Final Report

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Student Project No. [AHDB project number]

Title: The biology and integrated management of the bean seed fly

Integrated pest management of bean seed fly

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CONTENTS

1. Industry Summary

Growers of beans, peas and onions are at risk of damage from larval feeding by bean seed fly (BSF) in the UK, especially in spring. Growers do not have access to conventional insecticides to manage BSF in the UK so alternative management strategies are required. This PhD project has aimed to contribute towards an IPM strategy to reduce economic losses caused by BSF in the UK.

The research was split into four objectives to achieve the aim:

- Objective 1: Assess cultural and interference strategies to reduce damage by BSF
- Objective 2: Identify effective trapping methods for monitoring BSF
- Objective 3: Investigate the overwintering biology of BSF
- Objective 4: Create and validate a model to forecast the spring emergence of BSF

The objectives were achieved by conducting surveys, field trials, controlled experiments in a laboratory and computer modelling.

The findings of Objective 1 showed that growers can use cultural and interference strategies to reduce larval feeding on a crop. BSF were shown to be attracted to a bed within 24 hours of being cultivated by a bed former and the timing of sowing in relation to the timing of cultivation was shown to affect the level of damage caused by BSF. Growers should delay sowing in relation to the timing of cultivation by a minimum of three days. Alternatively, the crop can be covered with a fine mesh (0.6mm) if sowing cannot be delayed in relation to the cultivation date. Growers should cover the crop as soon as possible after sowing to reduce the duration of time that BSF can lay eggs in the soil surrounding the seeds.

The findings of Objective 2 can inform growers on how to effectively monitor BSF. Blue sticky traps wrapped around a lure (manufactured by AgBio Inc (2020) and Andermatt UK (2020)) were shown to significantly catch more BSF than sticky traps that were not wrapped around a lure ($P < 0.01$). Sticky traps can become covered in different species of insect (non-target species) as the duration of time in the field increases. The proportion of the trap visible to BSF reduces as more non-target species are caught. It was shown that the proportion of the surface of a blue sticky trap that was visible to BSF significantly affected the number of BSF to be caught on the trap ($P < 0.01$). Traps are likely to become less effective at trapping BSF as less of the surface of the trap becomes visible. The current advice for growers is to monitor BSF with a blue sticky trap wrapped around a lure. The trap should be monitored regularly (e.g. every 24 hours) and replaced when non-target species attach to the trap.

The findings of Objective 3 showed that *Delia platura* (one of two species in the BSF complex) differ from species in the *Delia* genus such as Cabbage Root Fly (CRF) and Onion Fly (OF) in their overwintering strategy. For example, the findings of Objective 3 suggest that a proportion of *D. platura* do not enter diapause (similar to hibernation) and the diapause of *D. platura* is shorter than CRF and OF. The findings from the experiments in Objective 3 can guide the development of the forecast in Objective 4.

The findings of Objective 4 have shown that the Spring emergence of BSF can be predicted using the accumulation of day degrees. The model predicts that the majority of the spring generation of BSF would have emerged once 313 day degrees have been accumulated from 1st January (using a base temperature of 3.9°C). Growers should take caution when sowing in the time period when 241 – 384 day degrees are being accumulated from 1st January (as 25% - 75% of the spring generation are predicted to emerge in this period). The recommendations provided in Objective 1 should be considered when 25 – 75% of the spring generation of BSF are emerging. Additionally, the recommendations provided in Objective 1 should be considered to protect a crop from subsequent generations of BSF.

2. Introduction

2.1. Bean Seed Fly: A pest of horticultural crops in the UK

Bean seed fly (BSF) refers to a complex of two species: *Delia platura* and *Delia florilega* (Kim & Eckenrode, 1984; Teverson, 2018). *D. platura* and *D. florilega* are flies (Diptera) in the family, Anthomyiidae and genus, *Delia*. The genus, *Delia,* contains multiple species that cause economic losses in horticulture, including the Cabbage Root Fly (CRF) (*Delia radicum*) and Onion Fly (OF) (*Delia antiqua*) (Savage et al., 2016).

BSF is distributed globally in temperate zones (Miles, 1948). In the USA and Canada, *D. platura* is named the ‘Seed-corn Maggot’ and *D. florilega* is named the ‘Bean Seed Maggot’ (van der Heyden, Fortier & Savage, 2020). It is reported to affect over 40 host crop species (BAYER Crop Science, 2021). In the UK, BSF damage is predominantly reported in legumes, such as vining peas (Collier & Howard, 2018) and alliums, such as bulb onions (Ellis & Scatcherd, 2007).

Female BSF are attracted to lay eggs when there are increased concentrations of volatiles associated with organic matter and germinating seeds (Guerra et al., 2017; Miller & McClanahan, 1960). Damage to a crop is caused when BSF larvae feed on germinating and emerging seeds and stems (Weston & Miller, 1989). In the UK, damage is primarily caused in spring when BSF emerge from an overwintering phase (Collier & Howard, 2018). Symptoms of infestation include lack of development from the cotyledons in beans (termed: ‘baldheadedness’), patchiness in emergence and plant death (Hill, 1973; Teverson, 2018).

There are no seed treatments approved to prevent BSF damage on legumes in the UK (Collier & Howard, 2018). From the beginning of 2022, Extensions for Minor Use (EAMUs) of seed treatments on alliums (product name: Force, active compound: tefluthrin) expired (Teverson, 2021). Alternative strategies under an Integrated Pest Management (IPM) approach are required to find a sustainable alternative to the sole use of chemicals.

2.2. The biology of bean seed fly

2.2.1. Classification

D. platura and *D. florilega* share very common morphological characteristics and life histories (Kim & Eckenrode, 1984; Savage et al., 2016). Previously they were considered one species due to morphological similarities between *D. platura* and *D. florilega*. DNA barcoding confirms their status as two species (Ding et al., 2015; van der Heyden, Fortier & Savage, 2020).

2.2.2. Development

The development of BSF is holometabolous (Gullan & Cranston, 2014). They have four distinct life stages: eggs, larvae, pupae and adults (Hall & Martín-Vega, 2019). BSF development is controlled by temperature (Throne & Eckenrode, 1986; Sanborn, Wyman & Chapman, 1982). Increasing environmental temperature (to an upper limit) has been shown to decrease the duration of the life cycle, as shown in Figure 1. Wild BSF are shown to have shorter development timings in warmer climates (Valenciano, Casquero & Boto, 2004).

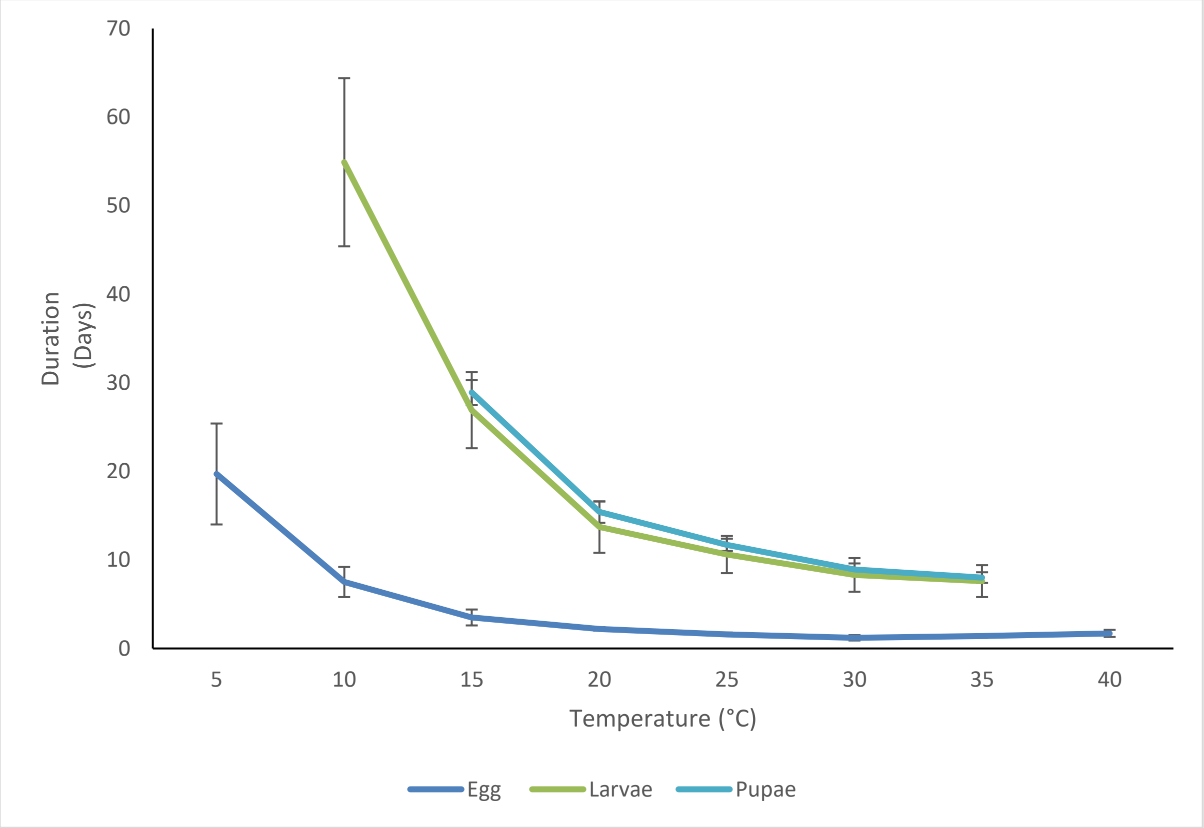

Figure 1. Average durations (days) of development for each life stage of *D. platura* kept at constant temperatures. Error bars show standard error of the mean. Data taken from Throne and Eckenrode (1986).

2.2.3. Diapause

As temperatures reach a lower limit, development duration increases (Figure 1). BSF are predicted to stop developing when temperature decreases below 3.9°C (Sanborn, Wyman & Chapman, 1982; Funderburk, Higley & Pedigo, 1984; Broatch et al., 2006). In the UK, it is assumed that BSF enter a period of arrested development (diapause) over the winter to survive prolonged temperatures below their threshold temperature.

2.3. Monitoring bean seed fly

An important part of an Integrated Pest Management (IPM) strategy involves monitoring the pest species (FAO, 2019). Monitoring methods, such as regular trapping within or surrounding a crop, can show if a pest species is increasing in numbers (Higley & Pedigo, 1985). The results of regular monitoring can indicate when intervention is required to control a pest species (Barzman et al., 2015).

2.3.1. Trapping methods

Growers and researchers have used a variety of traps to monitor BSF and examples of these are shown in Figure 2. In a study aimed to model BSF development under field conditions, baited cone traps were used to measure the phenology of *D. platura* (Funderburk, Higley & Pedigo, 1984). Sticky traps have been used in several studies (e.g. Broatch et al., 2006; Silver et al., 2018a) and baited sticky traps (containing extract from decomposing onions) are marketed to growers (AgBio Inc, 2020; Andermatt UK, 2020). Since 1999, the spring emergence of BSF at Warwick Crop Centre (WCC) has been monitored using yellow water traps.

