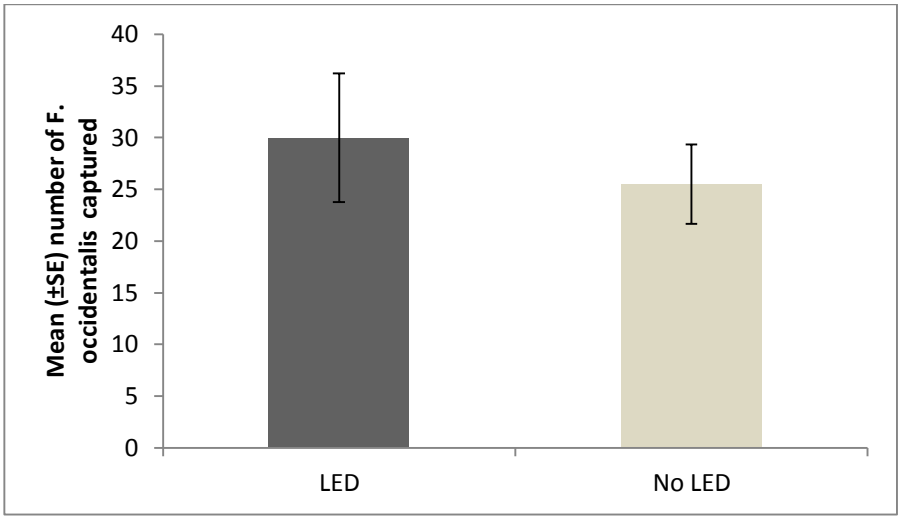

Figure 46. Mean (±SE) number of *F. occidentalis* captured on green (520 nm) LED and standard yellow sticky traps across study period at site 5 (21/08/13 – 02/10/13).

Frankliniella occidentalis: Blue (480 nm) LEDs (02/10/13 – 14/11/13) (site 5)

No significant differences were found between LED traps and standard yellow sticky traps in any of the individual batches (Fig. 47). No significant difference was found between the trap types across the entire trapping period (P=0.713) (Fig. 48) (Table 36).

Table 36. No. of *F. occidentalis* captured on blue (480 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3.

Batch number	***Dates***	***F statistic***	***P value***	***Standard traps. Mean (±SE)***	***LED traps. Mean (±SE)***
Batch 1	02/10/13 - 16/10/13	F1,10 = 0.138	0.718	37.17 (±10.81)	43.50 (±13.14)
Batch 2	16/10/13 - 31/10/13	F1,10 = 2.231	0.166	18.00 (±5.27)	9.50 (±2.14)
Batch 3	31/10/13 - 14/11/13	F1,10 = 0.38	0.848	5.33 (±1.26)	5.67 (±1.15)
Entire Study Period	02/10/13 – 14/11/13	F1,34 = 0.137	0.713	20.17 (±4.94)	19.56 (±5.88)

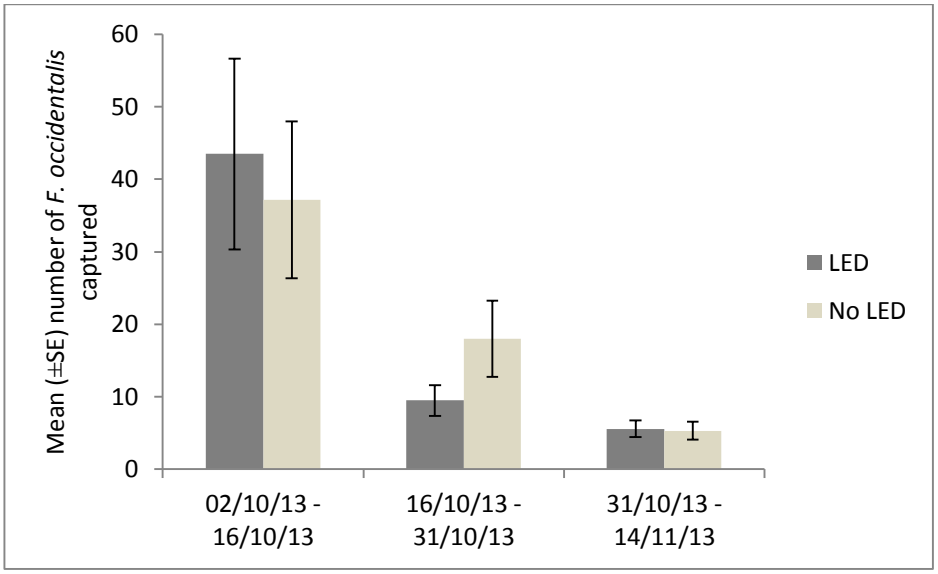

Figure 48. Mean (±SE) number of *F. occidentalis* captured on blue (480 nm) LED and standard yellow sticky traps at site 5.

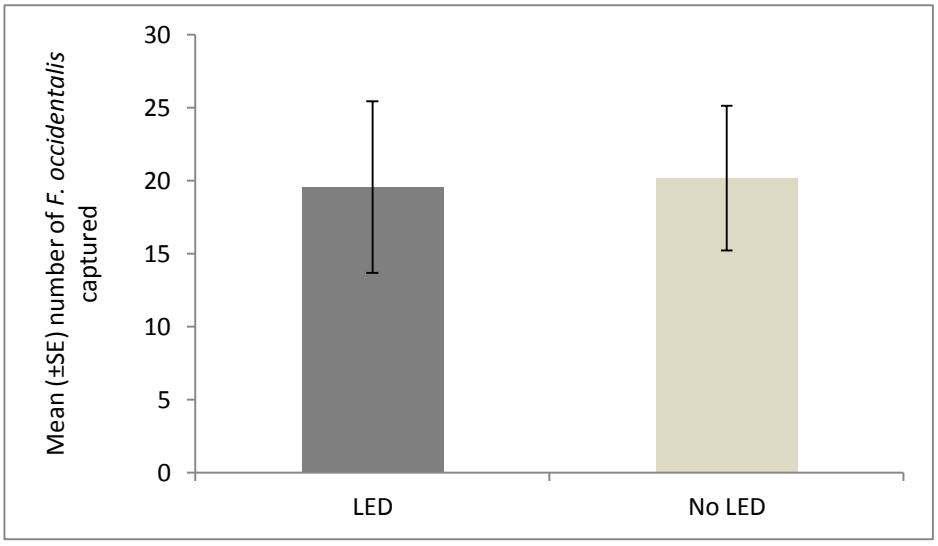

Figure 49. Mean (±SE) number of *F. occidentalis* captured on blue (480 nm) LED and standard yellow sticky traps across study period at site 5 (21/08/13 – 02/10/13).

Trialeurodes vaporariorum

Site 1

Trialeurodes vaporariorum: Blue (480 nm) LEDs (12/09/13– 03/10/13)

No significant differences were found between LED traps and standard yellow sticky traps in batch 1 (P=0.053) (Fig. 51), batch 2 (P=0.219) (Fig. 52), or batch 3 (P=0.792) (Fig. 53). There was a small but significant difference across the entire study period (P=0.05). Standard sticky traps captured 43.5% more *T. vaporariorum* than LED equipped sticky traps (Table 37) (Fig. 54).

Table 37. No. of *T. vaporariorum* captured on blue (480 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 1. Traps were changed weekly. *Significant at P < 0.05.

Batch number	***Dates***	***F statistic***	***P value***	***LED traps. Median (Q1, Q3)***	***Standard traps. Median (Q1, Q3)***
Batch 1	12/09/13	$F_{1, 18} = 4.293$	0.53	51.1 (41.5, 54.75)	69.5 (54.24, 104.25)
Batch 2	19/09/13	$F_{1, 18} = 1.624$	0.219	33 (19.75, 58.75)	43 (35.25, 80.5)
Batch 3	26/09/13	$F_{1, 18} = 0.072$	0.792	91.5 (54.25, 141.25)	101.5 (86.5, 117.75)
Entire study period	12/09/13–03/10/13	$F_{1, 58} = 4.002$	0.05*	53 (35.25, 89.25)	82.5 (46.5, 104.25)

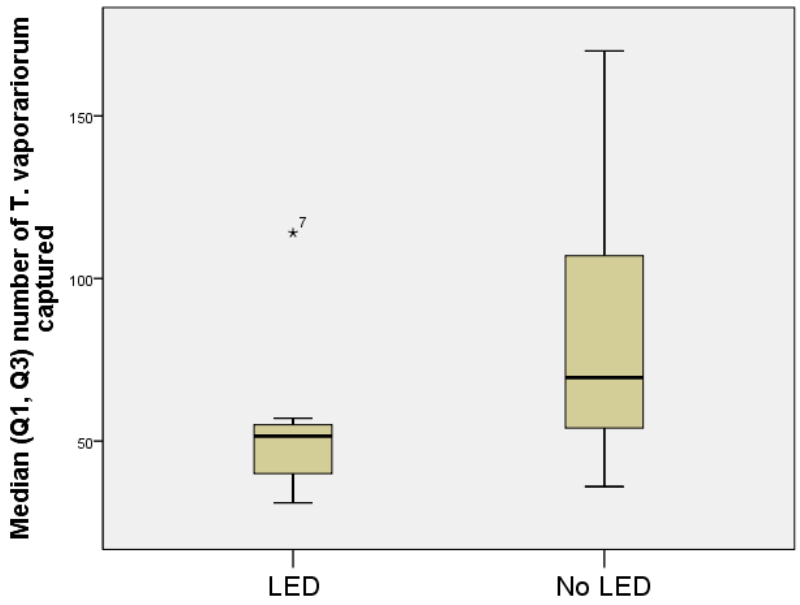

Figure 51. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *T. vaporariorum* captured on blue (480 nm) LED and standard yellow sticky traps in batch 1 at site 1 (12/09/13 – 19/09/13).

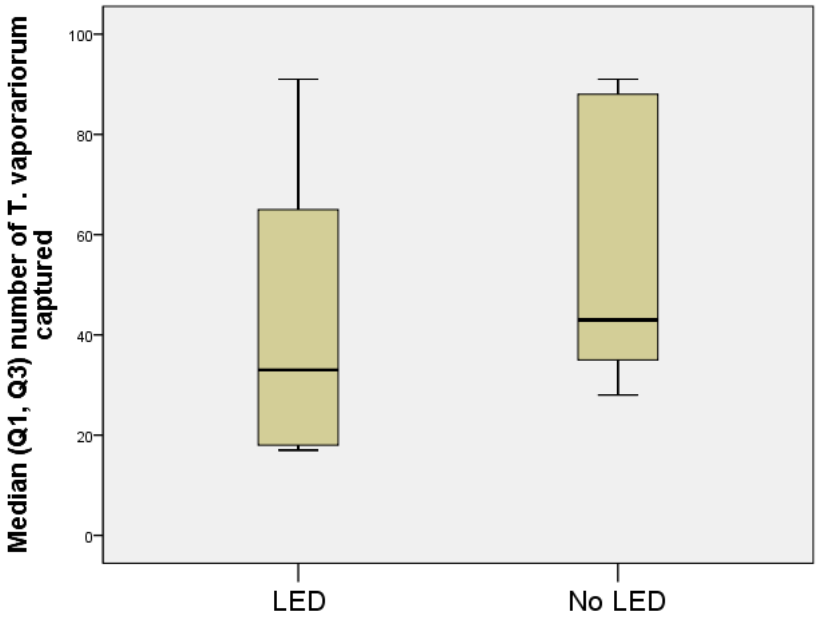

Figure 52. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *T. vaporariorum* captured on blue (480 nm) LED and standard yellow sticky traps in batch 2 at site 1 (19/09/13 – 26/09/13).

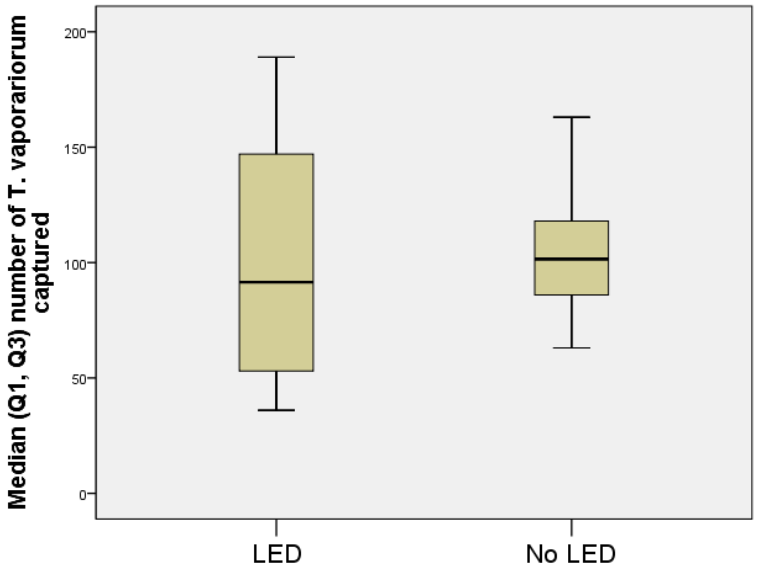

Figure 53. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *T. vaporariorum* captured on blue (480 nm) LED and standard yellow sticky traps in batch 3 at site 1 (26/09/13 – 03/10/13).

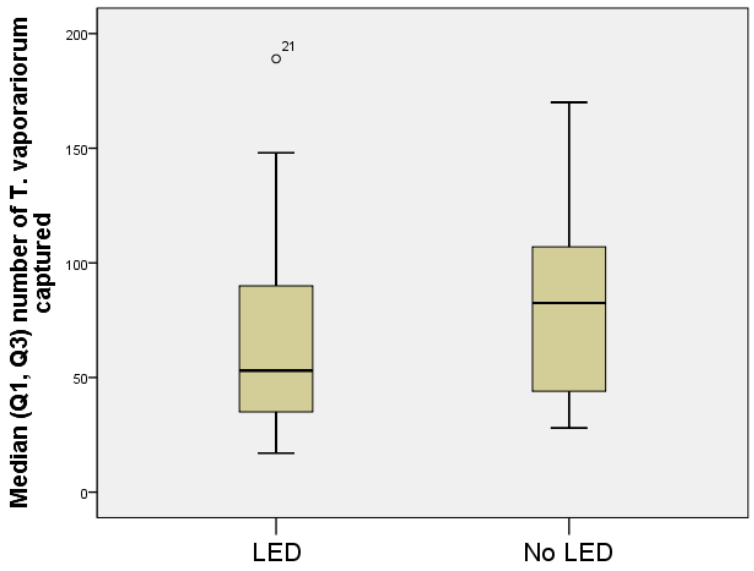

Figure 54. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *T. vaporariorum* captured on blue (480 nm) LED and standard yellow sticky traps across study period at site 1 (12/09/13– 03/10/13).

Site 5

Trialeurodes vaporariorum: Green (520 nm) LEDs (21/08/13 – 02/10/13)

No significant differences were found between LED traps and standard yellow sticky traps in any of the individual batches (Fig. 55). No significant difference was found between the trap types across the entire trapping period (P=0.518) (Table 38) (Fig. 56).

Table 38. No. of *F. occidentalis* captured on green (520 nm)) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3.

Batch number	***Dates***	***F statistic***	***P value***	***Standard traps. Mean (±SE)***	***LED traps. Mean (±SE)***
Batch 1	21/08/13 - 04/09/13	$F_{1,10} = 0.895$	0.366	27.67 (±3.37)	42.83 (±10.89)
Batch 2	04/09/13 - 18/09/13	$F_{1,10} = 2.325$	0.158	12.00 (±1.44)	9.17 (±3.29)
Batch 3	18/09/13 - 02/10/13	$F_{1,10} = 0.237$	0.637	36.83 (±8.71)	38.00 (±11.55)
Entire Study Period	21/08/13 – 02/10/13	$F_{1,34} = 0.518$	0.518	25.5 (±3.86)	30 (±6.23)

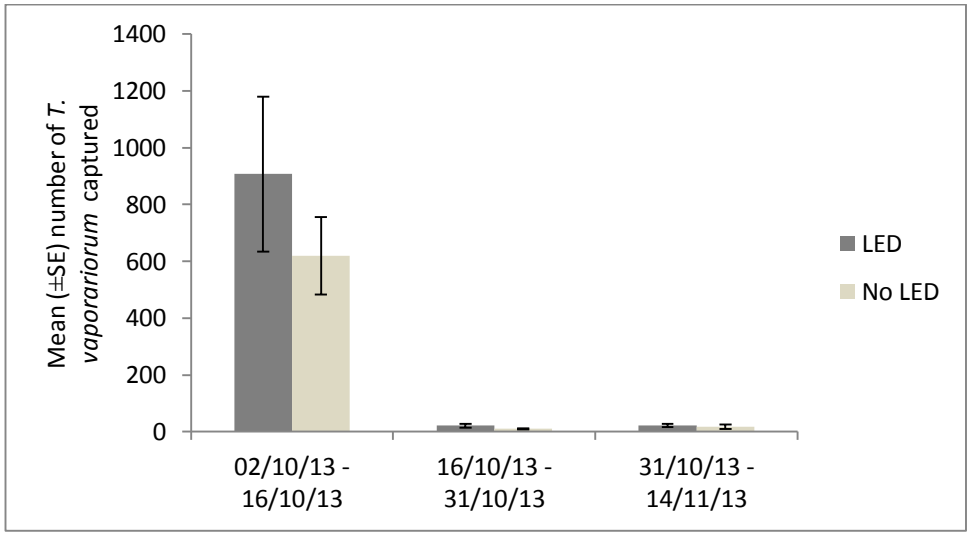

Figure 55. Mean (±SE) number of *T. vaporariorum* captured on green (520 nm) LED and standard yellow sticky traps at site 5.

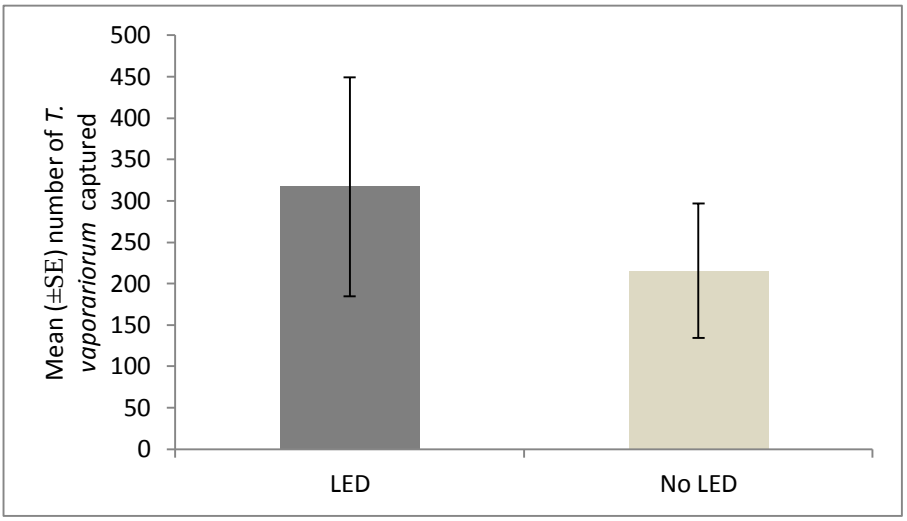

Figure 56. Mean (±SE) number of *T. vaporariorum* captured on green (520 nm) LED and standard yellow sticky traps across study period at site 5 (21/08/13 – 02/10/13).

Trialeurodes vaporariorum: Blue (480 nm) LEDs (02/10/13 – 14/11/13) (site 5)

No significant differences were found between LED traps and standard yellow sticky traps in any of the individual batches (Fig. 57). No significant difference was found between the trap types across the entire trapping period (P=0.501) (Table 39) (Fig. 58).

Table 39. No. of *F. occidentalis* captured on blue (480 nm)) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3. **Median (Q1, Q3).

Batch number	***Dates***	***F statistic***	***P value***	***Standard traps. Mean (±SE)***	***LED traps. Mean (±SE)***
Batch 1	02/10/13 - 16/10/13	$F_{1,10} = 0.163$	0.695	12.00 (±3.97)	14.17 (±3.61)
Batch 2	16/10/13 - 31/10/13	$F_{1,10} = 0.946$	0.354	6.67 (±1.76)	11.17 (±4.28)
Batch 3	31/10/13 - 14/11/13	$F_{1,10} = 0.271$	0.614	11.17 (±2.54)	14.33 (±5.52)
Entire Study Period	02/10/13 – 14/11/13	U = 311.5, Z= -0.682	0.501	9.94 (±1.68)	13.22 (±2.49)

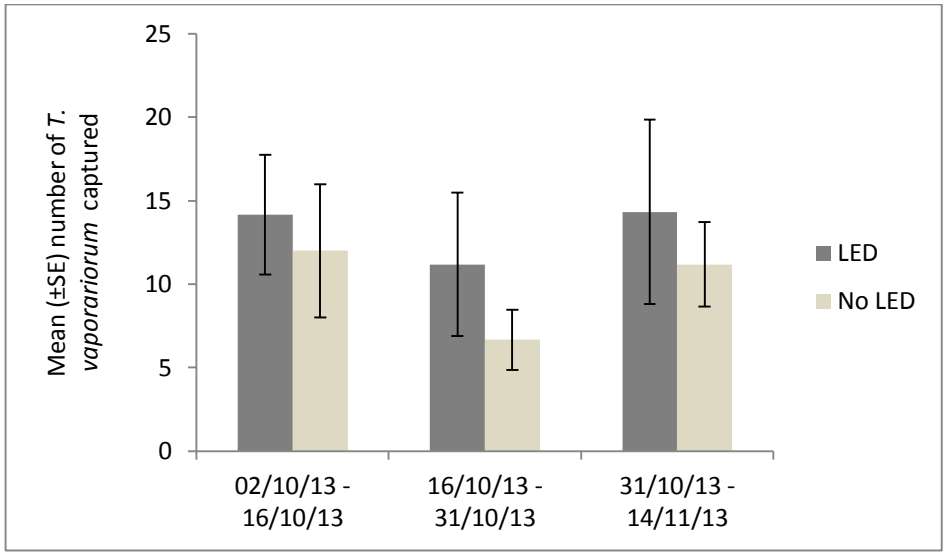

Figure 57. Mean (±SE) number of *T. vaporariorum* captured on blue (480 nm) LED and standard yellow sticky traps at site 5.

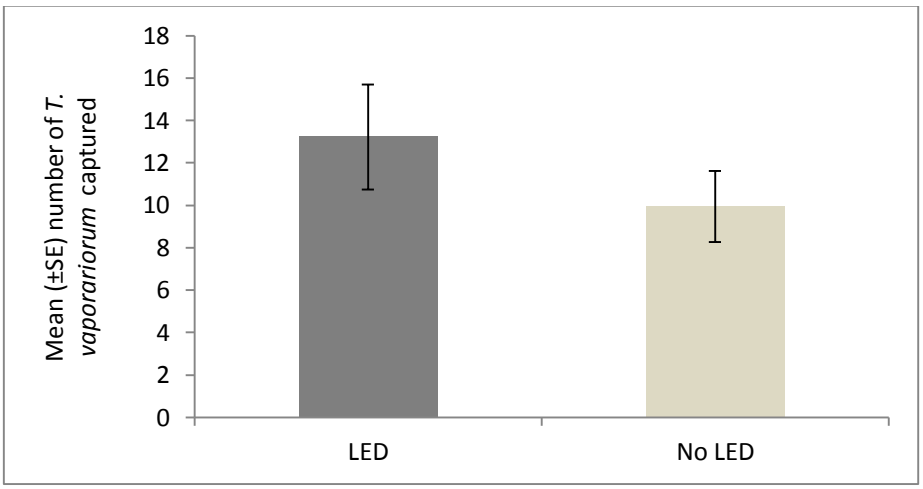

Figure 58. Mean (±SE) number of *T. vaporariorum* captured on blue (480 nm) LED and standard yellow sticky traps across study period at site 5 (02/10/13 – 14/11/13).

Site 6

Trialeurodes vaporariorum: Green (540 nm) LEDs (10/04/13 – 27/04/13)

There were no significant differences in the number of *T. vaporariorum* captured between the trap types ($F_{1,10} = 2.105$, P=0.177) (Fig. 59). LED traps captured a mean (±SE) of 61 (±8.22) and standard traps captured 14.44 (±1.7).

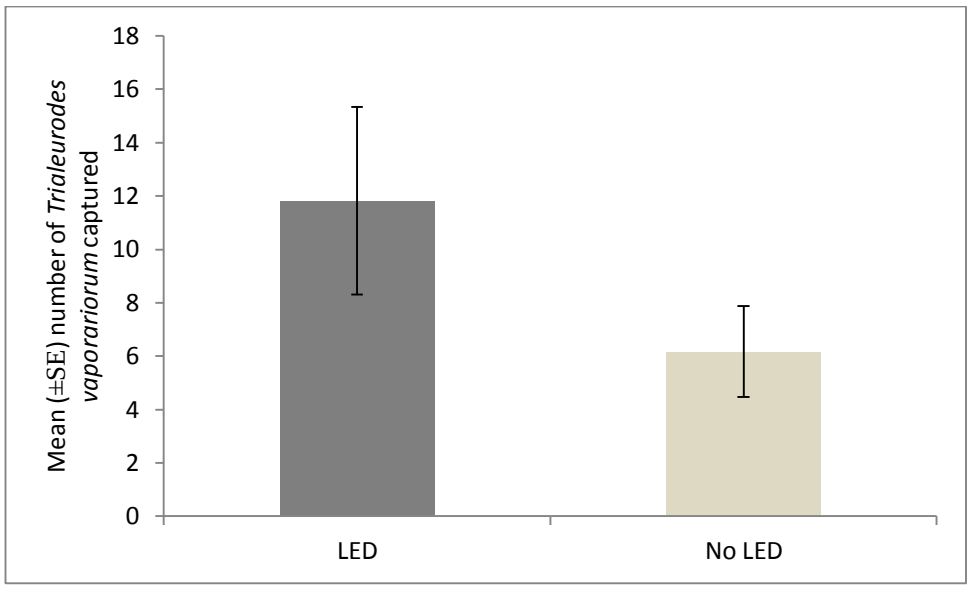

Figure 59. Mean (±SE) number of *T. vaporariorum* captured on green (540 nm) LED and standard yellow sticky traps across study period at site 7 (10/04/2013 – 27/04/2013).

Plutella xylostella

Site 3

Plutella xylostella: Green (540 nm) LEDs (11/10/12 – 22/11/12)

LED traps captured more *P. xylostella* than standard yellow sticky traps in batches one, two, and across the study period (Fig. 60, Fig. 61, and Fig. 62) (Table 40).

Table 40. No. of *P. xylostella* captured on green (540 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3. *Significant at P < 0.05.

Batch number	***Dates***	***LED traps. Median (Q1, Q3)***	***Standard traps. Median (Q1, Q3)***
Batch 1	11/10/12 - 08/11/12	2 (0, 3.5)	0 (0, 0)
Batch 2	08/11/12 - 22/11/12	1 (0, 1.75)	0 (0, 0)
Entire study period	11/10/12 – 22/11/12	0.5 (0, 0.5)	0 (0, 0)

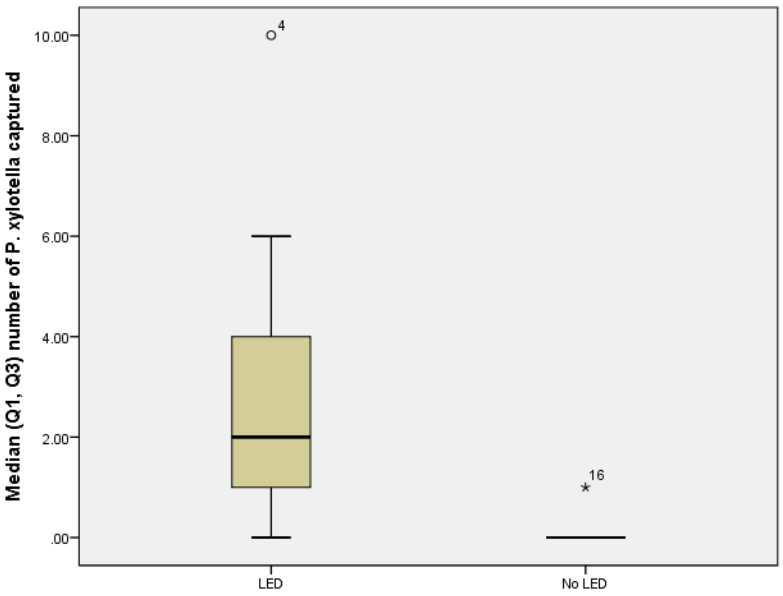

Figure 61. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of P. xylostella captured on green (540 nm) LED and standard yellow sticky traps in batch 1 at site 3 (11/10/12 - 08/11/12).

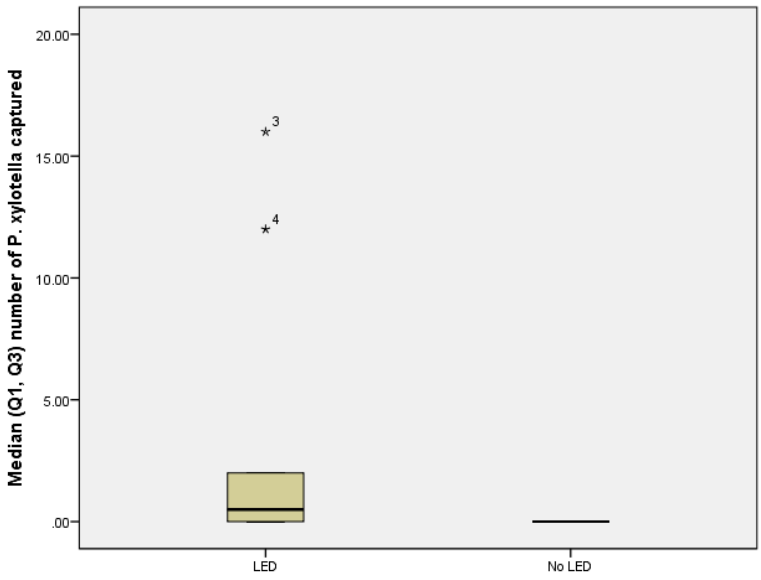

Figure 62. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *P. xylostella* captured on green (540 nm) LED and standard yellow sticky traps in batch 2 at site 3 (08/11/12 - 22/11/12).

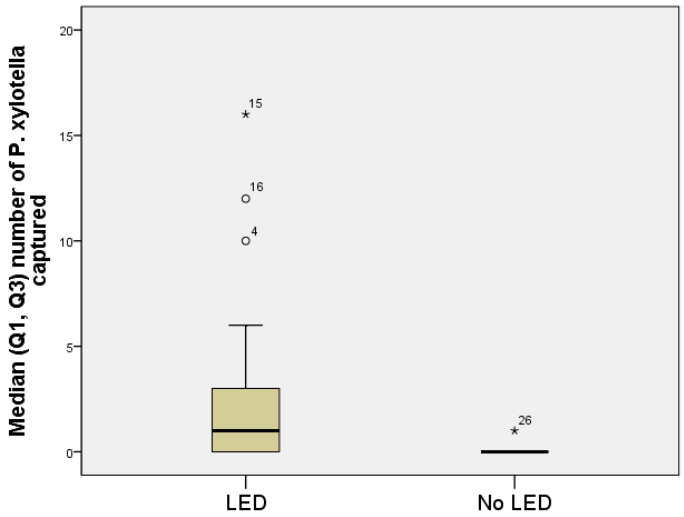

Figure 63. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *P. xylostella* captured on green (540 nm) LED and standard yellow sticky traps across the study period at site 3 (11/10/12 – 22/11/12).