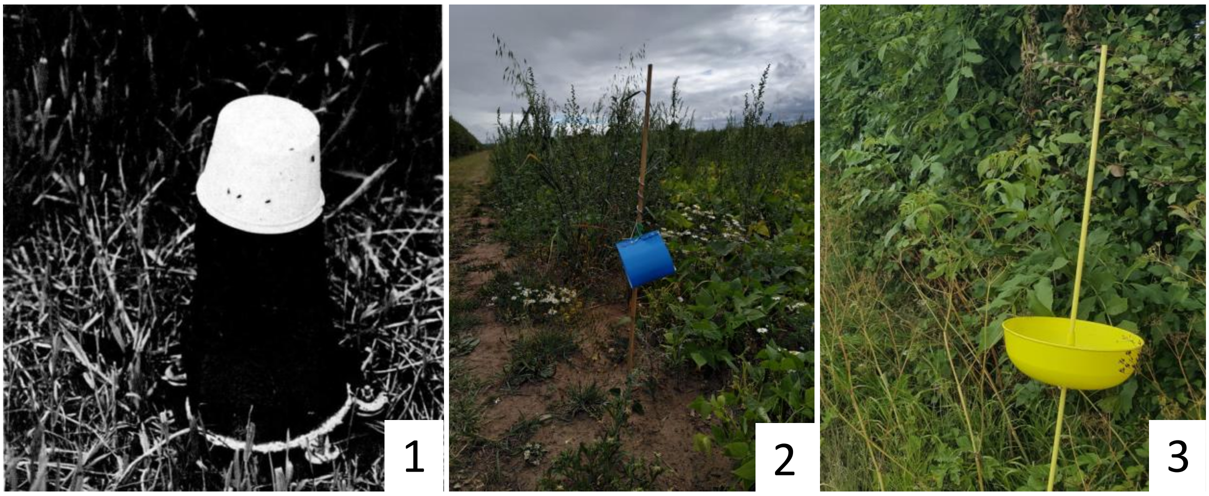

Figure 2. Passive traps used to monitor Bean Seed Fly. 1) Cone trap, taken from (Funderburk et al., 1984) 2) Baited blue sticky trap 3) Yellow water trap

Trapping methods vary in their selectivity for capturing BSF. In a study comparing yellow water and sticky traps, before the flowering stage of the ‘Tobin’ variety of Canola there were 10 times more female *D. platura* found on sticky traps compared to water traps (F = 84.8, $P < 0.001$) (Broatch & Vernon, 1997). Similar results were found for males and after the flowering stage. In a study by Finch (1990), significantly less *D. platura* were caught in a cone trap compared to a selection of other traps ($P < 0.05$).

2.3.2. Using sticky traps to monitor bean seed fly

Sticky traps use adhesive materials to entrap the insect. This method of entrapping the insect increases the difficulty of differentiating between *Delia* species (Finch, 1990). *D. platura* and *D. florilega* have microscopic anatomical differences, such as variations in the length of dorsal bristles on the first tarsomere of the midleg in males (Savage et al., 2016). Should the aim of trapping be to distinguish between species, anatomical characteristics may not be visible on specimens caught on sticky traps. Water traps could be more suitable.

Sticky traps can trap a variety of Anthomyiid species, making it challenging for growers to identify the pest and thus a suitable management strategy. In studies by Broatch and Vernon (1997) and Broatch et al. (2006), non-*Delia* species such as, *Botanophila fugax* and *Adia cinerella* and *Delia* species such as CRF, *Delia floralis* and *Delia planipalis* were found on sticky traps. It is difficult for non-specialists, such as growers, to differentiate between these species (Savage et al., 2016). Figure 3 shows the slight difference in size between BSF and CRF, further highlighting how common *Delia* species can be confused.

Figure 3. Bean seed fly (top row) and cabbage root fly (bottom row) placed on a yellow sticky trap

Sticky traps need to be selective for BSF over other common anthomyiid flies to allow growers to have greater accuracy in identifying BSF. Previously the colour and position of traps have been assessed for their effectiveness in catching *Delia* species. In a study assessing the colour of water traps for their selectiveness in catching CRF, blue water traps were shown to have increased BSF counts compared to a range of different colours (Finch, 1992). Blue water traps caught 8.9 BSF for every CRF. In a study investigating the angle of inclination of yellow sticky traps, horizontal yellow sticky traps caught more BSF than traps at other angles (Finch & Collier, 1989).

Sticky traps baited with compounds found in decomposing onion pulp are marketed to attract and trap more *D. platura* flies than sticky traps without a bait (Kuhar et al., 2006). The effectiveness of baited sticky traps for trapping BSF requires evaluating. There is a lack of empirical evidence to support the selectivity of baited traps for BSF. In a study by Vernon et al. (1989), the effect of trap colour and bait were cross analysed for their effectiveness in trapping OF. Evaluations of baits combined with variables such as sticky trap colour and angle of inclination could further inform best practice for monitoring BSF under an IPM strategy.

2.4. Forecasting bean seed fly

2.4.1. Forecasting insect pests

Forecasting is an integral part of an IPM strategy (Barzman et al., 2015). Insect forecasts are used to predict the timings of insect activity corresponding to environmental factors, such as temperature (Olatinwo & Hoogenboom, 2013). If large numbers of the pest species of concern are predicted to be active, management decisions can be made to reduce crop damage and economic losses. Management decisions may include the timing of insecticide applications (Finch, Collier & Phelps, 1996).

In the UK there are several forecasting systems/models to predict the phenology of species of economic concern. The ‘AHDB Pest Bulletin’ hosted forecasts for a variety of species including Carrot Fly (*Psila rosae*), Black Bean Aphid (*Aphis* spp.) and CRF (Collier, 2021a). Figure 4 shows forecasted egg laying activity of CRF in different regions of the UK. There is a predicted trend for eggs to be laid later in the year as latitude increases.

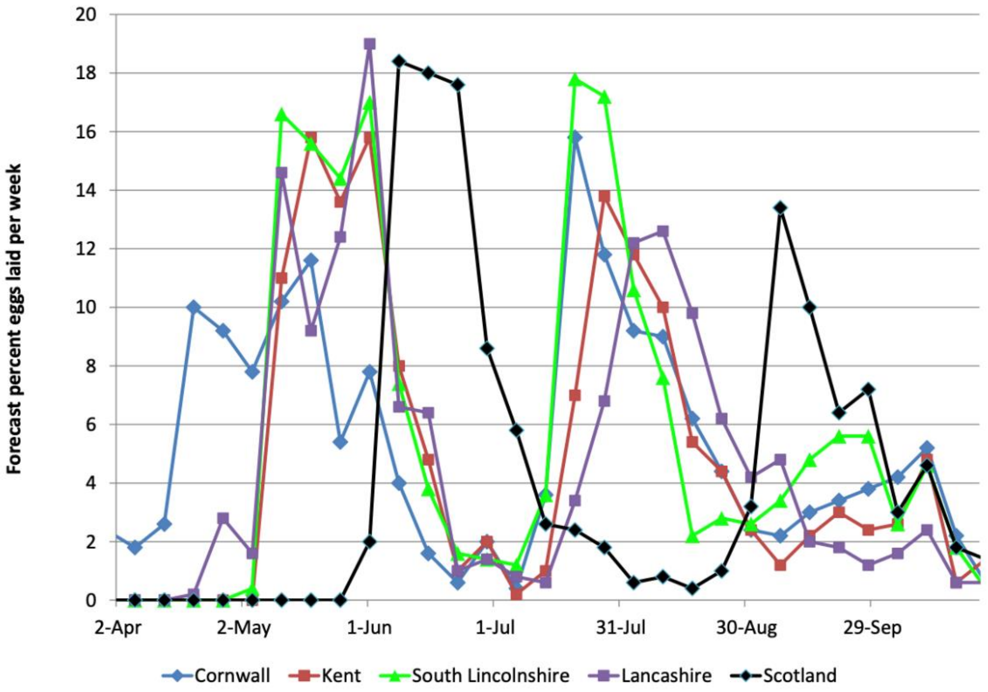

Figure 4. Predicted number of eggs laid per week by cabbage root fly in five regions of the UK in 2021. Taken from Collier (2021b)

Generally, pest insect forecasts such as those included in the ‘AHDB Pest Bulletin’, focus on the accumulation of temperature (thermal units) over time as the main ‘driver’ of insect development and activity (Phelps et al., 1993; Finch, Collier & Phelps, 1996). Many models that forecast dipteran pests focus on the accumulation of day-degrees from a predefined date when temperatures rise above a predetermined threshold temperature (Broatch et al., 2006; Funderburk, Higley & Pedigo, 1984; Liu, McEwen & Ritchey, 1982; Rowley et al., 2017; Son, Lee & Chung, 2007). Some models incorporate further parameters (Phelps et al., 1993; Rasche & Taylor, 2019). Examples of models developed in the UK include a Monte Carlo simulation programme to predict CRF activity and a day degree model to predict Saddle Gall Midge emergence (Phelps et al., 1993; Rowley et al., 2017).

2.4.2. Current models to forecast bean seed fly

There are a limited number of models to forecast BSF and they originate from the continent of North America. These models focus on the accumulation of day-degrees to estimate peaks in emergence and the proportion of a generation to emerge at specific time points. In research by Strong and Apple (1958), the threshold temperature for development of *D. platura* was estimated to be 10°C. They estimated accumulated day degrees for the peaks of four generations in Columbia County, Wisconsin, USA. In a later study, a lower threshold temperature for development of *D. platura* was estimated to be 3.9°C (Sanborn, Wyman & Chapman, 1982). The threshold temperature was obtained from a culture of *D. platura* originating from Geneva, New York, USA with added wild specimens from Wisconsin, USA.

In research by Funderburk et al. (1984), accumulated day degrees for the peaks of three generations of wild *D. platura* were averaged in central Iowa, USA using the threshold temperature of 3.9°C. The same threshold temperature was used to estimate proportions of emergence at specific time points of overwintering and F_1 generations of wild BSF in Central Alberta, Canada (Broatch et al., 2006). Forecasts developed from UK populations of BSF may be more reliable and accurate for UK growers.

2.5. Management practises to reduce damage by bean seed fly

Historically, a range of insecticides have been used to control BSF worldwide (Ditman et al., 1955; Ellis and Scatcherd, 2007; Reíd, 1940). In the UK, from January 2022, an EAMU for use of Force ST (active compound: 200g/L tefluthrin) on outdoor alliums to control for BSF was due to expire (Health and Safety Executive, 2019). This will mark the end to ‘silver bullet’ insecticidal approaches to controlling BSF damage in UK crops for the foreseeable future (Collier et al., 2020).

2.5.1. Cultural control

Cultural control involves altering agronomic practises in a growing system to reduce the chance of infestation by a pest species and damage to the crop (Cullen & Holm, 2013). Examples of cultural control strategies that have been researched for their efficiency in reducing damage by *Delia* species are shown in Table 1.

Table 1. Examples of cultural control strategies researched for *Delia* species

Species	Method	Reference
Various *Delia* species	Altering the seeding rate & row spacing	Dosdall et al. (1998)
Cabbage Root Fly (CRF)	Altering the length of time between harvest of the previous crop and planting of the new crop	Joseph, Godfrey & Bettiga (2017)
Cabbage Root Fly (CRF) & Onion Fly (OF)	Planting non-host plant species around host plant species	Finch, Billiald & Collier (2003)
Onion Fly (OF)	Altering the time of planting	Nault et al. (2011)
Onion Fly (OF)	Altering the time of planting & seeding rate	Hermize (2015)
Bean Seed Fly (BSF)	Non-tillage regimes & the effects of incorporating different species of cover crop	Hammond (1990)
Bean Seed Fly (BSF)	Altering the timing of planting	Silver, Hillier & Blatt (2018a)

Planting timing in relation to bean seed fly phenology

Altering the timing of planting can create asynchrony between crop and pest phenology (Dent & Binks, 2020). Once the pattern of emergence of a pest insect species is known, the crop species can be planted to avoid peaks in emergence of the pest species, reducing the levels of feeding on the crop by the pest species. This practise has been evaluated for its efficiency in reducing the levels of larval feeding by BSF on a crop, as shown in Table 1.