Plutella xylostella: Blue (480 nm) LEDs (02/09/13 – 20/09/13) (site 3)

No significant differences were found in batches 1 (P=0.130) (Fig. 64) or 2 (P=0.132) (Fig. 65, Fig. 66). Significantly more *P. xylostella* were captured by LED traps in batch 3 (P=0.004). A significant difference was found over the study period (P<0.001) (Table 41) (Fig. 67).

Table 41. No. of *P. xylostella* captured on blue (480 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 3. *Significant at 0.05.

Batch number	***Dates***	***Mann-Whitney U***	***P value***	***LED traps. Median (Q1, Q3)***	***Standard traps. Median (Q1, Q3)***
Batch 1	02/09/13 – 09/09/13	U = 17.5, Z = -1.596	0.130	8 (0.75, 14.25)	0 (0, 1)
Batch 2	09/09/13 - 16/09/13	U = 8.5, Z = -1.592	0.132	11 (2.75, 15.5)	0 (0, 0.75)
Batch 3	13/09/13 - 20/09/13	U = 1, Z = -2.823	0.004*	5.5 (3.5, 6.75)	0 (0, 0)
Entire study period	02/09/13 – 20/09/13	U = 75.5, Z = -3.515	<0.001*	6.5 (1, 14.25)	0 (0, 1)

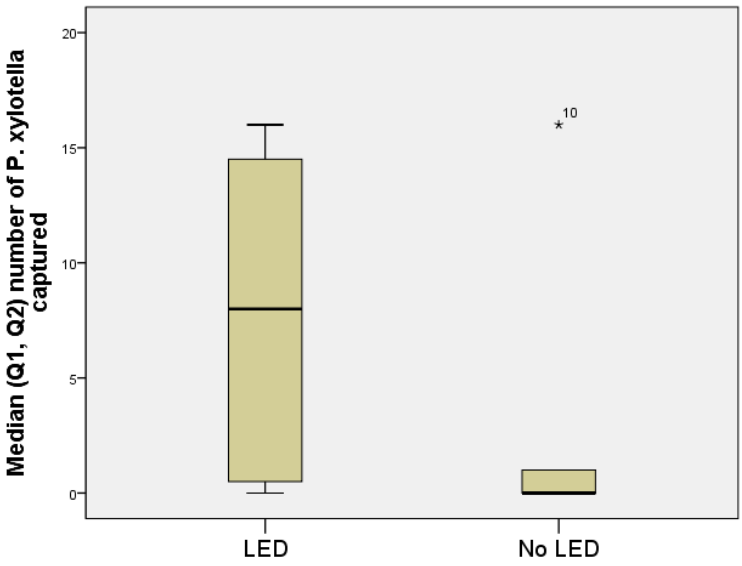

Figure 64. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *P. xylostella* captured on blue (480 nm) LED and standard yellow sticky traps in batch 1 at site 3 (02/09/13 – 09/09/13).

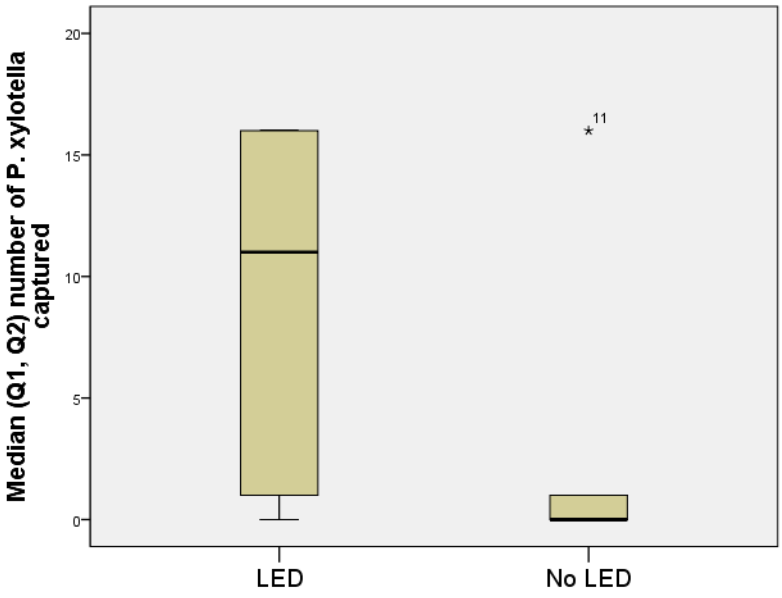

Figure 65. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *P. xylostella* captured on blue (480 nm) LED and standard yellow sticky traps in batch 2 at site 3 (09/09/13 - 16/09/13).

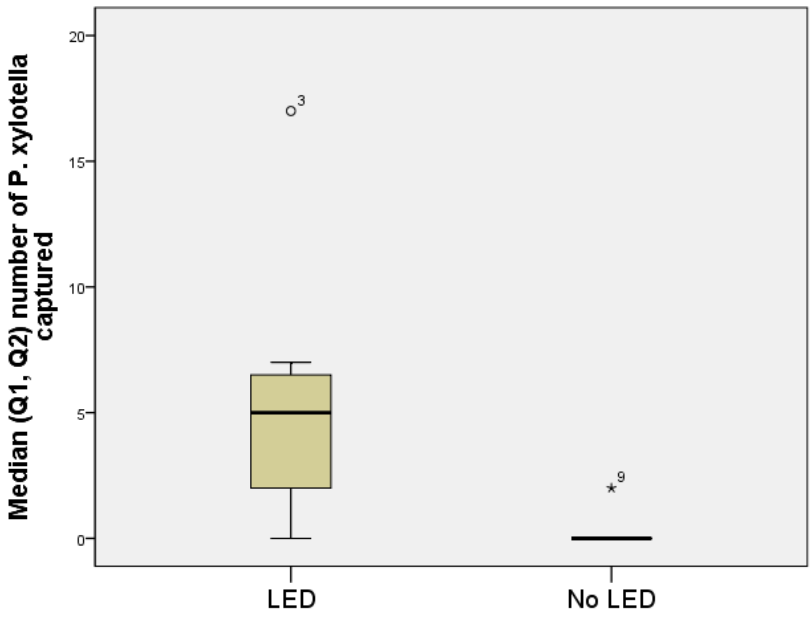

Figure 66. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *P. xylostella* captured on blue (480 nm) LED and standard yellow sticky traps in batch 3 at site 3 (13/09/13 - 20/09/13).

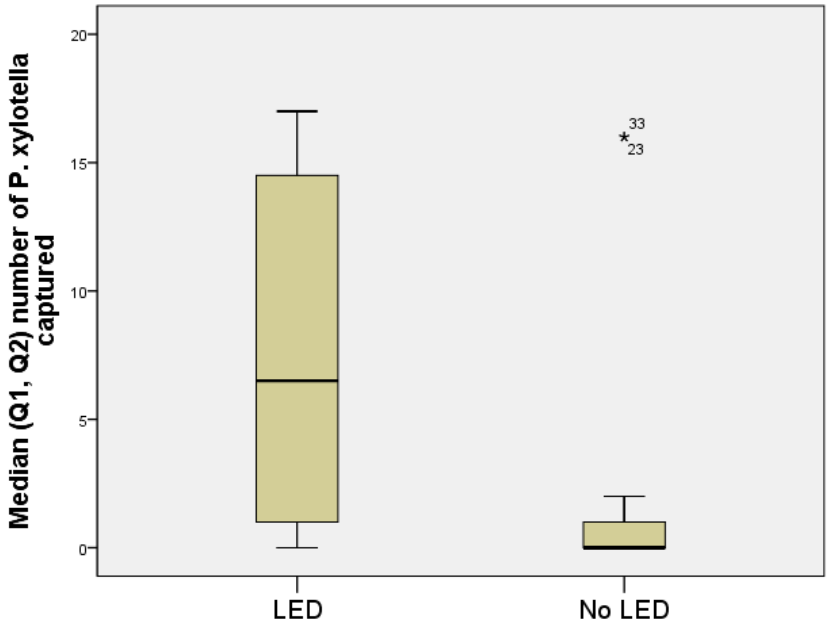

Figure 67. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *P. xylostella* captured on blue (480 nm) LED and standard yellow sticky traps across the study period at site 3 (02/09/13 – 20/09/13)..

Results Summary

- *B. differmis:* an increase in the number of *B. differmis* captured by green (540 nm) LED equipped yellow sticky traps was found at all three sites.
- *B. differmis:* a slight increase in the number of *B. differmis* captured by blue (480 nm) LED equipped yellow sticky traps was found.

- *F. occidentalis:* no significant increase in the number of *F. occidentalis* captured by yellow sticky traps equipped with green (540 nm) or blue (480 nm) LEDs was found.

- *T. vaporariorum:* a small increase in number of *T. vaporarioum* to yellow sticky traps equipped with green (540 nm) LEDs was found.
- *T. vaporariorum*: A small decrease in the number of *T. vaporariorum* captured by blue (480 nm) LED equipped yellow sticky traps was found.

- *P. xylostella*: A significant increase in the number of *P. xylostella* captured by green (540 nm) or blue (480 nm) LED equipped yellow sticky traps was found.

Discussion

Bradysia differmis

The main findings of this trap comparison were an increase in the number of *B. differmis* captured using both 540 nm and 480 nm LEDs. The difference in the numbers captured by the two trap types differed between sites, suggesting site specific factors influenced the success rate of the LED equipped traps.

There were notable differences in the number of *B. differmis* captured at the different study sites. Green (540 nm) equipped yellow sticky traps captured 29% more at site 1, 37.8% more at site 2 compared to the standard yellow sticky traps. Although no overall significant result was found for the entire study period at site one, 129.2% more *B.* differmis were captured at site 3 in batch 1. The absence of statistically significant results for batches 2 and 3 is likely a result in the sharp decline of the *B. differmis* population at this site due to pest control measures. It is probable the differences between the sites are due to either the growing methods used at the sites, the population sizes of *B. differmis*, or both.

Sites 1 and 2 grow their crops raised from the ground on benches, while site 3 grows their crops on the ground, using capillary matting covered in perforated plastic sheets. The capillary matting creates a humid environment where high populations of *B. difformis* are common (pers comm grower; Santos *et al.*, 2012; Yang, *et al.*, 2015). The high population of *B. difformis* at site 3 may result in more active individuals than those found at the other sites. As competition for resources will theoretically be greater than at sites 1 or 2, there may be greater levels of activity associated with a greater difficulty in finding food and egg laying sites. Additionally, a high population may encourage dispersal around the glasshouse seeking areas with less resource competition. This increase in flight time increases the chances of *B. difformis* being captured by the traps.

The findings at site 3 were similar to those of Chen *et al.* (2004b), where the capture of a related species, *Bradysia coprophila,* was increased by attaching a green (no wavelength given, presumably 530 nm based on other research by this group) LED to standard yellow sticky trap. Chen *et al.* (2004b) observed a difference of 136.6% over their study period. It is worth noting that Chen *et al.* (2004b) found a greater difference in the number of *B. coprophila* captured during the summer months, skewing the overall increase in capture and indicating seasonality may be a factor in the positive behavioural response of *B. coprophila* to these traps (Tauber and Tauber, 1981). Unfortunately, due to the short growing season within the UK, it was not possible to investigate seasonality during this project.

At site 3 no overall significant difference was observed between blue (480 nm) LED equipped traps and standard traps. However, there was a pattern of LED traps capturing more than standard traps, with significant differences in batches 4 and 6.

B. difformis feed on organic matter within the soil, rather than the plants themselves, so their positive behavioural response to the green (540 nm) wavelength may be explained by the expected presence of decaying organic matter beneath plants, as well as their egg laying behaviour. Eggs are laid near plant stems to provide their larvae easy access to food upon emerging (Malais and Ravenberg, 2003), so remaining close to an appropriate site may provide an advantage, particularly when considering the large number of eggs laid by the females (~50-1000) (Nielson 1997; Malais and Ravenberg, 2003).

A strong preference for light within the blue region of the spectrum would be unexpected for *B. difformis* given their ecology. An attraction to light in the blue region of the spectrum has been previously demonstrated in a related species *Bradysia pauper* (Ishitani *et al.*, 1997);

however, the light source used also provided output in the UV spectrum, which has commonly been found attractive to insects (Hu and Stark, 1977).

In summary it was shown that the addition of green (540 nm) LED's to yellow sticky traps increases their effectiveness for capturing *B. differmis*, with results varying greatly between the different sites. The addition of blue (480 nm) LED's was less successful, although a general pattern of an increased number of *B. differmis* captured was found this was not large enough to recommend using this wavelength to capture *B. differmis.* Further research is required to determine the factors contributing to the success of green (540 nm) LED equipped yellow sticky traps at site 3.

Frankliniella occidentalis

No significant differences were observed when comparing sticky traps equipped with either green (540 nm) or blue (480 nm) LEDs to standard yellow sticky traps.

The comparison between standard yellow sticky traps and those equipped with green (540 nm) LEDs produced similar results to those of Chen *et al.* (2004a), where no significant differences were found when comparing green (530 nm) and standard yellow sticky traps.

There were no significant differences found when comparing standard yellow stick traps to blue (480 nm) LED equipped traps. This is in contrast to results found by Chu *et al.* (2005) where a greater number of *F. occidentalis* were captured by blue sticky traps equipped with blue (460 nm) LEDs. These results may be explained by the use of yellow sticky traps here, and the distance dependent responses to light demonstrated by *F. occidentalis* (Chu *et al.*, 2005), where more *F. occidentalis* were captured when released 83cm away from the light source than 165cm away.

Chu *et al.* (2005) compared the positive behavioural response of *F. occidentalis* to a range of wavelengths by releasing *F. occidentalis* into a dark room 83cm and 165cm from the light source, and found that a much greater number of *F. occidentalis* were captured by UV traps when compared to other traps when the light sources were placed closer to the point of the release. This suggests that the response of *F. occidentalis* to light occurs over short distances. Although *F. occidentalis* are likely closer to the sticky traps (traps were located ~20-40cm above the crops (varying as plants grew and traps were changed)) used here than those released 83cm away by Chu *et al.* (2005), this distance dependent light response may exist at shorter distances. It is also important to note than if the *F. occidentalis* are below the sticky

trap, then then the light output of the LED will be greatly diminished, as the light is angled at 30°.

These distant dependent responses to light, coupled with the results found here, suggest that the yellow sticky traps and blue (480 nm) combination were ineffective as the relatively large reflectance area of the blue sticky trap may be required to lure *F. occidentalis* close enough for the blue LED light to create a difference in the number of *F. occidentalis* captured. Using a brighter blue LED may solve this issue, although there are safety concerns with using bright blue lights at eye level which prohibits this experiment in a glasshouse frequented by workers (Barker *et al.*, 2011; Kernt *et al.*, 2012). Although blue sticky traps are preferred for monitoring *F. occidentalis*, the decision to use yellow sticky traps here was made because the growers taking part in this project did not use blue sticky traps in their operations, and this project was designed to fit into their existing monitoring system.

Interestingly, a non-significant pattern of reduced capture efficiency was observed when equipping yellow sticky traps with blue (480 nm) LEDs at site 3. This may be due to a colour opponent mechanism (Döring and Chittka, 2007b), with the combination of these wavelengths reducing the positive behavioural responses exhibited by *F. occidentalis*. This pattern was not observed at site 5, and no other studies have combined yellow sticky traps and blue LED for capturing thrips. An alternative explanation may be an interaction between insects caught on the traps, with the large number of *B. difformis* captured on the traps reducing the positive behavioural response of *F. occidentalis* to the traps due to olfactory or visual cues. For example, by covering the trap and reducing the visibility of the coloured surface.

In summary it was shown that equipping yellow sticky traps with green (540 nm) or blue (480 nm) LEDs does not increase their effectiveness for capturing *F. occidentalis*, although a reduction in capture was observed at site 3 when attaching blue (480 nm) LEDs to yellow sticky traps.

Trialeurodes vaporariorum

No significant differences in the number of *Trialeurodes vaporariorum* captured were observed when comparing sticky traps equipped with either green (540 nm) or blue (480 nm) LEDs to standard yellow sticky traps.

A small, but not significant, increase in the number of *T. vaporariorum* captured was found in site 5 for traps equipped with green (540 nm) LEDs. Although this was consistent with findings by Chu *et al.* (2004), where a 31% increase was found using green (530 nm) LEDs, the strong spectral sensitivity to this wavelength, as well a previously demonstrated behavioural preference to LEDs in the 520-530 nm region suggest it may be possible to improve on these results and increase the number of *T. vaporiorum* captured (Mellor *et al.,* 1997; Jahan *et al.,* 2013). Site 5 grows their crops on narrow benches, resulting in the traps being placed directly above the crop, limiting their visibility to *T. vaporariorum.* A larger increase in the number of *T. vaporiorum* captured may have been found if the traps were placed in a better position.

The effectiveness of green LEDs for trap enhancement for capturing *T. vaporariorum* is clear, and Chu *et al.* (2004) found equipping a white-based plastic cup trap with a green (530 nm) LED increased the capture of *T. vaporariorum* by 90%. This large increase in capture clearly does not carry over to the combination of yellow sticky traps and green LEDs (of either 530 nm or 540 nm). This may simply be a matter of the angle of the light, as the clip on devices used both here, and by Chu *et al.* (2004), project light outwards over the crop, rather than towards it. A reflector used to angle the light downwards towards the crop may be a valuable addition to LED attachments for enhancing the capture of *T. vaporariorum*. Alternatively, sticky traps may be placed closer to the crop, although this is not always practical, which is supported by the findings by Gillespie and Quiring (1992) that traps close to the ground are more effective for capturing *T. vaporariorum.*

In summary it was shown that equipping yellow sticky traps with green (540 nm) LEDs offers a small increase in their effectiveness for capturing to *T. vaporariorum*; however, this is not enough of an increase to be of practical value. Blue (480 nm) LED equipped traps captured fewer *T. vaporiorum* than standard traps, though this difference was not significant.

Plutella xylostella

The main findings of this study are a significant increase in the capture of *P. xylostella* for yellow sticky traps equipped with green (540 nm) or blue (480 nm) LEDs.

P. xylostella have previously been shown to preferential select green LEDs (520 nm) when compared with a range of other wavelengths including blue (470 nm) and yellow (590 nm) (Cho and Lee, 2012). Sivapragasam and Saito (1986) suggest their preference for yellow when compared against blue, red, and clear sticky traps is a result of the high spectral

reflectance of yellow sticky traps within the green region of the light spectrum, i.e. the super-normal foliage type stimulus proposed by Prokopy and Owens (1983). Despite this attraction it is unusual for *P. xylostella* to be captured by the standard yellow sticky trap, as they typically fly below the crop canopy, rarely straying to the height sticky traps are typically placed (Hallet, 1986).

The addition of both LED types to yellow sticky traps at site 3, greatly increased the number of *P. xylostella* captured, although there was a high degree of variation between traps and an overall low number of *P. xylostella* were captured. The low number captured is to be expected, as the traps were placed at a commercial facility which does not grow plants of the Crucifer family, the sole plant group *P. xylostella* feeds on. The presence of *P. xylostella* in this facility may be due to nearby facilities growing members of this plant family, or migratory actions.

P. xylostella are capable of long distance migration, and have been reported to migrate distances over 3000km while flying continuously (Thygesen, 1968; Lokki *et al.*, 1978; Bretherton, 1982), although more recently it has been suggested that migration occurs only at night time, with an estimated flight endurance of at least 8 hours (Chapman *et al.*, 2002). While these migrations are windborne (Lokki *et al.*, 1978; Chapman *et al.*, 2002), considering the large distances it is likely that *P. xylostella* possess a compass sense to facilitate migration. Lunar and celestial navigation have been discounted in the moth *Autographa gamma* (Chapman *et al.*, 2008); however, this is not necessarily the case for *P. xylostella*. If lunar or celestial navigation are used by *P. xylostella*, the presence of LED lights would be expected to disrupt the flight patterns of individuals migrating through this facility.

This disruption in flight pattern is not necessarily related to migratory behaviour. Light trap effectiveness for the Noctuid moths (Lepidoptera: Noctuidae) is known to decrease as moonlight increases in brightness, without altering flight activity (Muirhead-Thomson 1991; Yela and Holyoak, 1997). This provides evidence that moonlight competes with the light traps, suggesting that moonlight alters flight patterns. Precise local navigation (e.g. foraging) via lunar and celestial cues have been demonstrated in other arthropods, and it is possible *P. xylostella* possess this ability (Ugolini *et al.*, 2002; Dacke *et al.*, 2011).

The positive behavioural response exhibited by *P. xylostella's* in response to green (540 nm) LED equipped traps may be the result of a direct attraction to this wavelength due to their diet and egg laying behaviour. *P. xylostella* eggs are laid on cruciferous plants, which are the

food source to their larvae (Macharia *et al.*, 2005). Many cruciferous plants green in colour, so an attraction to wavelengths in the green region of the spectrum would be advantageous.

The data from this study may appear to suggest that blue (480 nm) LEDs are more effective than green (540 nm) LEDs for luring *P. xylostella;* however, it is important to note that these experiments were conducted during separate growing seasons a year apart. A greater population of *P. xylostella* may have been present during the 2013 (blue LED) season, accounting for the greater numbers captured. Given the ecology of *P. xylostella* and the LED preference comparisons conducted by Cho and Lee (2012), the green (540 nm) LED is likely to be the more effective of the two LEDs used here.

In summary it was shown that equipping yellow sticky traps with green (540 nm) or blue (480 nm) LEDs increases their effectiveness for capturing *P. xylostella*, an affect which may be due to the disruption of flight navigation cues rather than an increase in attraction. This finding is of particular interest as the standard sticky traps were ineffective for monitoring *P. xylostella* at the study site, typically capturing no individuals. Further research is required to gain further understanding of this effect, in particular a direct comparison between green and blue LED equipped sticky traps, as well as traps with light angled downwards towards the crop.

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Chapter 6 Beneficial Insects

Abstract

Beneficial insects may be used as part of a pest management strategy, or to provide direct benefit to the crops. These insects are typically predators or parasitoids of pest insects, or pollinators such as domesticated honey bees (*Apis mellifera*). Light-emitting diodes (LEDs) have been shown to attract some species of beneficial insect, so an assessment of the impact trap enhancements may have on these species is essential.

To assess the impact of equipping yellow sticky traps with LEDs may have on beneficial insects, comparisons between standard yellow sticky traps and those equipped with green (540 nm) or blue (480 nm) LEDs were carried out at four commercial growing facilities. A mass release experiment using *Encarsia formosa* comparing standard yellow sticky traps against those equipped with green (540 nm) LEDs was also performed.

No significant differences were found between green (540 nm) LED equipped traps and those without for *E. formosa*, and a significant decrease in the capture of the shore fly parasitoid *Kleidotoma psiloides* Westwood (Hymenoptera: Figitidae) was observed.

Introduction

Beneficial insects can aid in pest management. These are typically parasitoids or predatory insects, some of which are commercially available. Beneficial insects are of particular use against pest species which possess, or rapidly develop, resistance to chemical pesticides such as *Plutella xylostella* and *Frankliniella occidentalis* (Gorman *et al.*, 2002; Quesada-Moraga *et al.*, 2005; Sarfraz *et al.*, 2005; Hu, *et al.*, 2014; Steinbach *et al.*, 2015). Beneficial insects may also provide direct benefit to the crop, for example pollination services provided by insects benefit crop yields, and have an estimated value of ~£400 million per annum within the UK (Klien *et al.*, 2007; POST, 2010). The majority of global agriculture pollination services are performed by bees (*Apidae*), with an estimated 80% being performed by domesticated honey bees (*Apis mellifera* (Linnaeus)) (Carreck and Williams, 1998). A number of beneficial insects will be discussed here.

Commercially available predatory beneficial insects include the predatory mite *Neoseiulus cucumeris* (formerly *Amblyseius cucumeris*), which are effective for controlling *F. occidentalis* on protected edibles and ornamentals (Dissevelt *et al*., 1995; Gillespie, 1989; Jacobson, 1997; De Courcy Williams, 2001; Shipp and Ramakers, 2003; Shipp and Wang, 2003). For example Wang and Shipp (2003) found that at an *F. occidentalis* density of 120.8±24.2 per m^2, the release of 1000 *N. cucumeris* per plant every four weeks was curative. On greenhouse Cyclamen crops which has been artificially infested with *F. occidentalis*, the introduction of *N. cucumeris* at 50, 200, and 350 mites per m^2 per week led to a reduction in the populations of *F. occidentalis* when compared with a control, with larger numbers of mites resulting in a larger decrease in population size (De Courcy Williams, 2001). Anthocorid flower bugs may also be used in *F. occidentalis control* (Van Lenteren, 2000; Silveria *et al.*, 2004), for example *Orius* spp. can be used to control *F. occidentalis* in protected edibles and ornamentals, such as sweet pepper, roses and chrysanthemums (Silveria *et al.*, 2004; Chow *et al.*, 2010; Weintraub *et al.*, 2011). Other predators include the predatory lacewing *Chrysoperla carnea* (Stephens, 1836) and the predatory beetle *Delphastus catalinae* (LeConte, 1852). *Chrysoperla carnea* is effective against a broad range of soft-bodied arthropod pests such as aphids, whiteflies, and thrips (Pappas *et al.*, 2007; Yadav and Pathak, 2010; Hassanpour *et al.*, 2015), while *Delphastus catalinae* are used to control whitefly (Gerling, Alomar, and Arnò, 2001; Lucas *et al.*, 2004; *Legaspi*, Simmons, and Legaspi, 2006 Ricon, Cañas, and Hoy, 2016).

The predatory mite *Stratiolaelaps scimitus* (formerly *Hypoaspis miles*) can be used for the management of fungus gnats in mushroom cultivars (Ydergaard, Enkegaard, and Brodsgaard, 1997; Jess and Bingham, 2004; Jess and Schweizer, 2009). There is evidence that this mite may be used to control *Bradysia* spp. in protected cyclamen and poinsettia crops (Chambers, Wright, and Lind, 1993). *Stratiolaelaps scimitus*, and *Hypoaspis aculeifer* (G. Canestrini, 1884), may also be used for controlling *F. occidentalis* in glasshouse conditions (Premachandra *at al.*, 2003; Cloyd, 2009).

Parasitoid options are more limited. *Encarsia formosa*, a parasitic wasp of whitefly, is considered to be more effective than chemical pesticides by growers (van Lenteren, *et al.*, 1996). *Encarsia formosa* is highly effective at controlling whitefly in protected edibles (van Roermund, van Lenteren, and Rabbinge, 1997; Hoddle, Van Driesche, and Sanderson, 1998; De Vis and van Lenteren, 2008), and may be used to provide control in protected ornamentals (McMahon *et al.*, 1992). In poinsettia glasshouses *E. formosa* provided similar results to chemical control for *Trialeurodes vaporariorum* (McMahon *et al.*, 1992); however, this should not be considered universal and a similar poinsettia glasshouse experiment found *E. formsa* failed to control *Bemesia argentifolii* when compared with chemical pesticides (Hoddle and Driesche, 1996). Poor performance was also found in cut gerbera (*Gerbera jamesonii* L.), where *E. formosa* released onto caged gerbera under glasshouse conditions failed to control *T. vaporariorum* (Berndt and Meyhöfer, 2007). On some protected herbs (e.g. mint, basil, sages) *E. formosa* are often found to be unreliable (Bennison *et al.* 2001). The parasitic wasp *Aphidius matricariae*, is known to parasitize 40 species of aphid (Giri *et al.*, 1982), and is highly effective for *Myzus persicae* management (Desneuz *et al.*, 2006).

Direct benefit to the crop may be provided by pollinators, such as honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris* L. (Linnaeus, 1758)) (Carreck and Williams, 1998; Velthuis and van Doorn, 2006). *Bombus terristris* are available in Europe, and are primarily used as pollinators in protected edible crops where they can increase the yield of the crops, for example the release of 100-120 worker bees in two 500m^2 glasshouses (50-60 bees per glasshouse) resulted in increased yields of tomato (17%) and eggplant (23%) when compared with a control group (Abak et al., 1995).

The effectiveness of beneficial insects varies considerably based on environmental conditions (Svendsen, *et al.*, 1999; Mohaghegh *et al.*, 2001; Yadav and Pathak, 2010), pest densities (Amiri-Jami and Sadeghi-Namaghi, 2014), prey type (Hassanpour *et al.*, 2011), and hunger levels (Hassanpour *et al.*, 2015). With this in mind the appropriate beneficial insect must be chosen for the appropriate environment. Furthermore, interactions between

beneficial insects must be considered, as inappropriate combinations can reduce their effectiveness. For example Chow *et al.* (2010) found that releasing both predators *Orius insidiosus* (Say) (Hemiptera: Orius) and *Amblyseius swirskii* on roses, proved no more effective for managing *F. occidentalis* than *A. swirskii* alone. This was believed to be due to *O. insidiosus* feeding on *A. swirskii*. Negative interactions have also been observed, with the dual release of the predatory mites *Phytoseiulus persimilis* Evans (Mesostigmata: Phytoseiidae) and *Neoseiulus cucumeris* proving less effective for control of *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) in strawberry crops, than when one species was released alone (Fitzgerald *et al.*, 2007).

The addition of LEDs to traps has been shown to increase the number of certain beneficial insects captured, so care must be taken when selecting a wavelength for trap enhancement, as there may be a negative impact on beneficial insects. For example, Chen *et al.* (2004), found that the addition of green (no wavelength given) LEDs to yellow sticky traps increase the capture of minute pirate bugs, parasitic wasps, and rove beetles. Furthermore, numerous species of bee are known to be attracted to UV and blue (Menzel and Shmida, 1993; Gumbert, 2000), so the addition of traps outputting strongly in these spectrums may reduce their effectiveness as pollinators. In some circumstances the attraction of beneficial insects to traps is desired, and a variety of traps can be a useful tool for monitoring the population levels of beneficial insects (Boetlpaepe, 1991; Felland *et al.*, 2005; Wallis and Shaw, 2008). In some beneficial species, the effectiveness of these traps can be altered by the use of different kinds of active light source (Nabli *et al.*, 1999). For example, in a comparison between black-light blue, blacklight, aquarium light, and cool white lamps (15W) *Ophion* sp. more were captured by black-light blue lamp traps (peak wavelength ~365 nm, 15W) and more *Chrysopa* spp. were captured by cool white lamps traps (15W) (Nabli *et al.*, 1999).

To ensure that the addition of LEDs to yellow sticky traps did not increase the capture of beneficial insects, these were recorded when found in sufficient quantities. Additionally, a mass release experiment was conducted with *Encarsia formosa* comparing standard yellow sticky traps to those equipped with green (540 nm) LEDs.

Materials and Methods

Study Sites

The sites and traps used here were the same as in the pest capture experiment (Chapter 5). Where biological control agents were found in sufficient numbers on the traps, the data was recorded, analysed, and presented here. Despite requests site 1 and site 5 did not provide lists of biological control agents used at their sites. Site 6 did not use biological control agents, as such *Encarsia formosa* was the only known biological control agent at this site as these were released as part of the mass release experiment.

Site 1: Experimental Design

Yellow sticky traps equipped with a single green (540 nm) LED powered by battery packs were compared against those without (Table 42). One half (side) of the trap was exposed for a week, this was then re-covered with the wax paper and the other half (side) was exposed. Each half (side) will be discussed as a separate batch. The crops were poinsettia, and were grown on benches in a 46.5×44m glasshouse.