In research by Silver et al. (2018a), later plantings of *Phaseolus vulgaris* (Variety: Gold Rush) showed significantly lower damage ratings for BSF than earlier plantings in the year ($P < 0.05$). In two fields on Prince Edward Island, Canada, damage ratings increased 7-fold and 6-fold when *P. vulgaris* seeds were planted earlier in the year in each field, respectively (First field: 15th June compared to 29th July. Second field: 25th May compared to 6th July). However, in both fields bean pod weights were significantly higher on the earlier planting date ($P < 0.05$) than the later planting date. Thus, whilst the later planting dates resulted in lower levels of damage by BSF, the earlier planting dates resulted in higher yields per plant. Planting date has been shown to significantly affect the yields of other varieties of *P. vulgaris* ($P < 0.05$) (Balasubramanian, Vandenberg & Hucl, 2004; Esmaeilzadeh & Aminpanah, 2015). In the research by Silver et al. (2018b), environmental conditions such as temperature and moisture levels in the earlier planting may have been more favourable for crop growth and yield than in the later planting. It is important to consider the balance between later planting for management of BSF infestation and earlier planting for more optimal environmental conditions (Silver, Hillier & Blatt, 2018b).

Cultivation and tillage

BSF are stimulated to lay eggs in areas of high organic matter, such as incorporated green manure and disturbed soils (Miller & McClanahan, 1960; Eckenrode, Harman & Webb, 1975; Hammond, 1990). It has been hypothesised that cultivation and incorporation of organic matter prior to planting will attract BSF to oviposit around the sown plants and increase the chance of larval feeding on the crop. In research by Hammond (1990), soybeans planted after alfalfa was incorporated into the soil were significantly more damaged by BSF than soybeans directly drilled into the alfalfa ($P < 0.05$). Yet, there was no significant difference in the proportion of plants damaged by BSF when the soybeans were directly drilled into bare soil compared to ploughed soil.

In research by Hammond and Cooper (1993), traps caught 40% less adults in soybeans planted 18 days after cover crop incorporation than soybeans planted 2 days after cover crop incorporation ($P < 0.05$). Their research showed a trend for there to be less ‘baldheaded’ soybeans (a symptom of BSF infestation (Teverson, 2018)) when planting occurred > 218 accumulated day degrees after cover crop incorporation.

After 218 accumulated day degrees from cover crop incorporation, BSF eggs laid at the time of cover crop incorporation were likely to be reaching pupation (Sanborn, Wyman & Chapman, 1982; Throne & Eckenrode, 1986). When soybeans were planted < 218 day degrees after cover crop incorporation, eggs laid at cover crop incorporation would have not reached pupation and there would be a larger larval feeding pressure on the soybeans.

Growers in the UK usually plant on the day of cultivation as this is the most economic option (Collier & Howard, 2018). If the soil has a high organic content due to the incorporation of organic matter from cultivation, growers are at a higher risk of experiencing economic loss due to BSF infestation (Hammond, 1990; Hammond & Cooper, 1993). Increasing the time between cultivation and planting may be an effective preventative measure under an IPM strategy.

2.5.2. Interference methods of control

Interference methods such as physical barriers can act as a preventative method under an IPM strategy by stopping a pest species from reaching the crop (Dent & Binks, 2020). Examples of interference methods researched for their efficiency in preventing damage by *Delia* species are shown in Table 2.

Table 2. Examples of interference methods researched for their efficiency in preventing damage by *Delia* species

Species	Method	Reference
Cabbage Root Fly (CRF)	Polyester row cover	(Matthews-Gehringer' And and Hough-Goldstein, 1988)
Cabbage Root Fly (CRF)	Vertical mesh barrier	(Blackshaw, Vernon & Prasad, 2012)
Onion Fly (OF)	Non-woven fibre barrier	(Hoffmann et al., 2009)
Bean Seed Fly (BSF)	Polyester row cover	(Hough-Goldstein, 1987)

Physical barriers

In research by Hough-Goldstein (1987), Reemay polyester material placed over plots sown with peas, watermelons and soybeans did not have a significant effect on plant damage by *D. platura*. The field was ploughed and disked the day prior to planting and the material was placed over the soil on the day of planting. The authors hypothesised that the disturbance to the soil via ploughing and disking could have attracted egg laying in the period prior to sowing and covering the crops (Miller & McClanahan, 1960).

Protecting newly sown crops that are susceptible to BSF damage with a physical barrier such as a polyester row cover may be a viable option for UK growers if the soil is covered once cultivation or cover crop incorporation occurs. This may reduce egg laying in the soil before the crop is planted, reducing the risk of larval feeding on the newly sown plants. Alternatively, a row cover may be viable if the period between cultivation and planting is reduced and the newly sown plants are covered immediately after planting. This method may reduce the period BSF have to access the soil to lay eggs.

2.6. Aims & objectives

UK growers require an alternative strategy to the sole use of insecticides to reduce larval feeding by BSF in horticultural crops. The overall aim of this project is to contribute towards an IPM strategy to reduce crop damage and economic losses caused by BSF in horticultural crops. The objectives will focus on the two lower levels of the IPM pyramid (avoidance/prevention and monitoring/forecasting) (Barzman et al., 2015; Gibb, 2015).

Objective 1 is to assess cultural and interference strategies to reduce damage by BSF. Cultural and interference strategies can prevent an insect from establishing in a crop (Cullen & Holm, 2013; Dent & Binks, 2020). BSF are attracted to lay eggs in areas of high organic matter and disturbed soils (Miller & McClanahan, 1960; Hammond, 1990). It is unknown if BSF are attracted to recently cultivated bare soil. Delaying sowing in relation to the cultivation date may affect the level of damage caused by larval feeding on the newly sown seeds.

Objective 2 is to identify effective trapping methods for monitoring BSF. BSF activity can be monitored by counting the number of BSF on blue sticky traps (Finch & Collier, 1989). Similar species such as CRF can easily be misidentified as BSF by growers (Figure 3). The current trapping method (blue sticky traps) may be improved by making the trap more selective to BSF over similar species such as CRF. Experiments will be designed to test treatments that may improve the number of BSF caught on blue sticky traps and the selectivity of the trap to BSF compared to similar species. The blue surface of the traps will become less visible as insects attach to the trap. It is unknown if the 'attractiveness' of blue sticky traps is affected as less of the blue surface is visible. An experiment will be designed to test the effect of covering traps in different proportions of black card on the count of BSF per trap. Experiments are shown Table 3.

Table 3. Experiments of Objective 2

Experiment	Treatment	Response variable
1	Lure (manufactured by Ag-Bio, Inc. and Andermatt)	BSF count, Muscoidea count, by-catch
2	Trap height	BSF count, Muscoidea count, by-catch
3	Trap orientation	BSF count, Muscoidea count, by-catch
4	Trap cover with black card	BSF count

Objective 3 is to investigate the overwintering biology of *D. platura*. BSF emerge in large numbers in Spring (Broatch et al., 2006). It would be beneficial to forecast the timing of emergence of BSF in Spring. More information is required on their overwintering biology, such as understanding of whether they enter diapause over the winter. A *D. platura* culture is maintained at Warwick Crop Centre. Experiments will be designed to gain more understanding on the overwintering biology of *D. platura*.

Objective 4 is to create and validate a model to forecast the spring emergence of BSF. Planting dates could be altered to avoid the Spring peak in BSF emergence if the emergence of BSF in Spring could be predicted. Models that accumulate day degrees are used to predict the emergence of BSF in the USA (Broatch et al., 2006; Strong & Apple, 1958; Funderburk, Higley & Pedigo, 1984; Sanborn, Wyman & Chapman, 1982). A model will be created to predict the emergence of BSF in the UK. The model will be improved using the knowledge gained from Objective 3.

3. Materials and methods

3.1. General methods

Sampling site

All field experiments took place at Warwick Crop Centre (WCC), an experimental farm approximately five kilometres east of Stratford upon Avon in Warwickshire, UK (52°N, -2°E).

Data analysis

All data was analysed using R Studio (Posit Team, 2023; R Core Team, 2023).

In Objective 1, Generalised Linear Mixed Models (GLMM) were used to analyse the data as the experimental design contained spatial and temporal autocorrelation. The data had a Poisson or negative binomial distribution as the data consisted of count data (Thomas, 2015). A bonferroni correction was used to conduct pairwise comparisons to reduce the risk of type one errors. The ‘lme4’ and ‘emmeans’ packages were used to analyse the data (Bates et al., 2015; Lenth, 2023).

In Objective 2, a generalised linear model (GLM) using bootstrapping resampling with 1000 samples was used to analyse the data with a negative binomial distribution and log link function. The data was analysed using the package ‘Mvabund’ in R Studio (Wang et al., 2017). GLMMs and zero-inflated GLMMs with a Poisson distribution and log link function were used to analyse the data. Pairwise comparisons used a Bonferroni correction. The ‘lme4’, ‘GlmmTMB’ and ‘emmeans’ packages were used (Lenth, 2023; Bates et al., 2015; Bolker, 2023).

In Objective 3, the data was shown to not be normally distributed. Kruskal Wallis tests were used to analyse the data.

In Objective 4, non-linear least squares regression was modelled using ‘drc’ and ‘aomisc’ packages (Onofri, 2020; Ritz et al., 2015).

3.2. Objective 1: Assess cultural and interference strategies for reducing damage by bean seed fly

3.2.1. Experiment 1: The effect of cultivation on bean seed fly count

Sampling sites

The experiment was split into two trials over two years. In 2020 and 2021 there were four and three trial sites, respectively. In 2020 and 2021, the experiment was repeated twice and three times, respectively.

Sampling methods

The arrangement of the trials changed in each year. Plan views of the trial sites are shown in Figure 5 and Figure 6. In 2020 and 2021, the water traps were yellow and white, respectively. On the day water traps were placed, they were filled with a liquid detergent and a Campden tablet was added. Contents were collected daily at approximately the same time. A bed was chosen at random to be cultivated for each site and replication in time. Timings of trapping and cultivation are shown in Table 4 and Table 5.

Identification

Due to time constraints it was not possible to use a microscope to confirm the identity of BSF in all the samples. Samples were assessed by a person experienced at identifying *Delia* species by eye. To check accuracy, sub-samples were identified to the BSF species complex level using the keys by Brooks (1951) and Savage et al. (2016).

Table 4. Sampling dates for both replications in 2020.

Start Date	Cultivation Date	Finish Date	Days of Sampling
13/07/2020	17/07/2020	22/07/2020	10
06/08/2020	10/08/2020	14/08/2020	9

Table 5. Sampling dates for the three replications in 2021.