There were further batches in study year 1 (2012), but due to corrosion of the battery packs data from later dates were unreliable and will not be included here. During year 2 battery packs were enclosed in water resistant plastic containers.

Table 42. Experimental design, LED specifications, and dates for comparison between green (540 nm) LED equipped and standard yellow sticky traps at site 1.

Study dates	*Batch dates*	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
09/08/12-23/08/12	09/08/12-16/08/12 16/08/12-23/08/12	21 standard and 21 LED equipped	540 nm (green) Avago Technologies, 5mm, 30° angle, power output 6.1mW	Randomised design (see appendix 2). Positions were not re-randomised when traps were changed.	Appendix 2

Site 5: Experimental Design

LED attachments at this site were constructed to allow the LED to be changed without replacing the entire device. LEDs were not soldered to the wire; rather, the LED anode and cathode were held in place against the wire solely using the terminal block screw.

Yellow sticky traps equipped with a single green (520 nm) or blue (480 nm) LED powered by battery packs were compared against those without (Table 43). Note that 520 nm LEDs were used at this site, rather than the standard 540 nm, the purpose of this was to gather data for this wavelength, wavelength from behaviour study. One half (side) of the trap was exposed for a set time, this was then re-covered and the other half (side) was exposed. Each half (side) will be discussed as a separate batch (Table 43). The plants were a collection of Scottish wild flowers grown for a student display, and were frequently changed. The glasshouse was 20×13m.

Table 43. Experimental design, LED specifications, and dates for comparison between green (520 nm) or blue (480 nm) LEDs and standard yellow sticky traps at site 5.

Study dates	*Batch dates*	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
21/08/13-02/10/13	21/08/13-04/09/13 04/09/13-18/09/13 18/09/13-02/10/13	6 standard and 6 LED equipped	520 nm (green) Multicomp, 5mm, 30° angle, luminous intensity 13cd	Traps were arranged in two rows of paired replicates (see appendix 2 for layout pattern)	
02/10/13-14/11/13	02/10/13-16/10/13 16/10/13-31/10/13 31/10/13-14/11/13	6 standard and 6 LED equipped	480 nm (blue) CREE, 5mm, 30° angle, power output 10.4mW	Traps were arranged in two rows of paired replicates (see appendix 2 for layout pattern)	

Site 6: Experimental Design

A mass release experiment was conducted at this site for *Encarsia formosa*. Yellow sticky traps were each equipped with a green LED (Avago Technologies, 5mm, 540nm, 30° angle, power output 10.4mW) on each side of the trap powered by a 9V ac/dc mains adaptor. These were compared against standard yellow sticky traps (Table 44). As Koppert traps were used here, both sides of the trap were uncovered.Experimental design was a paired treatment design with 6 replicates. Thirty cardboard strips, each with around thirty attached *E. formosa* pupae (Koppert Biological Systems, EN-STRIP), were suspended within the glasshouse on the 10th, and 17th May. Around 1600 *E. formosa* were released over the study period. The crops were a frequently changed assortment of Scottish flowering plants grown in pots on top of benches. The glasshouse was 30×12m.

Table 44. Experimental design, LED specifications, and dates for comparison between green (540 nm) LEDs and standard yellow sticky traps at site 6.

Study dates	*Batch dates*	*Number of traps*	*LED specifications*	*Trap formation/ experimental design*	*Distance between traps*
10/04/13	10/04/13-27/04/13	6 standard and 6 LED equipped	540 nm (green) Avago Technologies, 5mm, 540nm, 30° angle, power output 10.4mW	Traps were arranged in two pairs of rows (see appendix 2)	

Site 1: Statistical Methods

***Encarsia formosa*: Comparison of yellow sticky traps equipped with green (540 nm) LEDs and standard yellow sticky traps (09/08/12 – 23/08/12)**

The data from both batches and the combined data were non-normal in distribution.

Comparisons were performed using Mann-Whitney U tests.

***Kleidotoma psiloides*:** Comparison of yellow sticky traps equipped with green (540 nm) LEDs and standard yellow sticky traps (09/08/12 – 23/08/12)

The data from both batches and the combined data were non-normal in distribution. These data were transformed to Log10 to satisfy the assumption of normality required for ANOVAs. Comparisons were performed using One-way ANOVAs.

Site 5: Statistical Methods

***Encarsia formosa*:** Comparison of yellow sticky traps equipped with green (520 nm) LEDs and standard yellow sticky traps (10/04/13 – 27/04/13)

The data from batches 1 and 2 were normal in distribution; the comparisons were performed using a One-way ANOVA.

Site 6: Statistical Methods

Encarsia formosa: Comparison of yellow sticky traps equipped with green (540 nm) LEDs and standard yellow sticky traps (21/08/13-04/09/13)

The number of *E. formosa* captured was too low to reliably test for normality. The data were treated as non-normal and comparisons were performed using Mann-Whitney U tests.

Results

Encarsia formosa

Site 1

Encarsia formosa: Green (540 nm) LEDs (09/08/12 – 23/08/12)

No significant differences were found between LED traps and standard yellow sticky traps in batches 1 (P=0.203). Significantly more (80%) *E. formosa* were captured by LED sticky traps in batch 2 (P=0.032) (Table 45) (Fig. 68, Fig. 69). There was no significant difference across the entire study period (P=0.079) (Table 45) (Fig. 70).

Table 45. Green (540 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 1. *Significant at P < 0.05.

Batch number	***Dates***	***Mann-Whitney U***	***P value***	***LED traps. Median (Q1, Q3)***	***Standard traps. Median (Q1, Q3)***
Batch 1	09/08/12 – 16/08/12	U = 170, Z = -1.273	0.203	14 (7, 25)	9 (7, 17)
Batch 2	16/08/12 – 23/08/12	U = 135.5, Z = -2.149	0.032*	6 (3, 7)	3 (1, 5)
Entire study period	09/08/12– 23/08/12	U = 686, Z = -1.758	0.079	7 (4, 16.25)	6 (2, 10.5)

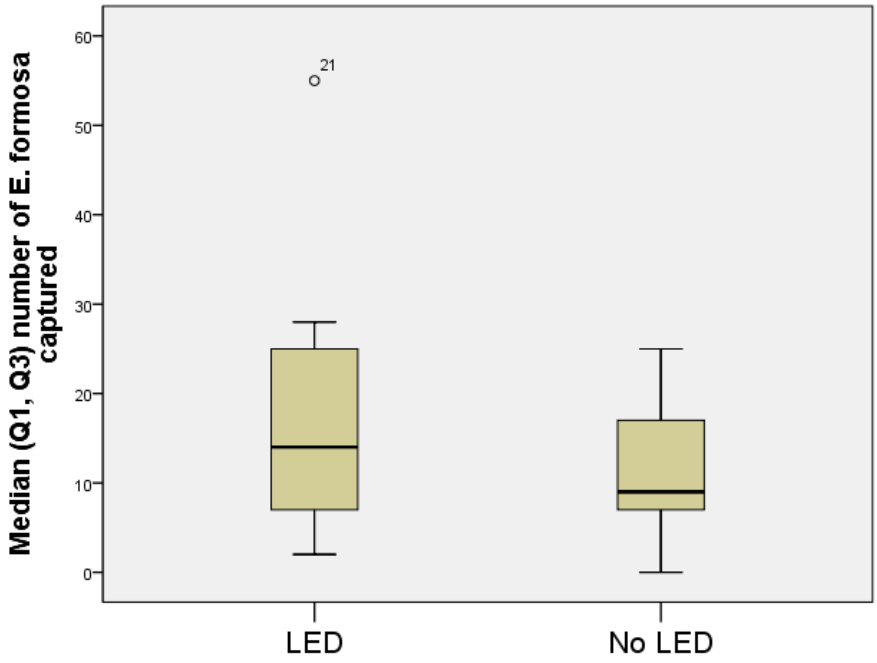

Figure 68. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *E. formosa* captured on green (540 nm) LED and standard yellow sticky traps in batch 1 at site 1 (09/08/12 – 16/08/12).

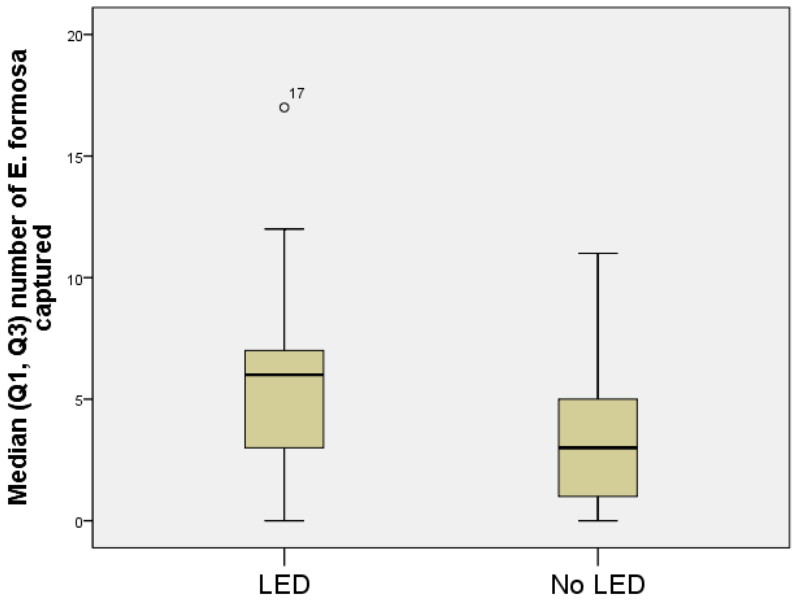

Figure 69. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *E. formosa* captured on green (540 nm) LED and standard yellow sticky traps in batch 2 at site 1 (16/08/12 – 23/08/12).

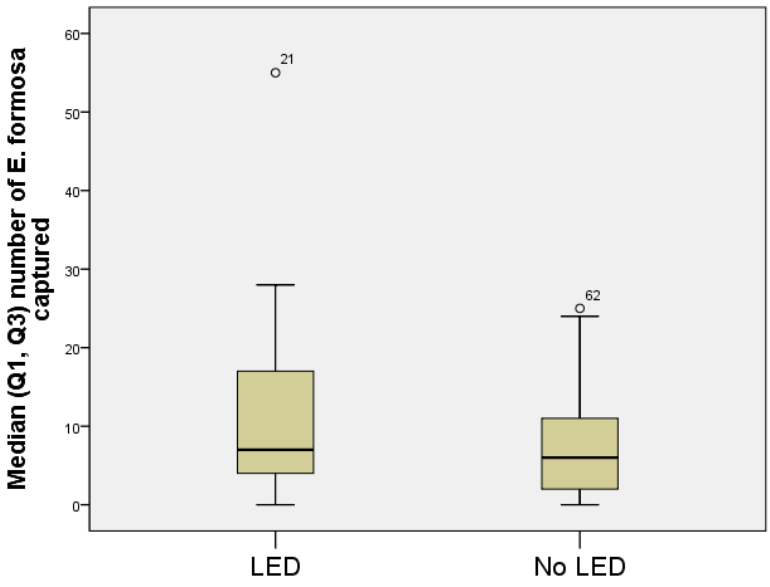

Figure 70. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *E. formosa* captured on green (540 nm) LED and standard yellow sticky traps across study period at site 1 (09/08/12– 23/08/12).

Site 5

Encarsia formosa: Green (520 nm) LEDs (21/08/13-04/09/13)

No significant differences were found between LED traps and standard yellow sticky traps in batches 1 (P=0.804) or 2 (P=0.604) (Fig. 71, Fig. 72). There was no significant difference across the entire study period (P=0.718) (Fig. 73).

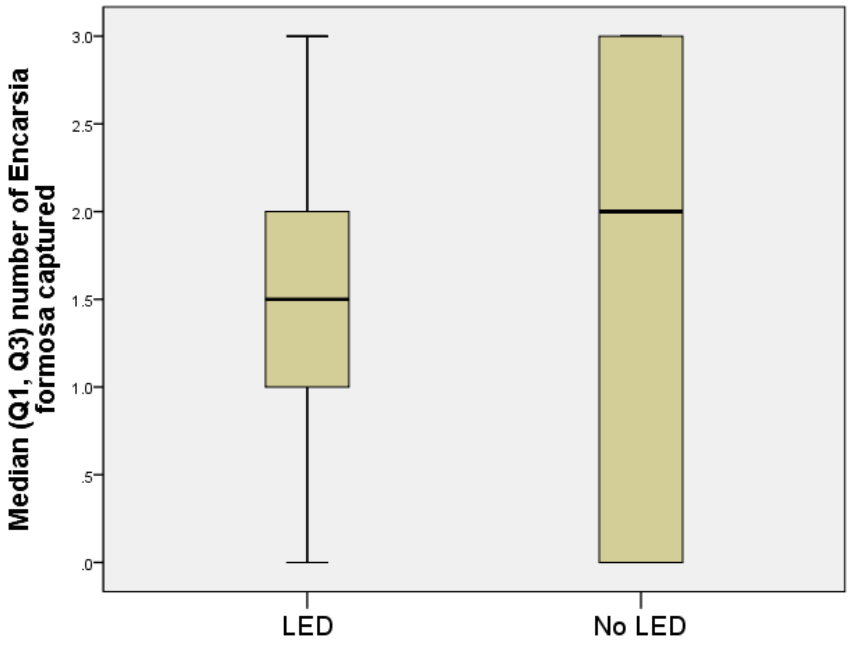

Figure 71. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *E. formosa* captured on green (520 nm) LED and standard yellow sticky traps in batch 1 at site 3 (21/08/13-04/09/13).

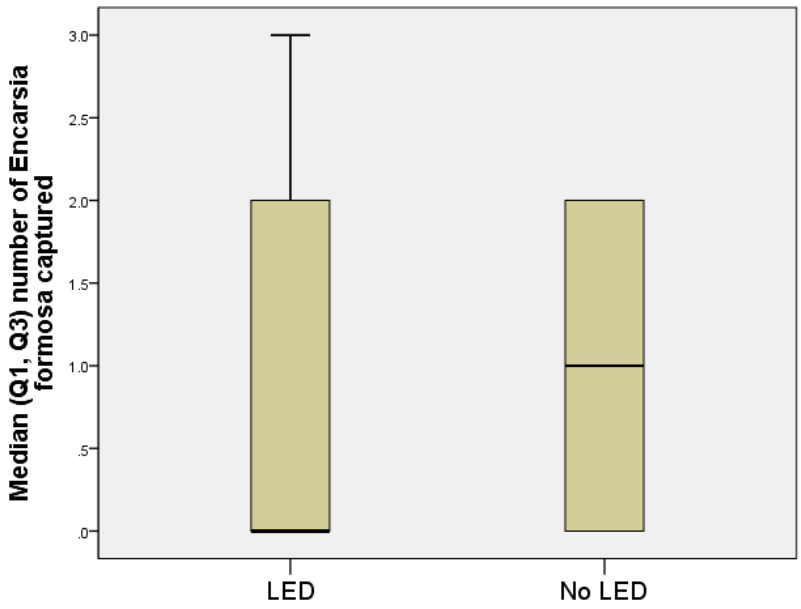

Figure 72. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *E. formosa* captured on green (520 nm) LED and standard yellow sticky traps in batch 2 at site 3 (04/09/13-18/09/13).