Start Date	Cultivation Date	Finish Date	Days of Sampling
28/06/2021	30/06/2021	02/07/2021	4
09/08/2021	11/08/2021	13/08/2021	4

06/10/2021	07/10/2021	08/10/2021	2

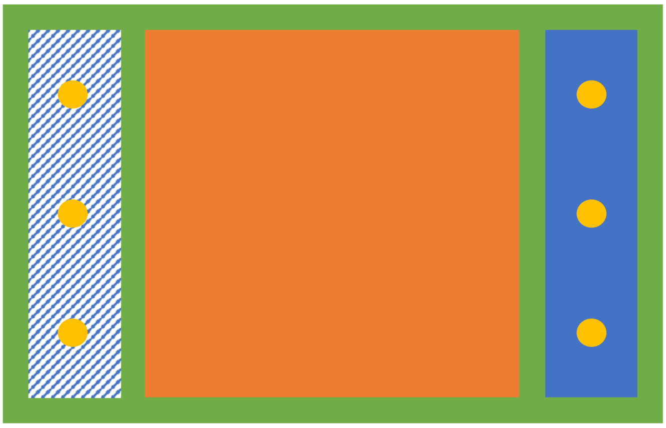

Figure 5. Plan view of sample site arrangement in 2020. The orange square refers to the patch of vegetation or bare soil. The blue and stripey rectangles refer to the beds. The yellow circles refer to the positions of the yellow water traps. Water traps were approximately seven meters apart.

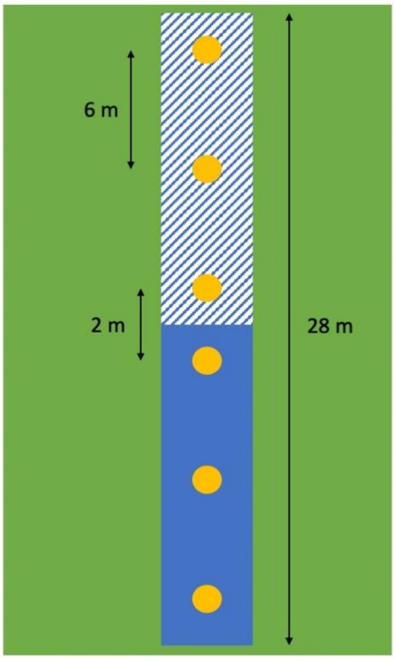

Figure 6. Plan view of sample site arrangement in 2021. The bed is split into two treatments shown by blue and stripey rectangles. The yellow circles refer to the positions of the white water traps. The water traps were placed 6m apart in each treatment.

3.2.2. Experiment 2: The effect of the timing of cultivation and covering of the crop in relation to sowing date on damage caused by bean seed fly on vining peas and dwarf French beans

Sampling sites

The experiment was split into two trials. Vining pea and French bean seeds were sown in the first and second trials, respectively. The vining pea trial was replicated three times in 2021 and once in 2022. The French bean trial was replicated three times in 2022. There was a different trial site for each year. Each trial took place in the same trial site within a year. In 2022, manure was evenly distributed over the trial site at a rate of 40 T/ha before the trials started.

The trial site contained four beds that were created by a bed former. The four beds were parallel to each other. The trial site was set up at least 21 days prior to the sowing date. The beds were approximately 1.83m in width. There were eight plots that were 2m long per bed. The gap between plots was increased from 0.5m to 2m to limit disturbance to the plots.

Trial design

The treatments are shown in Table 6. Treatment one plots were cultivated whilst the beds were being formed. A power harrow was used to recultivate plots. A fine mesh was used to cover individual plots. The fine mesh size was 0.6mm. The mesh was placed over the plot and soil was used to secure the ends of the mesh in the wheeling and spacing between plots.

Plots were assigned treatments per trial using a (4x4)/2 Trojan square (semi-Latin square) (Edmondson, 1998).

Table 6. Timing of cultivation and covering the plots in Experiment 2. Day 0 refers to the day of sowing.

Treatment	Timing of cultivation (days prior to sowing date)	Timing of covering
1	21 +	No covering
2	14	No covering
3	7	No covering
4	3	No covering
5	1	No covering
6	0	No covering
7	0	Day of sowing
8	0	Day after sowing

Trial timings

Timings of each trial and the varieties grown are shown in Table 7.

Table 7. Sowing dates of the crops for both trials in Experiment 2. Mean bean seed fly count recorded in a monitoring plot at Warwick Crop Centre (yellow water traps)

Year	Replicate	Crop	Variety	Sowing date	Mean fly count (trap/day)
2021	1	Vining pea	SV8112QH	15/04/2021	1
2021	2	Vining pea	SV8112QH	24/06/2021	5
2021	3	Vining pea	SV8112QH	23/09/2021	1
2022	4	Vining pea	Boogie	28/04/2022	2
2022	1	Dwarf French bean	Jameson	23/06/2022	0
2022	2	Dwarf French bean	Jameson	11/08/2022	0
2022	3	Dwarf French bean	Jameson	29/09/2022	2

Trial assessment

Assessments took place once the majority of vining peas had developed two leaves and the cotyledons of the dwarf French bean seedlings had separated. All seedlings (including those that did not emerge) present in the inner meter of the inner two rows per plot were assessed for BSF damage.

Table 8. Descriptions of assessments on vining peas and dwarf French bean plants per plot. Samples were taken from the middle meter of the middle two rows per plot.

Crop	Assessment	Description
Vining peas and dwarf French beans	Emerged plants	Count of all emerged plants.
Vining peas and dwarf French beans	Plants that did not emerge	Count of all plants or seeds found in the soil of the rows.
Vining peas and dwarf French beans	Plants containing larvae	Count of all plants containing larvae.
Vining peas	Tunnelling in seed	Count of all plants with clear tunnels in the seed. Plant was counted as having tunnelling in the seed if the seed contained larvae.
Dwarf French beans	No symptoms	Count of emerged plants with no lesions in the leaves
Dwarf French beans	Major lesions in leaves	Count of all emerged plants lacking > 50% of the unifoliate first true leaves.
Dwarf French beans	Baldheadedness	Count of all emerged French bean plants with no development from the cotyledons. No true leaf development.

3.3. Objective 2: Identify effective trapping methods for monitoring bean seed fly

The methods and findings from two experiments in Table 3 are presented in this report. The complete set of findings will be presented in the PhD thesis.

3.3.1. General methods

Identification

BSF were counted per trap without a microscope. Proportions of the suspected BSF were identified under a microscope (Experiment 3: 25% & Experiment 4: 10%). A key was used to identify BSF (Savage et al., 2016). Male *D. platura* and *D. florilega* were differentiated. Female BSF were not differentiated as female *D. platura* and *D. florilega* cannot be differentiated by morphological traits (Savage et al., 2016).

3.3.2. Experiment 3: The effect of lures attached to blue sticky traps on the count of bean seed fly on the trap

Sampling sites

Two blue sticky traps were placed in four different locations at WCC from 19th June 2020 – 17th July 2020. Traps were placed approximately 20m apart. The positions of traps placed at WCC are shown in Figure 7.

Figure 7. Approximate locations of traps placed at Warwick Crop Centre for Experiment 3 1: Hedgerow base. 2: Haricot bean field perimeter. 3: Wheat field perimeter. 4: Maize field perimeter.

Trap arrangement

In each location a lure was attached to one trap. Lures were purchased from AgBio-Inc (AgBio Inc, 2020). The lures contained volatile attractants (2-phenylethanol & *n*-valeric acid) associated with decomposing onion pulp (Kuhar et al., 2006). Sticky traps were blue. They were 25 cm long and 10cm wide. Sticky traps were wrapped around the plastic pouch that contained the lure. Traps were attached to the top of 1m long bamboo canes. The film was removed from the outer side of the trap to collect insects. Trap arrangement is shown in Figure 8.

Figure 8. Arrangement of sticky traps for experiment 3. A: No lure. B: Lure.

3.3.3. Experiment 4: The effect of the proportion of a blue sticky trap covered with black card on the count of bean seed fly on the trap

Trial design

All sampling occurred at one sample site at WCC between 10/10/2022 – 09/11/2022. There were four treatments as shown in Table 9. Black card ($4cm^2$) was attached in a random pattern to the sticky surface of one side of each blue sticky trap to cover 25 – 75% of the trap. An example is shown in Figure 9. A control trap had no black card attached to the surface of the trap. Traps were collected after 24 hours.

Table 9. Treatments for Experiment 4

Treatment	Cover (%)
1	0
2	25
3	50
4	75

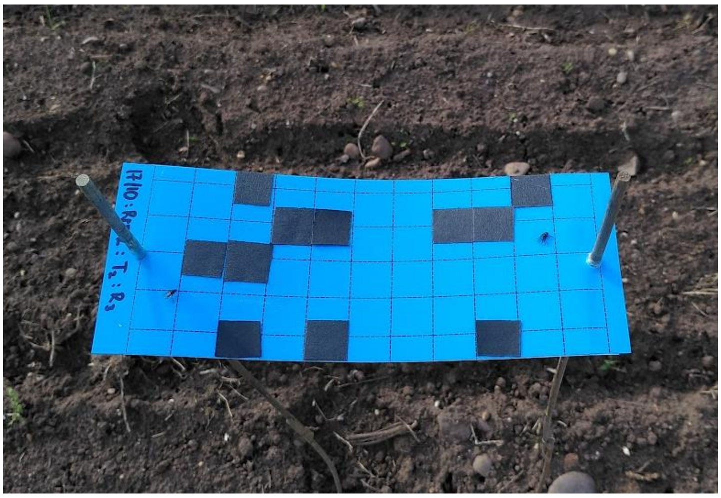

Figure 9. Blue sticky trap with 25% of the sticky surface covered with black card.

The treatments were replicated four times in space using a 4 x 4 Latin square that was re-randomised for every repeat in time. The trial was repeated three times. Traps were positioned horizontally between two bamboo canes approximately 90cm above the ground. Traps were 3m apart in each column and 3.5m apart in each row.

3.4. Objective 3: Investigate the overwintering biology of bean seed fly

3.4.1. General methods

Creating a bean seed fly culture

Wild *D. platura* eggs and larvae were collected from baited pots at WCC. They were reared through subsequent generations in controlled conditions using similar methods to Harris, Svec & Begg (1965) and Webb & Eckenrode (1978).

Estimating development in field conditions

The accumulation of day degrees was used to estimate the development of *D. platura* under field conditions. The threshold temperature of 3.9°C (Broatch et al., 2006) was subtracted from hourly soil temperatures above or equal to 3.9°C. If hourly temperatures were below 3.9°C, 0 degree hours were accumulated. These were summed for each day and then divided by 24 to give day degrees. It is estimated that is takes 267 day degrees for eggs to develop into pupae (Throne & Eckenrode, 1986). Temperatures were recorded at a 10cm depth in the soil at WCC. The temperature was recorded at the same site for all experiments and was not recorded in the specific trial sites.

Estimating development in controlled conditions

Durations (days) for eggs to develop into pupae at constant temperatures have been published (Throne & Eckenrode, 1986). The average number of days for *D. platura* to develop from eggs to pupae are shown in Table 10. Developing *D. platura* were placed at the temperatures and durations shown in Table 10 whilst in controlled conditions.