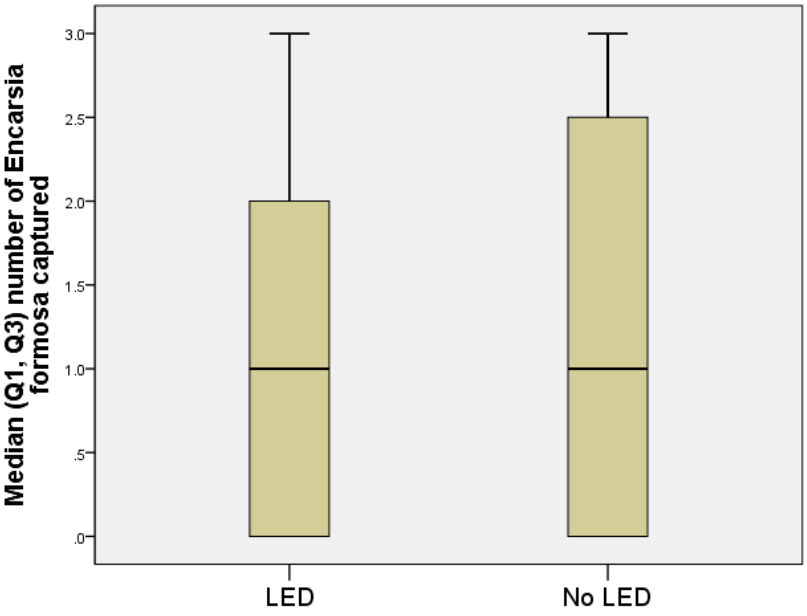

Figure 73. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *E. formosa* captured on green (520 nm) LED and standard yellow sticky traps across study period at site 3 (21/08/13-18/09/13).

Site 6

Encarsia formosa using green (540 nm) LEDs (10/04/2013 – 27/04/2013)

No significant differences were found between between the number of *E. formosa* captured between LED and standard traps (P=0.320) (Fig. 74).

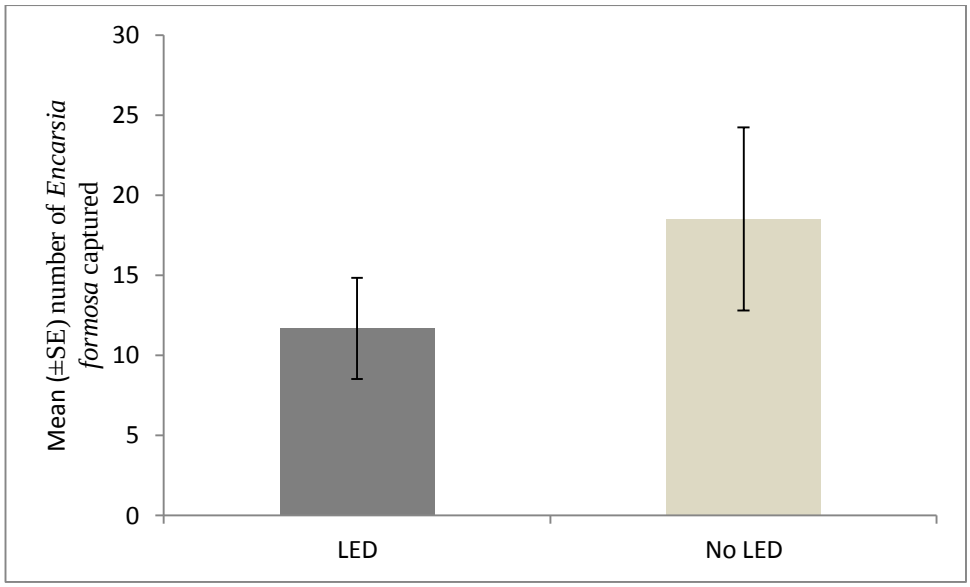

Figure 74. Mean (±SE) number of *E. formosa* captured on green (540 nm) LED and standard yellow sticky traps across study period at site 7 (10/04/2013 – 27/04/2013).

Kleidotoma psiloides

Site 1

Kleidotoma psiloides: Green (540 nm) LEDs (09/08/12 – 23/08/12) (site 1)

No significant differences were found between LED traps and standard yellow sticky traps in batches 1 (P=0.09) or 2 (P=0.544) (Fig. 75, Fig. 76). LED traps captured significantly fewer *K. psiloides* across the study period ($F_{1,81}$ = 24.649, P<0.001), with LED traps capturing a median of 61.5 (26, 108.75) and standard traps capturing 103.5 (45.25, 153.25), a 68.29% difference (Table 46) (Fig. 77).

Table 46. Green (540 nm) LED equipped yellow sticky traps compared with standard yellow sticky traps at site 1. *Significant at 0.05. **Median (Q1, Q3).

Batch number	***Dates***	***F statistic***	***P value***	***LED traps. Median (Q1, Q3)***	***Standard traps. Median (Q1, Q3)***
Batch 1	09/08/12 – 16/08/12	$F_{1,40} = 3.024$	0.09	32 (17, 64)	51 (26, 123)
Batch 2	16/08/12 – 23/08/12	$F_{1,40} = 0.375$	0.544	101 (60, 148)	115 (68, 215)
Entire study period	09/08/12– 23/08/12	$F_{1,81} = 24.649$	<0.001	61.5 (26, 108.75)	103.5 (45.25, 153)

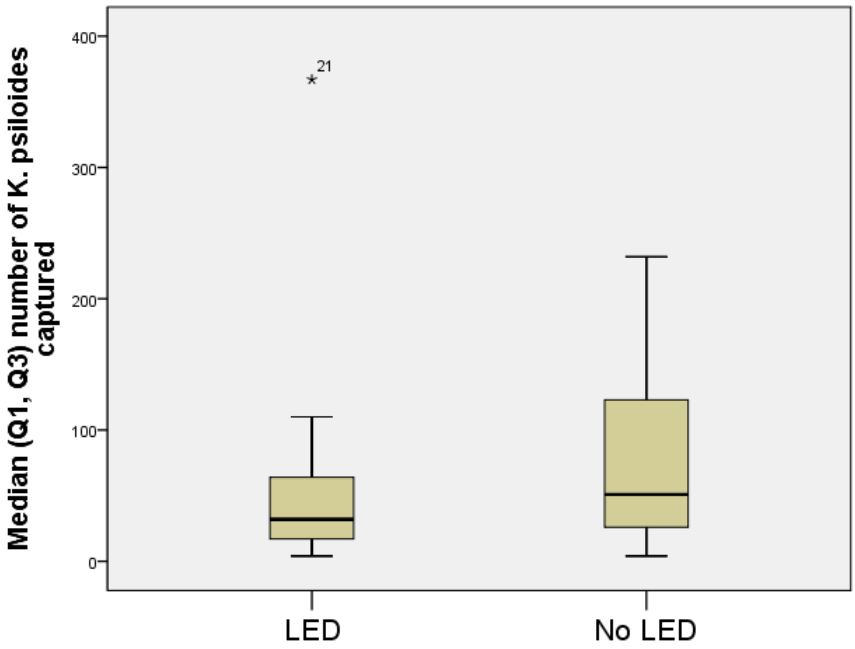

Figure 75. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *K. psiloides* captured on green (540 nm) LED and standard yellow sticky traps in batch 1 at site 1 (09/08/12 – 16/08/12).

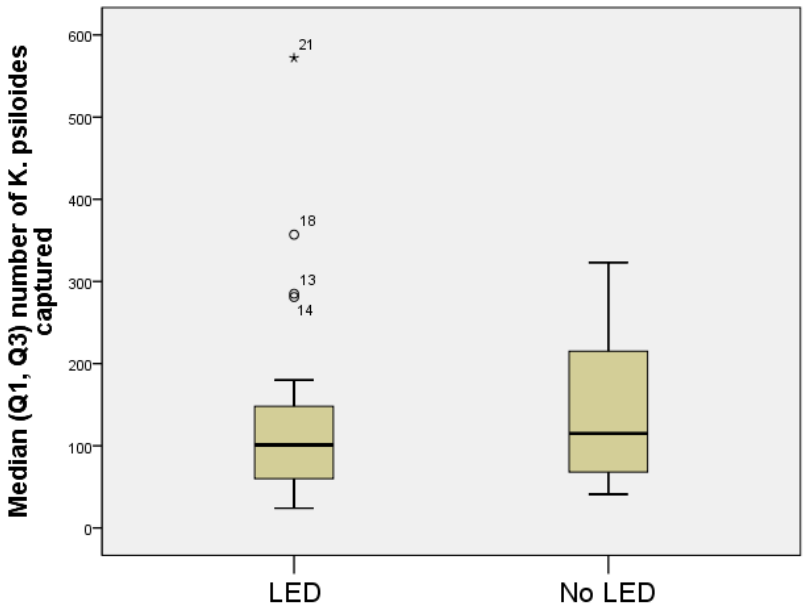

Figure 76. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *K. psiloides* captured on green (540 nm) LED and standard yellow sticky traps in batch 2 at site 1 (16/08/12 – 23/08/12).

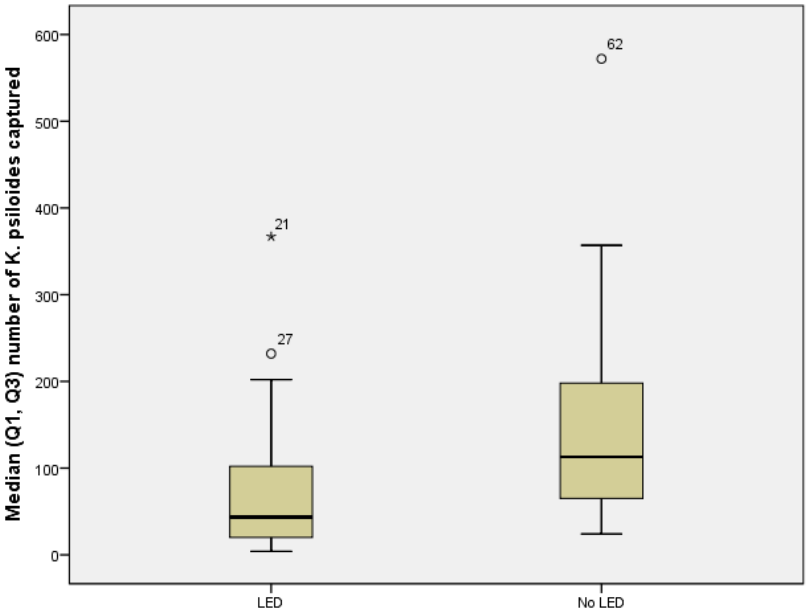

Figure 77. Median, interquartile range, and and the smallest and largest sample values (adjusted for extreme values) of *K. psiloides* captured on green (540 nm) LED and standard yellow sticky traps across study period at site 1 (09/08/12– 23/08/12).

Results Summary

- *Encarsia formosa:* there was no overall change in the number of *E. formosa* capatured. Although one batch of green (540 nm) equipped yellow sticky traps at site 1 showed a significant increase in attraction, results from sites 5 and 7 using green 5 (520 nm) and green (540 nm) showed a slight decrease in attraction. This is a positive result, as attracting this beneficial insect to traps is undesireable.

- *Kleidotoma psiloides:* There was a significant decrease in the number of *K. psiloides* captured by yellow sticky traps equipped with green (540 nm) LEDs over the study period. This is a positive result, and a reduction in the capture of this beneficial insect may enable better control of shorefly.

Discussion

Encarsia formosa

The main findings of this study were that there were no differences in the the number of *E. formosa* captured by sticky traps equipped with green (520 nm) or (540 nm) LEDs and standard yellow sticky traps.

The findings of this study confirm that the addition of green (520 or 540 nm) LEDs to yellow sticky traps should not have a direct negative effect on the use of *E. formosa* for use as a biological control agent. This is consistent with what is known about how *E. formosa* locates its hosts.

Encarsia formosa appears to locate its host using a combination of olfactory cues (from whitefly and their host plants) and random searching throughout the crop for whitefly signs, for example the presence of larvae, pupae, or adult whitefly (van Lenteren *et al.*, 1996; Guerrieri, 1997; Birkett *et al.*, 2003). This search behaviour may result in an increased number of *E. formosa* captured by sticky traps containing whitefly. Unfortunately, due to the lack of success in increasing the effectiveness of sticky traps for capturing *T. vaporariorum* in these experiments, it is not possible to determine this at this time. Future studies should seek to determine whether there is a correlation between the number of *T. vaporariorum* and *E. formosa* captured on sticky traps.

Kleidotoma psiloides

The main findings of this trap comparison were that green (540 nm) LED equipped yellow sticky traps captured significantly fewer *K. psiloides* over the study period.

Very little is known about this naturally occurring parasitic wasp of shore flies (family: Ephydridae). The results from this study along with anecdotal evidence gained from a grower in England suggest that *K. psiloides* are attracted to the colour yellow (per comms grower). This would imply that at a site with a high population of *K. psiloides*, yellow sticky traps may be detrimental to their use as a control for shore fly. The addition of a green (540 nm) LED to these traps may go towards counteracting this. Further research at sites with a high population of *K. psiloides*, as well as research determining how effective *K. psiloides* are as a biological control agent, are required before any recommendations can be made.

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Chapter 7 Relative Spectral Preference

Abstract

Colour is typically considered to be the most important visual stimulus for phytophagous insects, and the presentation of a particular wavelength can result in behaviour responses which are beneficial to monitoring strategies. Coloured traps are used to capture a broad range of insect pest species, including whitefly and thrips. A better understanding of these insects visual system may enable more appropriate wavelength selection when considering the development and implementation of a monitoring system.

Here, the relative spectral preferences of two pest species, western flower thrips (*Frankliniella occidentalis* Pergande (Thysanoptera: Thripidea)) and Glasshouse whitefly (*Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae)), were determined using a choice test comparing a range of wavelengths in 20 nm steps against a control wavelength.

It was found that *F. occidentalis* showed a peak spectral preferences at 360, 420, and 480 nm, and *T. vaporariorum* at 320, 340, and 380 nm.

Introduction

It is typically assumed that colour is the most important visual stimulus for phytophagous insects, likely due to the supposed poor visual acuity of insects (Matteson, *et al.*, 1992; Land, 1997). Numerous phytophagous species appear to possess trichromatic vision (Matteson, *et al.*, 1992; Kirchner *et al.*, 2005; Döring and Chittka, 2007b). The presentation of a particular wavelength, or combination of wavelengths, can result in behavioural response and colour is successfully used to trigger a positive behavioural response in a broad range of insects for trapping, including whitefly and thrips (Gillespie and Quiring, 1987; Gillespie and Quiring, 1992; Yaku *et al.*, 2007; Brødsgaard 2009; Górska-Drabi *et al.*, 2011; Broughton and Harrison 2012) (see chapter 1, Sticky Traps, and chapter 2).

The colour and brightness of light are subjective, and are a result of the individual's physical visual system, as well as their cognitive functions (Briscoe and Chittka 2001; Skorupski and Chittka 2009). When performing a comparison between wavelengths using an active light source, it would be ideal to compare two light sources outputting subjectively equally; however, this is not possible without a detailed knowledge of the subject's visual system, and would be impractical for multi-species studies. At the time of writing there appear to be no studies which adjust for the subjective properties of vision in insect wavelength preference comparisons. Light output can be measured using a photometer, and adjusted to be objectively equal (Brown *et al.*, 1998; Stukenberg *et al.*, 2015); however, this applies only at a particular distance from each light source as light wavelengths refract at different angles (Tilley, 2000) (see chapter 2, Introduction to Light, Refraction and Dispersal).

Brown *et al.* (1998) measured the spectral preference, i.e. the light wavelength preferentially selected when presented with multiple wavelengths, of the parasitoid wasp *Trybliographa rapae* (Westwood) (Hymenoptera: Cynipidae) by introducing groups into a T-shaped 190mm glass tube (presumably a circular tube) with a 5mm internal diameter, and a 25mm side arm (entrance). This tube had a control light source (570 nm) at one end, and a test wavelength between 240-670 nm at the other. Groups of 10 parasitoids were introduced into the chamber, dark adapted for 30 minutes, then given 5 minutes to select a wavelength. This was repeated three times for a wavelength, which was considered to be a replicate. Up to three wavelengths being tested per day. A new group of parasitoids were used each day. The use of groups raises concerns of biases arriving from group behaviour, particularly with the same group of insects being used repeatedly. For example, male *F. occidentalis* produce aggregation pheromones which are known to attract both male and female *F. occidentalis* (Hamilton *et al.*, 2005; Zhang *et al.*, 2011).