Table 10. Average durations (days) for *D. platura* eggs to develop into pupae. Data taken from Throne & Eckenrode (1986)

Temperature (°C)	Duration (Days)
10	62
15	30
20	16

Determining diapause

It takes 15 days for pupae of *D. platura* to emerge as adults at a constant temperature of 20°C and 16H photoperiod (Throne & Eckenrode, 1986). Pupae were placed in jars containing moist vermiculite in a controlled environment room at 20°C and a photoperiod of 16 hours. Adult flies per jar were counted every 2 – 4 days. Flies were assumed to not be in diapause when they emerged up until 15 days at 20°C. Flies were assumed to be in diapause when they emerged after 15 days at 20°C.

3.4.2. Experiment 5: Measuring the development of bean seed fly in field conditions at Warwick Crop Centre

Sampling sites

A survey was conducted in 2020 and the experiment was split into two trials across 2021 and 2022. In autumn 2020, pupae were collected at intervals from various fields at WCC. In late summer and autumn 2021 and 2022, eggs from the *D. platura* culture were placed in field conditions at WCC. The trial site remained the same in 2021 and 2022.

Timings

Pots of wild BSF and *D. platura* from the culture were placed outside until they developed into pupae in late summer and autumn 2020 and 2021, respectively. These dates are shown in Table 11. The pupae were filtered after approximately 267 day degrees had been accumulated from the date that they were placed in the field as eggs. The emergence of the adult flies was recorded using the methods described in 3.4.1: General methods.

Table 11. Dates of placing and filtering pots of pupae that had developed in field conditions. Survey: 2020 - 2021 Trial one: 2021 – 2022 Trial two: 2022 – 2023. Average hourly temperature (soil at 10cm depth) is calculated from the date of eggs being laid or placing eggs in field conditions and the date of filtering the pupae.

Trial	Treatment	Replicates	Placing in field	Placing in tygan house	Filtering date	Pupae (per pot)	Average hourly temperature (°C)
Survey	1	1	01/09	04/09	23/09	35	17±2
Survey	2	1	18/09	22/09	27/10	40	12±2
Survey	3	1	29/09	02/10	12/11	11	10±2
Survey	4	1	09/10	13/10	27/11	25	9±2
Survey	5	1	13/10	19/10	04/12	16	9±2
1	1	15	31/08 - 02/09	N/A	22/09	74±24	18±3
1	2	6	23/09 - 24/09	N/A	26/10	60±42	13±3
1	3	15	12/10 - 13/10	N/A	10/12	35±30	8±4
1	4	10	03/11 - 05/11	N/A	12/01	38±22	6±3
1	5	15	24/11	N/A	22/02	27±13	5±2
2	1	7	31/08	N/A	23/09	137±34	17±2
2	2	7	14/09	N/A	12/10	128±15	13±3

2	3	7	21/09	N/A	25/10	135±31	12±2
2	4	7	28/09	N/A	02/11	123±37	12±2
2	5	7	05/10	N/A	09/11	138±9	12±2
2	6	7	12/10	N/A	22/11	102±29	11±2
2	7	7	19/10	N/A	20/12	98±33	7±5

3.4.3. Experiment 6: Measuring the development of *D. platura* under controlled conditions

Treatments

The experiment was split into two trials. The results from Trial 1 informed the treatments for Trial 2. Pots containing eggs from the culture were placed in a controlled environment at 10°C for 45 days. The photoperiod was eight hours. Pupae were filtered from the pots placed at 10°C for 45 days and placed in jars of vermiculite. Jars were placed at 0°C in a fridge with no photoperiod for different durations. Treatments are shown in Table 12.

Table 12. Durations pupae spent at 0°C

Trial	Treatment	Days (at 0°C)
1	Control	0
1	1	50
1	2	100
1	3	150
2	Control	0
2	1	8
2	2	20
2	3	29
2	4	40
2	5	50
2	6	75

Recording emergence

The emergence of adult flies was recorded over time per treatment and replicate using the methods described in 3.4.1: General methods. The pupae were left for 100 and 50 days for Trial 1 and 2, respectively. The pupae that had not produced flies were assumed to be deceased or in diapause. To test if the pupae were in diapause and needed more time at 0°C to complete diapause, the jars were placed at 0°C for a further 100 and 50 days for Trial 1 and 2, respectively.

The jars were then placed at 20°C for 50 and 50 days for Trial 1 and 2, respectively. Emergence was recorded every 2 – 4 days. After this period, pupae which did not hatch were dissected to check if they were dead.

3.5. Objective 4: Create and validate a model to predict the spring emergence of bean seed fly

3.5.1. Experiment 7: Day degree accumulation to predict the development of bean seed fly

Sampling method

BSF activity has been recorded twice weekly in spring and summer at WCC between 1999 – present. Three yellow water traps were placed along a transect approximately 10m apart. The number of BSF per trap was counted without using a microscope. BSF counts from 2014 – 2019 were used for this analysis as hourly temperatures were not available from before 2014. Data from 2015 was not used as there was not a clear time point for the start of the emergence of the spring generation.

Identifying peaks in emergence

The number of BSF caught in each trap was averaged per trap for each sampling date. The average number of BSF caught per trap was divided by the number of days between the sampling date and the previous sampling date (average number of BSF caught per trap per day). The start, peak and end date of the spring generation was estimated for each year BSF were monitored. The definitions in Table 13 were used to estimate the start, peak and end of the Spring generation.

Table 13.Definitions used to estimate the dates of the beginning, peak and finish of the spring generation of BSF each year.

Point of Measurement	**Definition**
Beginning	The time point from when an incline in BSF trap counts occurs at the beginning of the year.
Peak	The time point in the emergence of the generation when the largest count of BSF occurs
Finish	After the peak, the time point at which the steep decline in BSF trap counts decrease or an incline begins. If a steep decline is followed by a steep incline, this is not considered as the finish point.

Modelling cumulative emergence

The average number of BSF caught per trap per day were converted into cumulative emergence for the spring generation per year. Cumulative emergence was converted into percentage cumulative emergence, so that on the ‘beginning’ and ‘finish’ dates of the spring generation, 0% and 100% of BSF for the spring generation were predicted to have emerged, respectively.

4. Results

4.1. Objective 1: Assess cultural and interference strategies for reducing damage by bean seed fly

There was a significant increase in BSF count (per water trap) approximately 24 hours after cultivation (referred to as day one) in both trials (Trial 1: $\chi^2 = 15.909$, df = 1, $P < 0.001$) (Trial 2: $\chi^2 = 13.244$, df = 1, $P < 0.001$) as compared to beds that were not cultivated. There were no significant differences between BSFcount (per water trap) in each bed per site approximately 24 and one hour prior to cultivation and 48 hours after cultivation in both trials. BSF counts per day are shown in Figure 10 and Figure 11.

There were minimal differences found in the field trial that assessed the effect of timing of cultivation and covering of the plot in relation to the sowing date on damage caused by BSFin vining peas. Timing of cultivation and covering the plots was shown to significantly affect counts of vining pea plants containing larvae as shown in Figure 12 ($\chi 2 = 38.267$, df = 7, $P < 0.001$). Significantly more plants contained larvae in plots cultivated the day before sowing than plots cultivated on the day of sowing and covered one and 24 hours after sowing ($P < 0.01$).

There was a significant effect of timing of cultivation and covering of the plot in relation to the sowing date on damage caused by BSF in dwarf French beans as shown in Figure 13 ($\chi 2 = 27.592$, df = 7, $P < 0.001$). Significantly more plants showed ‘baldheaded’ symptoms when plots were cultivated on the day of sowing and were not covered than plots cultivated on the day of sowing and covered one hour after sowing and plots that were not covered and cultivated 3 – 21 days prior to sowing ($P < 0.05$). Significantly more plants showed ‘baldheaded’ symptoms when plots were cultivated on the day of sowing and covered 24 hours after sowing than plots that were not covered and cultivated 3 – 21 days prior to sowing ($P < 0.05$).

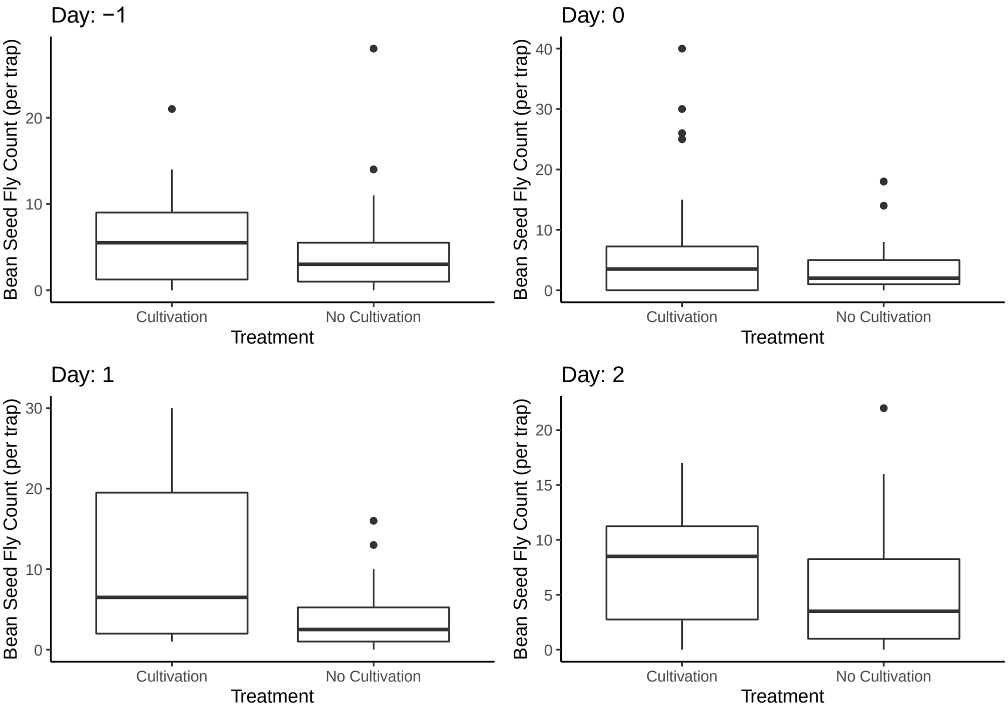

Figure 10. Bean Seed Fly count per trap per day in 2020. Day -1 and 0 refers to 24 hours and one hour prior to cultivation, respectively. Day 1 and 2 refers to 24 and 48 hours after cultivation, respectively.

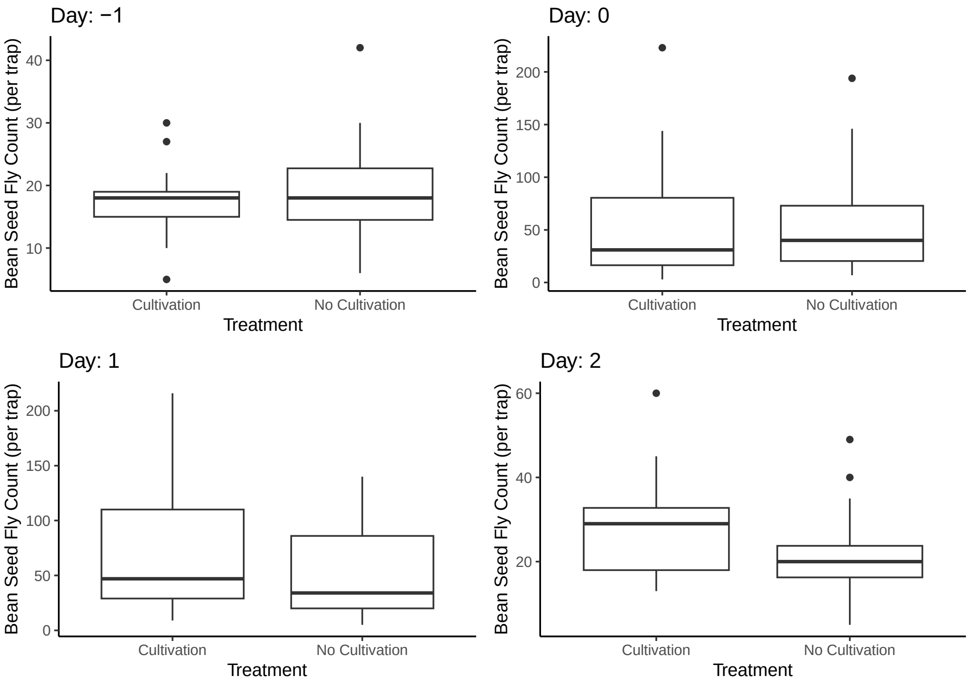

Figure 11. Bean Seed Fly count per trap per day in 2021. Day -1 and 0 refers to 24 hours and one hour prior to cultivation, respectively. Day 1 and 2 refers to 24 and 48 hours after cultivation, respectively.

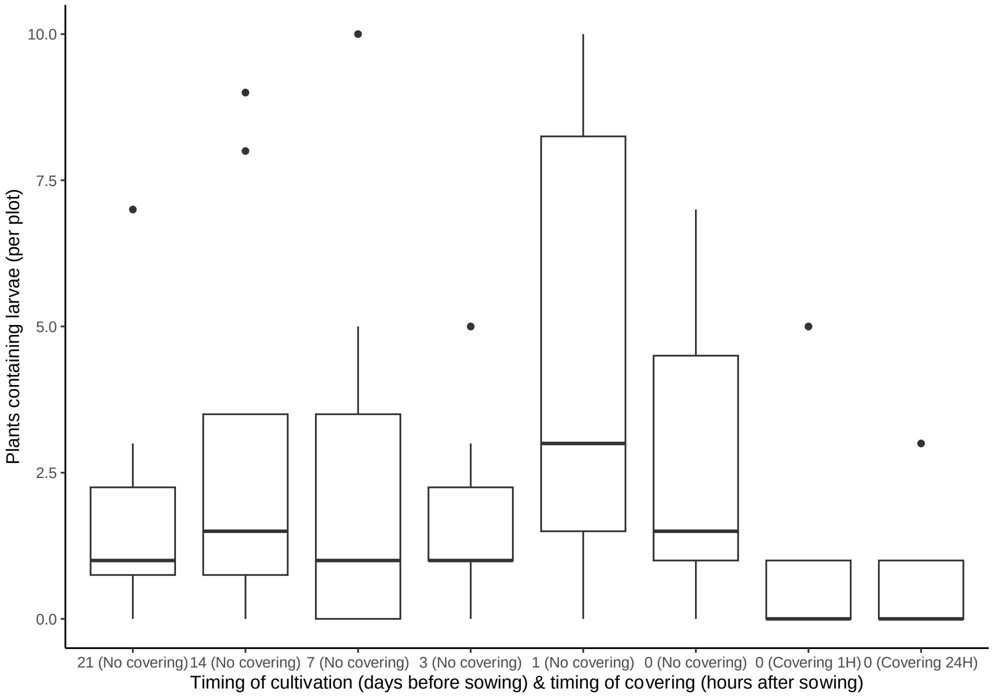

Figure 12. Counts of vining pea plants that contained larvae per plot for repeats one and two of the field trial.

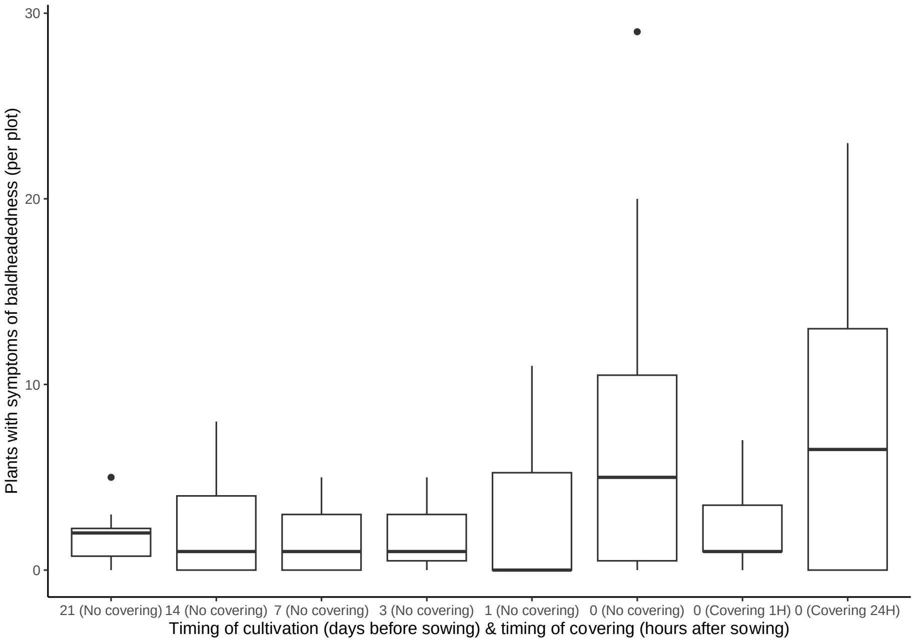

Figure 13. Counts of dwarf French bean plants to show symptoms of ‘baldheadedness’ per plot for the three repeats of the field trial.

4.2. Objective 2: Identify effective trapping methods for monitoring bean seed fly

Blue sticky traps with a lure attached caught significantly more BSF than blue sticky traps not containing a lure ($F = 3.916$, $P < 0.01$) as shown in Figure 14. On average blue sticky traps with a lure attached caught 34 BSF per week and blue sticky traps with no lure attached caught 12 BSF per week.

The count of BSF per square did significantly differ between blue sticky traps with 0 – 75% cover with black card ($\chi^2 = 13.8$, $Df = 3$, $P < 0.01$). There were no significant pairwise comparisons. The counts of BSF per square (as non-integers) are shown in Figure 15.

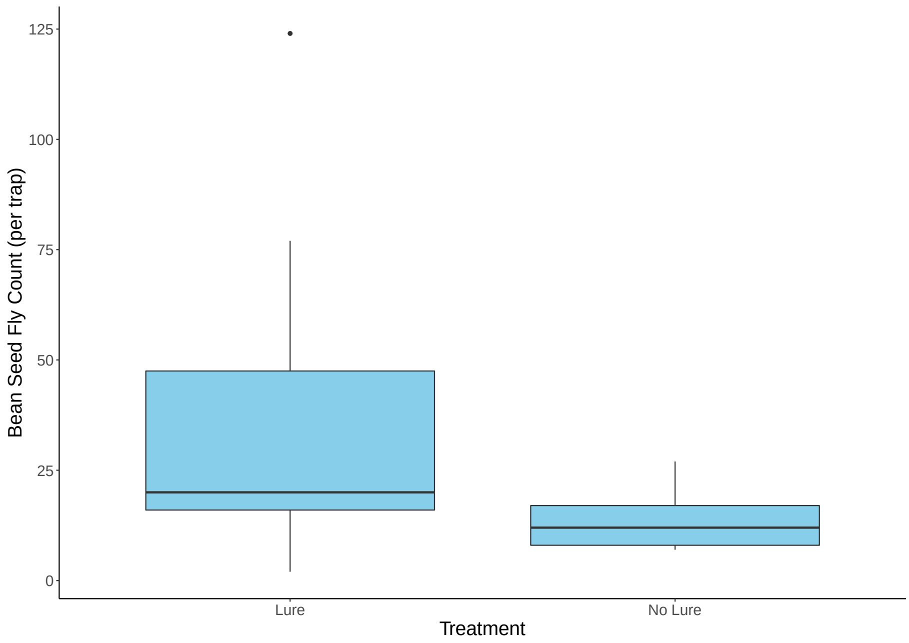

Figure 14. Bean Seed Fly count per trap on blue sticky traps with and without a lure attached. Counts have not been rounded to integers.

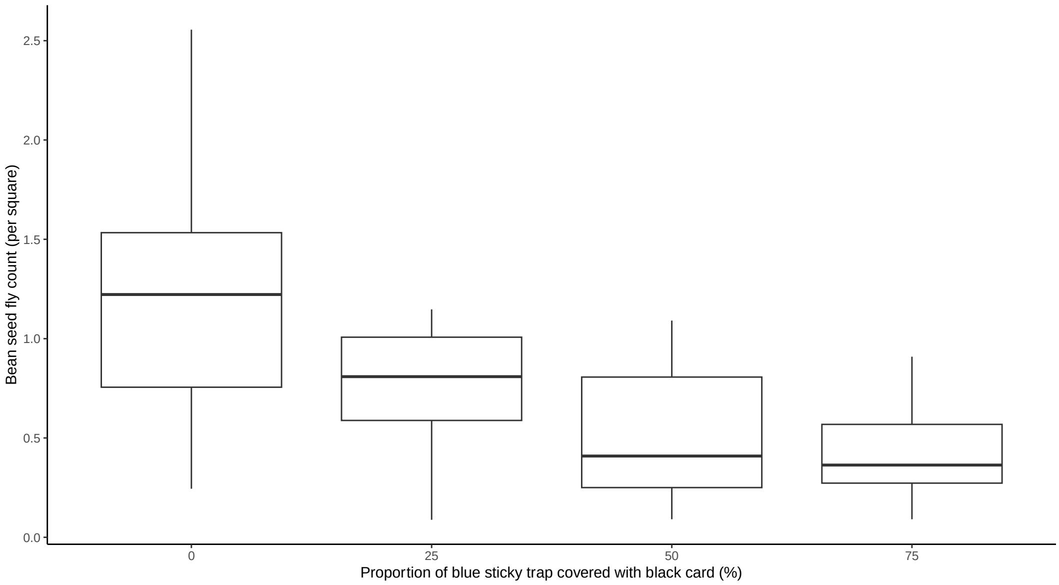

Figure 15. Bean seed fly count per square. Counts have not been rounded to integers.

4.3. Objective 3: Investigate the overwintering biology of bean seed fly

In 2020, < 50% of wild pupae emerged by day 15 at 20°C when they were laid between 18/09/2020 – 22/09/2020 and developed under field conditions. It can be assumed that the majority of eggs laid in this time period went into diapause after pupation. 100%, 56%, 59% and 92% emerged by day 15 at 20°C when eggs were laid between 01/09 – 04/09, 29/09 – 02/10, 09/10 – 13/10 and 13/10 – 19/10, respectively. Proportions are percentages of the number of flies to emerge by day 150 at 20°C.

In 2021 and 2022, there was a significant effect of the date of placing eggs from the culture under field conditions during late summer and autumn on the proportion of flies to emerge by day 15 at 20°C ($P < 0.0001$). In 2021, < 50% of pupae emerged by day 15 at 20°C when they were placed under field conditions as eggs on 24/09/2021 as shown in Figure 16. In 2022, < 50% of pupae emerged by day 15 at 20°C when they were placed under field conditions as eggs between 14/09/2022 – 12/10/2022 as shown in Figure 17. It can be assumed that the majority of eggs laid in these time periods went into diapause after pupation.