Capturing and manoeuvring small insects can be challenging, particularly when attempting to work with a specific number of individuals. Numerous anaesthetic options are available to mitigate these difficulties; however, the decision to use an anaesthetic should be undertaken with care. The three most common methods are reducing the insect's body temperature, CO_2, and ether, each of which have their own advantages and disadvantages (Ashburn and Thomson, 1978). Research into the negative impact of these anaesthetics in insects has primarily focused on *Drosophila melanogaster* Meigan (Diptera: Drosophilidae (Barron, 2000; Badre *et al.*, 2005; Nilson *et al.*, 2006). While the short term anaesthetic effects of all three methods are reversible (Badre *et al.*, 2005; MacMillan and Sinclair, 2011), the recovery times for the species studied here (*Frankliniella. occidentalis* and *Trialeurodes vaporariorum*) are not known, and periods greater than 24 hours have been suggested for *D. melanogaster* (Barron, 2000). So the use of these anaesthetics would be unsuitable for behavioural studies of this kind. Although the long term effects (e.g. reduced fecundity) (Perron *et al.*, 1972) do not raise any immediate concerns for a behavioural study, this should a consideration if maintaining a culture is required.

The addition of an active light to source to an existing trap design has proven successful for increasing their effectiveness for trapping some phytophagous species (Chu *et al.*, 2003; Nombela *et al.*, 2003; Chen *et al.*, 2004a; Chen *et al.* 2004b; Stukenberg *et al.*, 2015). Few studies appear to account for the species spectral sensitivity, and simply choose a light source within a particular wavelength range found previously effective as a trap colour. Determining a particular species relative spectral preference will enable more effective wavelength selection when improving traps using active light sources.

Materials and Methods

Maintenance of study species for choice tests

Frankliniella occidentalis

Frankliniella occidentalis were reared on chrysanthemums in plastic enclosures in an insectary maintained at 20±1°C. Florescent lighting was operated on a 16/8h light/dark cycle.

One chrysanthemum plant was purchased fully grown in a pot of soil from Sainsbury's. The chrysanthemum was placed on a tray inside of a ~60*60*60cm cage (not thrips proof), and water was provided to the plant via the tray. Once per week, a new chrysanthemum pot was purchased and placed in the tray next to the current chrysanthemum to allow the *F.*

occidentalis to migrate to the new flowers. At the end of the week, the old chrysanthemum were disposed of, and a new chrysanthemum pot was placed in the tray next to the existing chrysanthemum. The chrysanthemum were watered every 2-3 days.

Trialeurodes vaporariorum

Trialeurodes vaporariorum were captured in a nearby glasshouse and maintained on Moneymaker tomato plants and cucumber, within a mesh enclosure in an insectary maintained at 22±1°C. Florescent lighting was operated on a 16/8h light/dark cycle.

Moneymaker tomato plants and cucumber (unknown variety) were purchased fully grown in a pot of soil from a local garden centre. The plants were placed on a tray inside of a ~60*60*60cm cage (not thrips proof), and water was provided to the plant via the tray. Watering was performed every 2-3 days.

Relative spectral preference of insects

Relative spectral preference was measured by placing an individual within a 20mm linear clear square plastic tube with a 5mm internal diameter, which was marked into three equal sections (Fig. 78). The ends of the tube were sealed using clear plastic tape, which was determined via photometer to have no discernible filter or refraction effect on the light. This tube was contained within a wooden box (Fig. 79), which was intended to block out any ambient light. The lid to the box was not permanently secured, and was held in place between the raised ends of the box by friction. Similarly, the plastic tube was also held in place by friction within the box, and was removable. At either end of the box was a source of monochromatic light, which was able to enter the box through a small hole. At one end a control light wavelength was produced via an LED, the other end of the test chamber was illuminated by a test wavelength produced by a 100 W xenon arc lamp (Osram XBO100W/2 OFR) housed in a Xe-100 lamp housing device (UV- Gröbel, Ettlingen, Germany) filtered through band pass filters in 20nm steps and transferred by a liquid light guide (Fig. 80). An LED was used as the control light source because too much light was lost when attempting to split the Xenon arc light to provide both control and test wavelengths. The short length (20mm) of the chamber was selected to reduce the impact of the differing refraction angles of the light wavelengths, issues of light refractions caused by using two different lights sources, and the internal reflection of light within the tube. The control wavelength was one which the subject species was determined to be sensitive to via electroretinograms performed by others (Matterson *et al.*, 1992; Mellor *et al.*, 1997).

The photon flux of the two light sources was equal in the centre of the chamber. This was achieved by measuring the power (mW) of the test wavelength using a photodiode (Thor Labs, S120VC attached to a PM100USB compact console), this was converted to photon flux (Formula 3). The plastic tube was removed whilst using the photodiode. The appropriate wattage (mW) for the test wavelength was then calculated, and an iris (Thor Labs, ID8 – Post-mounted iris diaphragm) (Fig. 81), was used to adjust the amount of light able to enter the chamber. Room windows were covered using blackout cloth, and the room temperature was maintained at 17±3°C.

Conversion of 1mW of 500nm light

$E = hc \div \lambda$

$E = (6.63\times10^{-34} \times 3\times10^{8}) \div 500\times10^{-9}$

$E = 3.978\times10^{-19}$ Joules

Where E = energy (J), *h* = Planck's constant ($6.63x10^{-34}$J/s), C = speed of light ($3x10^{8}$ m/s), λ = wavelength (nm)

Power = E÷s

W = J÷s

$1mW = 1\times10^{-3}$ J/s

Where Power = rate of energy transferred (J/s), E = Joules, s = time in seconds, W = Watts

Therefore photons per second for 500nm light

$1\times10^{-3} \div 3.978\times10^{-19} = 2.514\times10^{15}$ photons/sec

Formula 3. Example formula demonstrating the conversion of 1mW of 500nm light into photon flux.

The subject was introduced to the centre of the tube through a hole in the top of the tube which was then sealed using a plastic square. The wooden box was sealed and the room lights were immediately turned off to prevent light contamination. After a period of time (differing by species) had passed the room lights were turned on and the segment of the tube the subject was located in was recorded and considered to be their choice. Individuals which had not moved from the centre segment were not included in the statistical analysis. Two different linear chambers were used, and were alternatively rotated 180° and swapped out.

The chambers were cleaned using 100% ethanol and water at the end of each day. Subjects were not anaesthetised for collection as this may have influenced their behaviour (Barron, 2000). A maximum of 10 data points were collected for each wavelength. Statistical analyses were performed using Fisher's exact test. The Fisher's exact test is a contingency table test, which can be used as alternative to the Chi Square test when the sample size is small (Witmer, 2003). This differs from the Chi Square test in that the P value is determined exactly, whereas the Chi Square test provides an approximation, which can be misleading when the sample size is small (Witmer, 2003). The null hypothesis tested here is that the different light sources do not affect the insect's decision to move within the chamber.

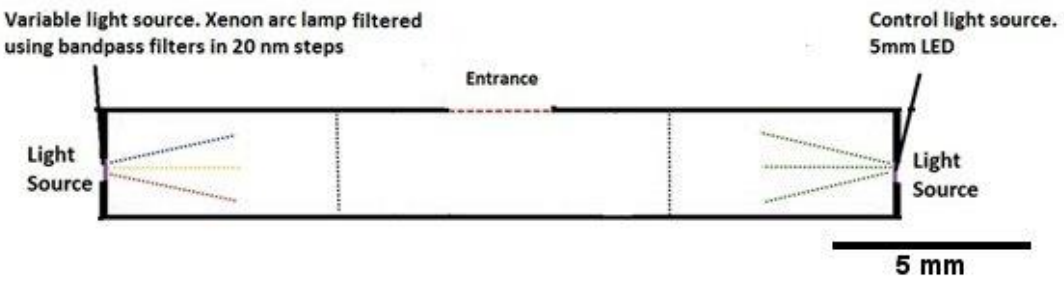

Figure 78. Choice chamber.

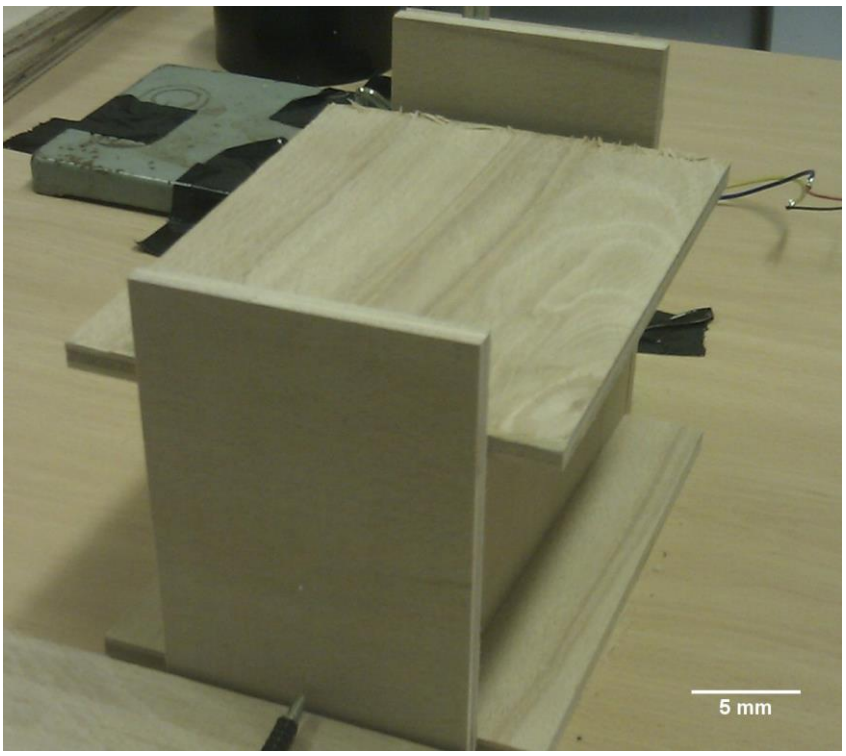

Figure 79. Wooden box test chamber was contained in. *NB: This photo was taken before the box was nailed together, and the holes were drilled to permit light to enter.*

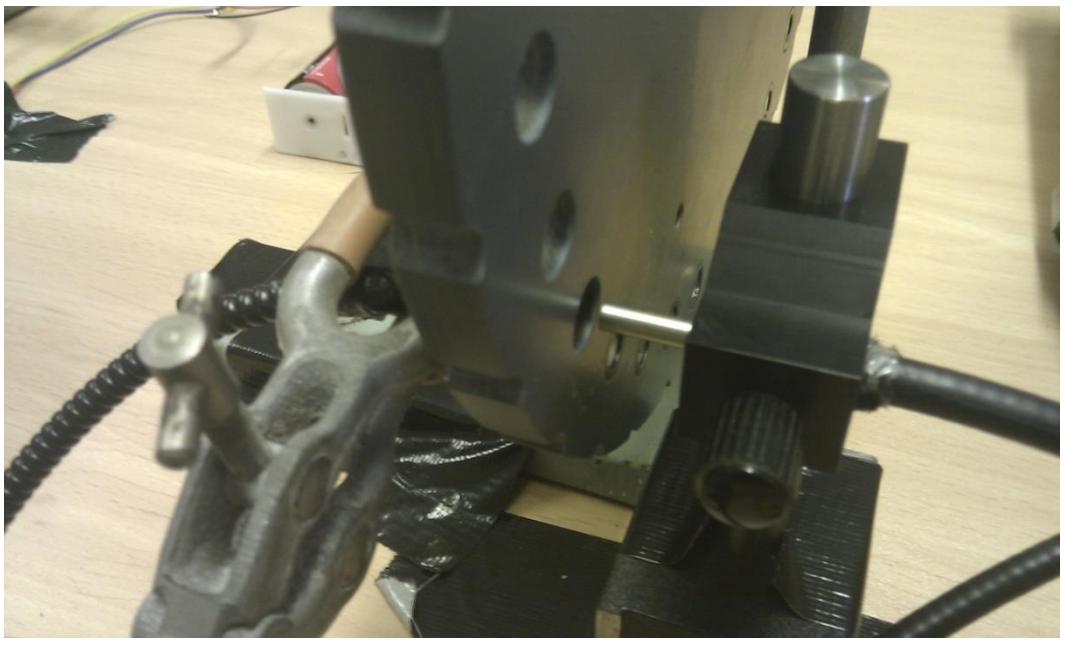

Figure 80. Liquid light guides direct light through the bandpass filter wheel.

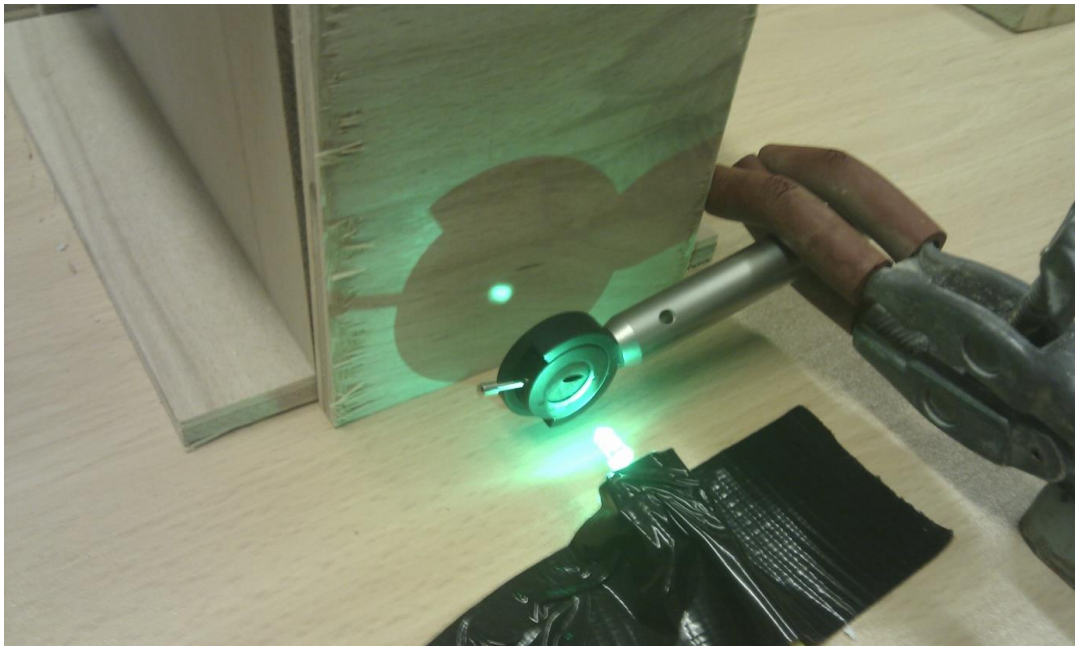

Figure 81. Light from the control wavelength LED was controlled using an iris. NB: This photo was taken before the box was nailed together, and the holes were drilled to permit light to enter.

Frankliniella occidentalis

Individual female insects were captured by holding a small plastic vial above them, and taking advantage of their tendency to "jump" by flicking their abdomen when disturbed. Females were used due to the difficulty of sexing males, i.e. a small female may be mistaken for a male. Subjects were placed into a 5mm internal diameter tube. The control light source was a green LED (Avango Technologies, 5mm, 540 nm, 30° angle, power output 10.4mW). The control wavelength was selected as *F. occidentalis* electroretinograms have demonstrated a relatively high sensitivity to this wavelength (Matterson *et al.*, 1992). The test wavelengths were in 20 nm steps between 340-620 nm.

Trialeurodes vaporariorum

Individual female insects were captured by holding a small plastic vial below them, and taking advantage of their tendency to drop when letting go of the underside of a surface. Females were used due to the difficulty of sexing males, i.e. a small female may be mistaken for a male. Subjects were placed into a 5mm internal diameter tube. The control light source was a green LED (Multicomp, 5mm, 520 nm, 30° angle, luminous intensity 13cd). The

control wavelength was selected as *T. vaporariorum* had a relatively high sensitivity to this wavelength (Mellor *et al*., 1997). The test wavelengths were in 20 nm steps between 320-620 nm.