The majority of *D. platura* were likely not to have entered diapause in Experiment 6 as > 50% of *D. platura* emerged by day 15 at 20°C across all treatments and trials. The time pupae spent at 0°C had a significant effect on the proportion of adult flies to emerge by day 15 at 20°C in Trial 1 of Experiment 6 as shown in Figure 18 ($W = 0.736$, $P < 0.001$). Significant differences were observed between the control treatment (0 days at 0°C) and 50 – 150 days at 0°C. A similar pattern was shown in Trial 2 of Experiment 6 as shown in Figure 19. The time pupae spent at 0°C had a significant effect on the proportion of adult flies to emerge by day 15 at 20°C in Trial 2 ($P < 0.001$). Significant differences were observed between the control treatment and 8 – 75 days at 0°C. Significant differences were observed between pupae that spent 8 days at 0°C and 20 – 75 days at 20°C.

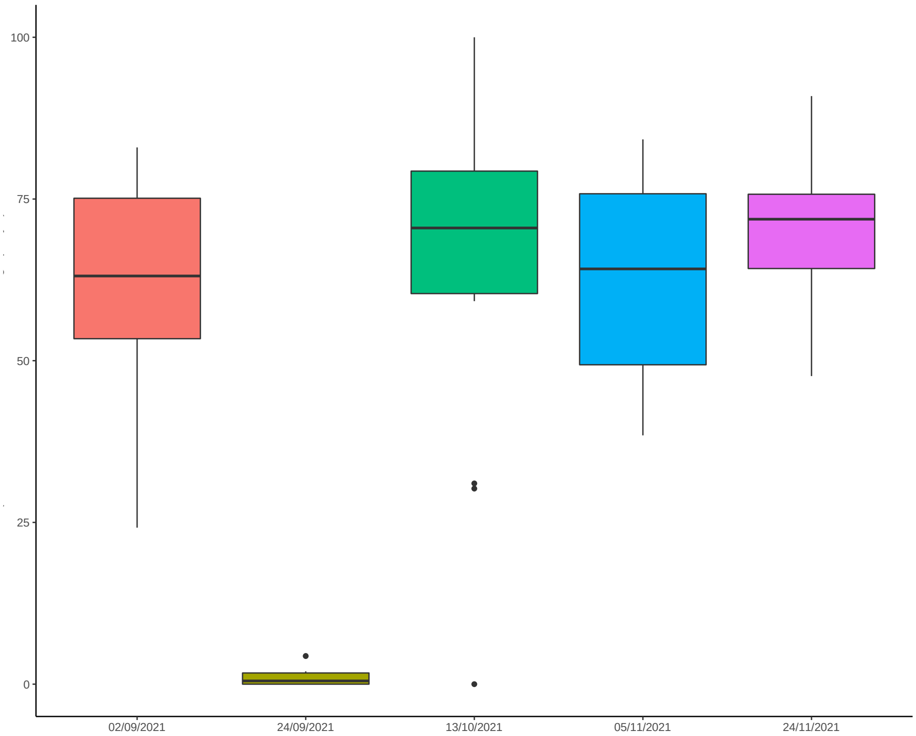

Figure 16. Proportion of adult flies to emerge per jar (y axis) when eggs were placed under field conditions between September – November 2021 (x axis). Proportion is of the overall flies to emerge per jar by day 50 at 20°C.

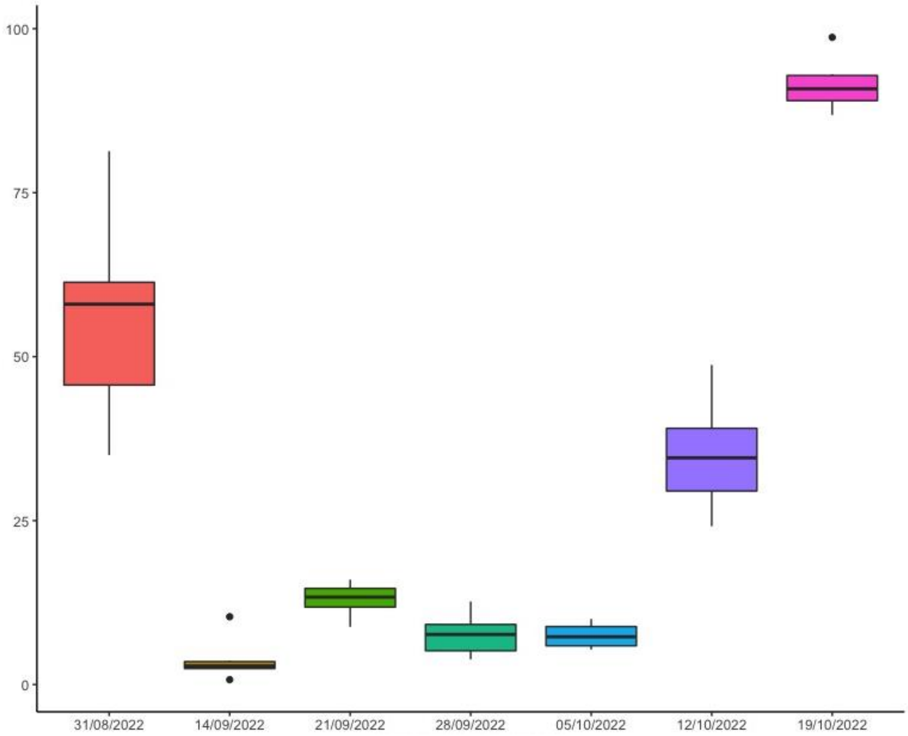

Figure 17. Proportion of adult flies to emerge per jar (y axis) when eggs were placed under field conditions between September – October 2022 (x axis). Proportion is of the number of pupae per jar.

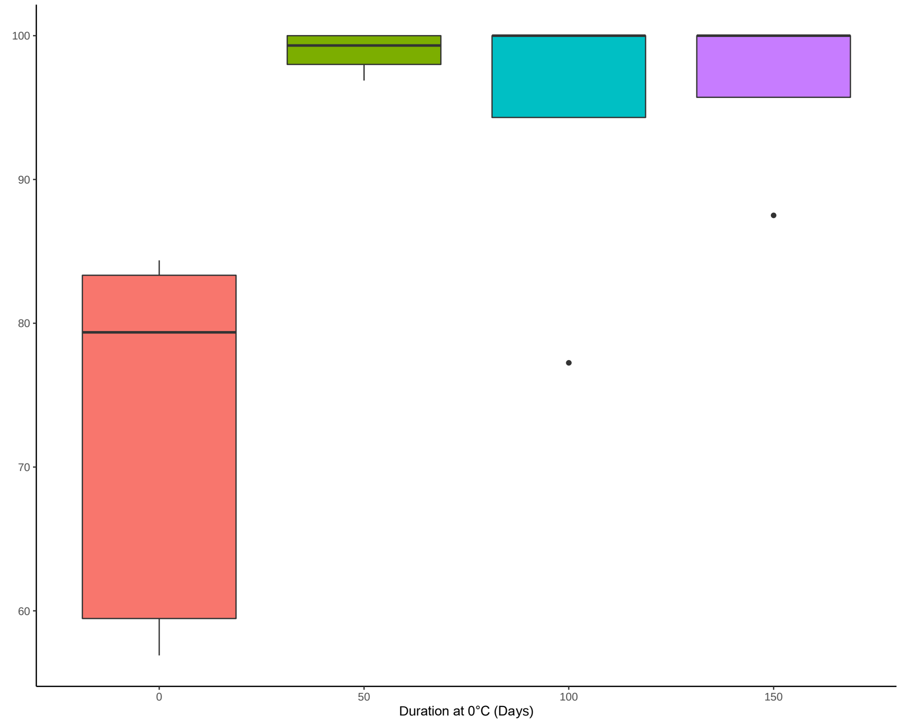

Figure 18. Proportion of adult flies to emerge per jar by day 15 at 20°C (y axis) when pupae were placed at 0°C for different durations (x axis). The proportion is the percentage (%) of flies to emerge from the total number of flies to emerge per jar by day 100 at 20°C.

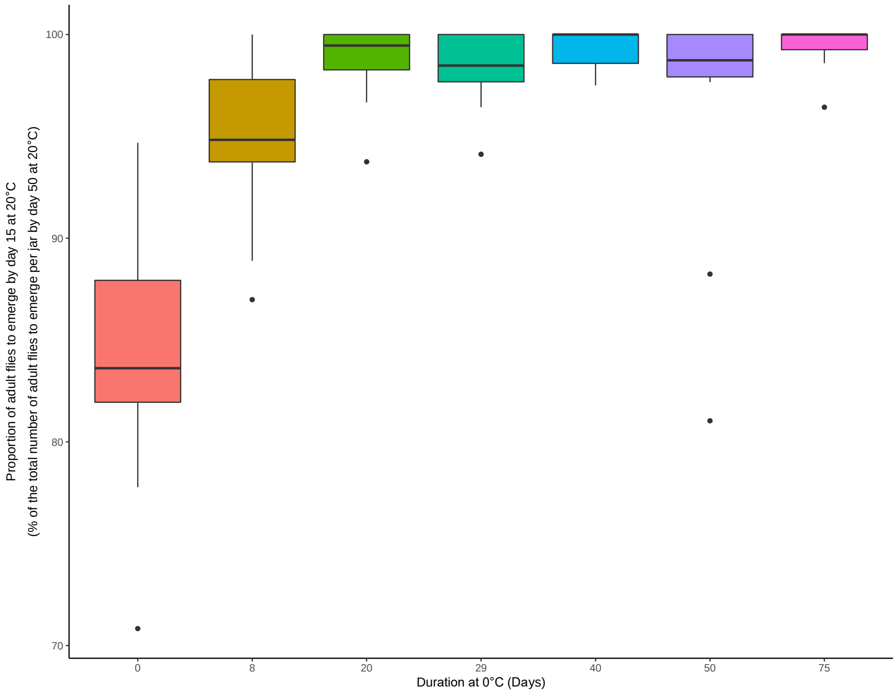

Figure 19. Proportion of adult flies to emerge per jar by day 15 at 20°C (y axis) when pupae were placed at 0°C for different durations (x axis). The proportion is the percentage (%) of flies to emerge from the total number of flies to emerge per jar by day 50 at 20°C.

4.4. Objective 4: Create and validate a model to predict the spring emergence of bean seed fly

On average 207±58 and 275±72 day degrees were accumulated from 1st January for the spring generation of BSF to start and peak in emergence at WCC, respectively. The Weibull (Type 2) model showed the best fit to the data and had the lowest residual standard error (goodness of fit) of all the models applied to the data. The residual standard error was 22.993. The estimated accumulated day degrees from 1st January for 10 – 100% emergence of the Spring generation are shown in Table 14.

Table 14.Estimates of accumulated day-degrees from the 1st January for proportions of emergence of adult flies of the Spring generations of Bean Seed Fly at Warwick Crop Centre for years 2014, 2016, 2017, 2018 and 2019.