Results

Frankliniella occidentalis

There were significant differences found between the control wavelength and 360 nm, 380 nm, 420 nm, 480 nm, and 500 nm (Table 46). A visual representation based on these data (Fig. 82) shows peaks of relative attractiveness at 360 nm, 420 nm, and 480 nm.

Trialeurodes vaporariorum

There were significant differences found between the control wavelength and 320 nm, 340 nm, 380 nm, 440 nm, 600 nm, and 620 nm (Table 47). A visual representation based on these data (Fig. 83) shows a high degree of relative attractiveness between 320-400 nm, with an additional peak at 480 nm.

Table 46. P values showing differences between control, and test, wavelengths for *F. occidentalis*. The number of decisions equals the numbers of time the subjects chose a wavelength (i.e. moves from the central area), and was a maximum of 10. *Significant at P < 0.05, ** Significant at P < 0.005.

Wavelength (nm)	**Number of decisions**	**Decisions where test wavelength was chosen**	**P value**	**Wavelength (nm)**	**Number of decisions**	**Decisions where test wavelength was chosen**	**P value**
340	8	7	0.0703	500	9	8	0.039*
360	10	10	0.0019**	520	5	5	0.0625
380	9	8	0.039*	540 (Control)	N/A	N/A	N/A
400	9	5	1	560	8	4	1
420	10	9	0.0214*	580	7	5	0.4531
440	7	6	0.125	600	6	3	1.3125
460	9	7	0.1796	620	4	1	0.625
480	9	9	0.039*	N/A	N/A		N/A

Table 47. P values showing differences between control, and test, wavelengths for *T. vaporariorum*. The number of decisions equals the numbers of time the subjects chose a wavelength (i.e. moves from the central area), and was a maximum of 10. *Significant at P < 0.05.

Wavelength (nm)	**Number of decisions**	**Decisions where test wavelength was chosen**	**P value**	**Wavelength (nm)**	**Number of decisions**	**Decisions where test wavelength was chosen**	**P value**
320	7	7	0.0156*	480	6	3	0.5078
340	7	7	0.0156*	500	5	3	1.3125
360	9	7	0.1796	520 (Control)	N/A	N/A	N/A
380	8	7	0.0703	540	5	2	1
400	9	6	0.5078	560	5	1	0.375
420	8	4	1	580	5	1	0.375
440	9	1	0.039*	600	5	0	0.0625
460	8	3	0.7265	620	5	0	0.0625

Results Summary

- *Frankliniella occidentalis* showed peak spectral preference at 360, 420, and 480 nm wavelengths when compared against 540 nm.

- *Trialeurodes vaporariorum* showed peak spectral preference at 320, 340, and 380 nm wavelengths when compared against 520 nm.

Discussion

Frankliniella occidentalis

The main findings of this study were peak attractions at 360, 420, and 480 nm, suggesting these wavelengths may be effective for increasing the effectiveness of traps for capturing *F. occidentalis*.

F. occidentalis showed a spectral preference for every other wavelength tested with the exceptions of 560, and 620 nm. *F. occidentalis* is known to show a preference for blue sticky traps (Brødsgaard, 2009; Broughton and Harrison 2012), although a preference for white has been observed in field crop experiments (Hoddle *et al.*, 2002). This preference for blue may be due to an open space response, which hypothesizes insects fly to open spaces, which are generally brighter (Hu and Stark, 1977). Alternatively, this may be due to their attraction to flowering plants, some of which reflect within the blue region of the light spectrum, although this is typically not the case for flowers grown by commercial growers (FReD, 2014).

The high spectral preference of *F. occidentalis* to 360 nm is promising, and this may be an effective wavelength for increasing the effectiveness of traps for capturing *F. occidentalis* in future when the cost of UV LEDs decreases, and light output increases.

Trialeurodes vaporariorum

The main findings of this study were peak attractions at 320, 340, and 380 nm, suggesting these wavelengths may be effective for increasing the effectiveness of traps for capturing *T. vaporariorum*. Green (520 nm and 540 nm) performed well, and were the most attractive wavelengths outside of the UV spectrum.

The high spectral preference to wavelengths within the UV spectrum is promising, and suggests the addition of a UV LED to a yellow sticky trap could improve their effectiveness for trapping *T. vaporariorum.* At the current time, the light output of UV LEDs is far below that of standard 5mm LEDs within the visible light spectrum, and it is unclear whether the attraction of *T. vaporariorum* from a distance is viable. Stukenberg *et al.* (2015) found the combination of green (517 nm) and UV (368 nm) to be an effective combination for increasing the capture of *T. vaporariorum* on yellow sticky traps, although this was in small (1.6x1.1x.19m) gauze cages. This combination deserves further investigation under field conditions, as the more powerful green LED may be effective at drawing *T. vaporariorum* closer to the trap from a distance, where they will then gain vision of, and exhibit a positive behavioural response triggered by the UV light source. A further advantage of such a combination, is the ability to take advantage of the high attraction to UV light, without having to use bright UV light sources which may be hazardous to worker's eyes (Pfeifer *et al.*, 2005; Chalam *et al.*, 2011).

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Chapter 8 General Discussion

The main finding of this research were an increase in the number of *Bradysia difformis* captured and *Plutella xylostella* by equipping yellow sticky traps with both green (540 nm) and blue (480 nm) LEDs. No significant changes in the number of *Frankliniella occidentalis* or *Trialeurodes vaporariorum* captured was observed for either LED type, although a non-significant pattern of reduced capture efficiency for *F. occidentalis* was observed at site 3 for yellow sticky traps equipped with blue (480 nm) LEDS. No negative impacts were found regarding the beneficial parasitoid *Encarsia formosa* and a positive impact was found with the reduction of the trapping of the parasitoid *Kleidotoma psiloides*. Relative spectral preference experiments found *F. occidentalis* to have peak spectral preferences at UV (360 nm), blue (420 nm), and blue (480 nm). *T. vaporariorum* showed peak spectral preferences at UV (320 nm, 340 nm, and 380 nm) and green (520 nm).

These finding suggest that sites suffering from simultaneous infestations of both *B. difformis* and *P. xylostella* would benefit most from the use of these LED attachments. Green (540 nm) appears to be the more effective of the two wavelengths tested in this regard for *B. difformis* while blue (480 nm) were more effective for *P. xylostella*, although direct comparisons would need to be performed to confirm this.

The attraction of *Frankliniella occidentalis* to blue (480 nm) found in the behaviour experiments was not reflected in the trap comparisons. As discussed in chapter 5, this may be due to a distance dependent behaviour response (Chu *et al.*, 2005), with the blue (480 nm) LEDs used here did not output enough light to trigger a positive behavioural response in *F. occidentalis* from a distance. As growers do not typically use blue sticky traps, expanding the range of pests captured by yellow sticky traps remains desirable, and may be achievable with alterations to the attachment design, e.g. a more powerful LED or the inclusion of a chemical lure (Hamilton *et al.*, 2005). Each of these alterations comes with downsides, such as the risk of bright blue LEDs to workers eyes (Barker *et al.*, 2011; Kernt *et al.*, 2012), and the unintended increase in capture of beneficial insects to the traps (Broughton and Harrison, 2012). While blue light can be filtered using amber/yellow glasses, this may not be practical, and could have unintended consequences, e.g. a reduction in blue light triggers the production of melatonin, and may lead to tired workers (Burkhard and Phelps, 2009).

The combination of the field work and behaviour experiment suggests that green (540 nm) LEDs could be effective at increasing the effectiveness of yellow sticky traps for capturing *Trialeurodes vaporariorum*. There was a small but non-significant increase in capture found

at site 5 and 7 for yellow sticky traps equipped with blue (480 nm) LEDs, which is consistent with the peak in relative spectral preference found at 480 nm in the behavioural experiment. This effect was not observed at site 1 where a small decrease in the number of *T. vaporariorum* was found; however, due to the difficulties explained in the methods with this particular batch of traps, these data may be unreliable.

The high spectral preference of *T. vaporariorum* to 320, 340, and 380 nm demonstrated in the behavioural experiments is promising, and these may be effective wavelengths for increasing the effectiveness of traps for capturing *T. vaporariorum.* At the present time UV LEDs are not recommended for trap enhancements as they are expensive. The cost to power ratio of UV LED is expected to continue to improve (Muramoto *et al.,* 2014), and these may prove to be effective for trap enhancement in future, although care must be taken to avoid damage to workers' eyes (Pfeifer *et al.,* 2005; Chalam *et al.,* 2011). Stukenberg *et al.* (2015) found green (517 nm) and UV (368 nm) to be more effective than either wavelength alone for attracting *T. vaporariorum*, which may enable effective use of UV LED for trap enhancement, without the safety concerns. Alternatively, the development of a UV specific trap may be beneficial; for example, a modified version of Chu and Henneberry's (1998) CC- trap with downwards angled UV light, and an opaque body, may be an effective tool for capturing *T. vaporariorum* while minimising the risk to workers. Care would have to be taken to ensure that the UV light reflected from the crop did not reach harmful levels.

Equipping yellow sticky traps with green (540 nm) or blue (480 nm) LEDs did not increase the number of *Encarsia formosa* captured by the traps. This is a promising result, particularly as equipping yellow sticky traps with green (530 nm) LED does not appear to increase the number of *Eretmocerus mundus*, a parasitoid of the tobacco whitefly *Bemisia tacaci*, captured by the traps (Chu *et al.,* 2004; Muñiz *et al.,* 2005), and suggests that the use of green LEDs (530 nm and 540 nm) in pest monitoring are compatible with two common whitefly parasitoids. Furthermore, fewer *Kleidotoma psiloides* were captured on yellow sticky traps equipped with green (540 nm) LEDs, demonstrating that LED attachments can be used to reduce the negative impact yellow sticky traps may have on beneficial insects. Based on the large number of *K. psiloides* captured at site 1, one would expect a large population of shorefly at this site. Unfortunately, despite the high number of shorefly parasitoids at this site, very few shorefly were captured by either trap type, despite previous success with yellow sticky traps by others (Tilley *et al.,* 2000). Further research to determine whether the addition of green (540 nm) LEDs to yellow sticky traps could simultaneously increases shorefly capture while reducing the number of *K. psiloides* is desirable.

Future work should seek to compare LED wavelength and trap combination against one another. A comparison between green (540 nm) and blue (480 nm) equipped yellow sticky traps was proposed for a tomato glasshouse located at site 2; however, this did not go ahead due to staffing problems at this site. Comparisons such as this will provide valuable information on the relative effectiveness of these LEDs for trap enhancement. This is particularly important when considering the issue of subjective brightness, where the brightness of the light source is dependent on the individual's sensitivity to these wavelengths (Briscoe and Chittka 2001; Skorupski and Chittka 2009). The implications of this are that a direct comparison of LED specifications may have little value when considering how effective they may be in the field.

As the available options for LED wavelength of sufficient power to be viable for the purposes of trap enhancement are limited, combinations of wavelength should be employed in future, for example Stukenberg *et al.* (2015) found green (517 nm), UV (368 nm), and the combination of the two to increase the capture of *T. vaporariorum*, although this was in small (1.6x1.1x.19m) gauze cages. By using a more powerful LED to influence the insects' behaviour from farther away, bringing them closer to the more effective, but less powerful, LED.

Future behaviour experiments should aim to further refine the results found here for *T. vaporariorum* and *F. occidentalis*, as well expand this to a broader range of pest species. Of particularly interest would be those who appear to exhibit positive behavioural responses to non-standard sticky trap colours, such as blossom thrips (*Frankliniella schultzei*) and a preference for red (Yaku *et al.*, 2007). Similar experiments using beneficial insects should also be performed, either by determining their relative spectral preference as found here, or by exposing subjects to a single light wavelength in a linear chamber, to determine whether they exhibit photo-negative or photo-positive responses (i.e. do they move away or towards from a wavelength). The staggered emergence of *Encarsia formosa* from their eggs provide advantages for behavioural experiments of this kind. *Encarsia formosa* eggs are sold attached to card, with the *E. formosa* expected to emerge over the course of 1-2 weeks (Hoddle, *et al.*, 1998; Malais and Ravensberg, 2003), this card can be placed within a chamber with sticky card and an active light source at either end, and the number of *E. formosa* at each end can be counted after a two week period. The disadvantage of performing the experiment in this manner is that it would not be possible to use a xenon arc lamp to produce the light, as these cannot be left turned on for extended periods of time, and LEDs do not cover the the entirety of the visible spectrum with notable gaps within the blue region

(Avago 2016). In order to gather sufficient data for analysis in a reasonable time, multiple experiments would have to be run concurrently, unfortunately it was not possible to do this here as this experiment requires a dark room, with an appropriate temperature (25°C), to store the chambers for the duration of the experiment (Malais and Ravensberg, 2003).

The practical implications of this work are not yet known, and for species where a much larger number of captured by LED traps, as the expectation of the population size of a pest species based on the number captured on a sticky trap will differ, there may be uncertainty in when to engage in control measures. With this in mind, it would be advantageous to perform experiments to ascertain the relationship between the number of insects captured by LED equipped sticky traps and the population of the pest species.

In conclusion, both green (540 nm) and blue (480 nm) LED attachments are promising enhancements for the monitoring of both *B. difformis* and *P. xylostella*, and have no known negative impact on the use of beneficial insects. A range of UV wavelengths show promise for attracting *F. occidentalis* and *T. vaporariorum*, although a redesign would be necessary to take advantage of this while minimising the risk to workers' eyes (Pfeifer *et al.*, 2005; Chalam *et al.*, 2011).

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Appendices

Appendix 1: Questionaire

LED Trap Questionnaire

1. Company and Contact Information

Company Name:

Contact Name:

Phone Number:

E-mail address:

Address (address traps should be mailed to):

2. Glasshouse (Polytunnels) and Traps

1. Do you have a glasshouse or polytunnel in mind? Which material does this use?

2. Which crop types do you have within this glasshouse/polytunnel? (If crops rotate, would it be possible to give some information of the crop types and frequency of rotation?)

3. Are there any pests you're currently having a problem with?

4. How many sticky traps do you current have in this glasshouse/polytunnel? Is it possible to increase this if necessary? (Realistically the max sticky trap number I could manage at one site is around 40.)

5. Can we be supplied with a basic plan of the glasshouse with dimensions (these do not need to be exact), which also show the current (and potential, for if this needs to be increased) locations for sticky trap placement?

6. Do you record temperature or humidity in your glass houses/polytunnel? If so would it be possible for us to have this information?

3. Sticky traps

1. What colour sticky traps do you currently use?

2. How frequently do you change your sticky traps?

3. Would it be possible to power the LED traps from the mains? (This likely isn't possible, it's quite a lot more work than it sounds).

4. If battery packs are used, will these need waterproofing?

4. Additional information

1. Do you use beneficial insects which may be attracted to these traps? (If so, could you list these?)

2. Is there any additional information you think may be relevant?

3. Which times of the year do you begin to notice pest problems? Which pests are these?

4. Which lighting system do you use?

Appendix 2: Trap layout

1		15			29	
	8			22		36
2		16			30	
	9		P	23		37
3		17	A		31	
	10		T	24		38
4		18	H		32	
	11			25		39
5		19			33	
	12			26		40
6		20			34	
	13			27		41
7		21			35	
	14			28		42

Figure 82. Trap layout at site 1 (09/08/12 – 23/08/12). Traps are numbers 1-42, and yellow highlighted traps were equipped with green (540 nm) LEDS. Traps were ~1m apart, with the exception of those with the path in between, which was ~3m.

1		2
3	Gap between rows of crops	4
5		6
7		8
9		10

Figure 83.Example trap layout for sites 2, 3, 5, and 6. Traps were arranged in two rows of pairs (i.e. 1 and 2, 3 and 4, etc), with each pair consisting of an LED equipped yellow sticky trap, and a standard yellow sticky trap.