Proportion of adult flies from the Spring generation to emerge (%)	Accumulated day-degrees
10	178
25	241
50	313
75	384
90	442
100	523

5. Discussion

5.1. Objective 1: Assess cultural and interference strategies for reducing damage by bean seed fly

5.1.1. The effect of cultivation on bean seed fly counts

There were significantly increased counts of BSF in beds 24 hours after cultivation as compared to beds that were not cultivated ($P < 0.001$). A bed former was used to cultivate the soil in Experiment 1. Similar findings have been shown in studies that have investigated the effect of alternative ground working methods to the use of a bed former on counts of *D. platura*. For example, counts of *D. platura* have been shown to significantly increase in plots that have been ploughed and disked compared to plots that have not been ploughed and disked ($P < 0.05$) (Hammond, 1990; Hammond & Stinner, 1987). In research by Funderburk et al. (1983), increased *D. platura* counts were recorded in plots that were ploughed and the seedbed was disked than plots that were not tilled ($P < 0.001$). The results from these previous experiments have suggested that the common ground working practise of ploughing followed by disking attracts *D. platura* flies to the area that is being ploughed. The results shown in Experiment 1 support the previous findings and provide new evidence for the effect of bed forming on BSF counts.

Counts of BSF do not provide information about egg laying activity and any resulting crop damage. The findings of Experiment 1 confirmed the need for an experiment that investigated the effect of cultivation on crop damage caused by the BSF.

5.1.2. The effect of cultivation and covering crops with fine mesh netting on damage caused by the bean seed fly

The findings shown in 3.2.2: Experiment 2: The effect of the timing of cultivation and covering of the crop in relation to sowing date on damage caused by bean seed fly on vining peas and dwarf French beans indicate that larval feeding by BSF can be reduced by managing the timing of cultivation in relation to sowing date, or covering the crop with a fine mesh when the timing of cultivation cannot be delayed. There were significantly more seeds containing larvae in plots that were cultivated the day before sowing than in plots that were cultivated on the day of sowing and covered one and 24 hours after sowing in the trial on vining peas ($P < 0.01$) (Figure 12). There were significantly more plants showing ‘baldheaded’ symptoms in plots that were cultivated on the day of sowing and not covered or covered 24 hours after sowing than plots that were not covered and cultivated 3 – 21 days before sowing ($P < 0.05$) (Figure 13).

The findings shown in 4.1: Objective 1: Assess cultural and interference strategies for reducing damage by bean seed fly differ from previous findings by Hammond & Cooper (1993). In research by Hammond & Cooper (1993), the authors found inconsistent effects of the timing of planting in relation to cover crop incorporation on the proportion of 'baldheaded' soybeans. The findings shown in Figure 12 and Figure 13 provide new evidence for the timing of cultivation and covering the crop with a fine mesh in relation to sowing date reducing damage by BSF.

There were low levels of damage observed throughout the four trials on vining peas. This may have been because there were low numbers of BSF recorded in traps whilst the four replicates of the trial were conducted, as compared to previous years (Table 7). Increased numbers of larvae are required to cause noticeable damage in vining peas (20 per seed) than in beans (10, 5 and 5 per seed for kidney, lima and snap beans, respectively) (Vea, Webb & Eckenrode, 1975). It is likely that there were less significant findings shown in the vining peas than the dwarf French beans because there were low activity levels of BSF at WCC.

5.2. Objective 2: Identify effective trapping methods for monitoring bean seed fly

5.2.1. The effect of attaching lures to traps on the number of bean seed fly caught on the trap

In 4.2: Experiment 3, the presence of a lure had a significant effect on the number of BSF caught on blue sticky traps ($P < 0.01$) (Figure 14). In research by Kuhar et al. (2006), yellow sticky traps with a lure attached caught 2 – 12 fold more BSF than yellow sticky traps with no lure attached. The lure used by Kuhar et al. (2006) was the same type of lure as used in 4.2: Experiment 3, containing the compounds: 2-phenylethanol and *n*-valeric acid that are associated with decomposing onion pulp (AgBio Inc, 2020). In research by Ishikawa & Matsumoto (1984), on average, traps caught 376±43, 52±6 and 14±2 *D. platura* when they had a chemical lure (containing 2-phenylethanol (0.2%) + *n*-valeric acid (0.05%)), decomposing onion pulp and no lure attached, respectively. There were no statistical analyses shown for these previous findings. The findings from 4.2: Experiment 3 provide statistical significance (1% confidence interval) for the presence of a lure (containing 2-phenylethanol and *n*-valeric acid) increasing the number of BSF caught on blue sticky traps.

5.2.2. The effect of traps with different areas covered in black card to mimic cover by insects on the number of bean seed fly caught on the trap

In 4.2: Experiment 4, the proportion of the trap covered in black card had a significant effect on the number of BSF caught per square ($4cm^2$) per blue sticky trap ($P < 0.01$) (Figure 15). In research by Finch (1991), the authors showed that less BSF are attracted to yellow water traps as higher proportions of the surface are concealed with black paint. The findings shown in 4.2: Experiment 4 show a similar pattern; the BSF caught on a blue sticky trap reduces as there is less blue surface visible on the trap. Less of the colour blue is visible to BSF near the trap as more insects attach to the sticky surface of a blue sticky trap. The findings by Finch (1991) and of Experiment 4 support the hypothesis that a trap surface is less attractive to BSF as more of the trap surface is concealed by insects.

5.3. Objective 3: Investigate the overwintering biology of bean seed fly

5.3.1. The effect of egg laying date on the proportion of bean seed fly to enter diapause

The findings from Experiment 5 in 2020 suggest that a proportion of wild BSF enter diapause at WCC and this occurs in early autumn. BSF pupae that developed from eggs laid between 18/09 – 22/09 showed the lowest proportions of BSF adults to emerge by day 15 at 20°C. It can be hypothesised from the findings in 2020 that BSF enter diapause in late September under field conditions at WCC. The findings from 2021 and 2022 support this hypothesis. The findings suggest that eggs from the culture that were placed under field conditions on 24/09/2021 and between 14/09/2022 – 12/10/2022 went into diapause (Figure 16 - Figure 17).

The average hourly soil temperatures for the time period between egg laying or placing the eggs in field conditions and filtering the pupae are shown in Table 11. In research by Throne & Eckenrode (1986), 22% and 59% of *D. platura* and *D. florilega*, respectively, were assumed to have entered diapause when they were reared at a constant 15°C. For a related species, the OF, > 50% of larvae that develop at 14°C and a photoperiod of 16h enter diapause (Ishikawa, Tsukada & Matsumoto, 1987). During the time the BSF were developing from eggs to pupae, the temperature could have been low enough to affect the initiation of diapause.

Additional environmental factors to temperature could have affected diapause initiation. For a similar species such as the CRF, diapause is initiated by a combination of decreasing temperature and photoperiod (Soni, 1976; Collier & Finch, 1983). The majority of eggs laid or placed under field conditions from mid-October onwards did not enter diapause. BSF may have evolved to enter diapause when temperature and photoperiod are between certain limits.

5.3.2. The effect of duration at a sub-threshold temperature on the completion of diapause

In Experiment 6, Trial 1 showed that *D. platura* require up to 50 days at a sub-threshold temperature (< 3.9°C (Broatch et al., 2006)) to complete diapause ($P < 0.001$) (Figure 18). In Trial 2, shorter intervals were investigated. Trial 2 showed that *D. platura* require between 8 – 20 days to complete diapause (Figure 19). Significant differences were shown in the proportion of adult flies to emerge by day 15 at 20°C when pupae were not placed at 0°C or pupae were placed at 0°C for eight or 20 days ($P < 0.001$). In the OF, 150 days is required at a threshold temperature of 5.6°C to complete diapause and start development (Ishikawa, Tsukada & Matsumoto, 1987; Nomura & Ishikawa, 2000). The findings of Experiment 6 show that *D. platura* require a shorter duration (days at a sub-threshold temperature) than OF to complete diapause.

5.4. Objective 4: Create and validate a model to predict the spring emergence of bean seed fly

5.4.1. Predicting the spring emergence of bean seed fly by accumulating day degrees

The spring emergence of BSF can be predicted by accumulating day-degrees. There is a significant relationship between accumulated day degrees and the spring emergence of BSF ($P < 0.0001$). A 3-parameter Weibull (Type 2) model showed the best fit for the spring emergence of BSF as a function of accumulated day degrees from 1st January. The methods used to calculate the estimates shown in Table 14 are a foundation for creating the first model to predict BSF activity in the UK.

Predicted values from the model need to be compared to observed values to understand how reliable the model will be for predicting the emergence of BSF in spring. It is likely that the model should be useful in predicting the spring emergence of BSF as Weibull and similar models have been successful in predicting insect emergence. Logistic models have been successfully used to model *D. platura* emergence in Canada (Broatch et al., 2006). In research by Rowley et al. (2017), a Weibull model showed the best fit for the emergence data of a dipteran pest species, *Haplodiplosis marginate* (Saddle Gall Midge) compared to Probit and Binomial GLM models.

The findings discussed in 5.3: Objective 3: Investigate the overwintering biology of bean seed fly suggest that a proportion of wild BSF and *D. platura* from the culture do not enter diapause and those that enter diapause have a short diapause. It may be beneficial to accumulate day degrees from an earlier date than 1st January. Or, it may be beneficial to accumulate day degrees from the most recent peak in trap counts of BSF as it can be assumed that a proportion of BSF do not enter diapause and continue to develop throughout autumn and winter at WCC.

5.5. Recommendations for growers

5.5.1. Cultural and interference control

The findings of Experiment 1 show that BSF are attracted to the seedbed within 24 hours of cultivation, therefore growers should take caution when sowing seeds when cultivation has occurred recently.

The findings of Experiment 2 show that there are increased symptoms of damage by BSF when cultivation occurs one day before (Figure 12) or on the day of sowing (Figure 13) vining peas and dwarf French beans, respectively. For example, there were significantly less ‘baldheaded’ dwarf French beans per plot when cultivation was delayed for at least three days ($P < 0.05$). Growers should delay sowing by at least three days in relation to the date of cultivation to reduce the risk of damage by BSF.

The findings of Experiment 2 show that there are decreased symptoms of damage by BSFwhen the plot is covered with a fine mesh. For example, there were significantly less ‘baldheaded’ dwarf French bean plants per plot when the plot was covered with a fine mesh approximately one hour after sowing than plots cultivated on the day of sowing and not covered ($P < 0.05$). However, there were significantly more ‘baldheaded’ dwarf French bean plants per plot when the plot was cultivated on the day of sowing and covered with a fine mesh approximately 24 hours after sowing than plots that were not covered and cultivation occurred three days before sowing ($P < 0.05$). It is likely that BSF laid eggs in the plots before the fine mesh covered the plots as Experiment 1 showed that there are increased BSF counts within 24 hours of cultivation. Growers should cover their crop with a fine mesh if sowing cannot be delayed. The fine mesh should be placed over the crop immediately after sowing to reduce the risk of damage caused by BSF.

5.5.2. Monitoring bean seed fly

The findings of Experiment 3 show that blue sticky traps with a lure attached (AgBio Inc, 2020) catch increased numbers of BSF compared with blue sticky traps that do not have a lure attached (Figure 14). The findings of Experiment 4 suggest that blue sticky traps will catch less BSFas more of the surface is covered by insects (Figure 15). The traps were placed outside for 24 hours in Experiment 4. Growers should use a blue sticky trap with a lure attached to the trap to monitor BSF. Growers should be cautious that traps will attract less bean seed fly as more insects are caught on the trap. It will be more difficult to differentiate BSF from other insect species as more insects are caught on the trap.

There are findings on trap height and orientation from the PhD project that are not presented here. The guidance on trapping may change once the data from these experiments is analysed.

5.5.3. Forecasting bean seed fly

The majority of the spring generation of BSF are likely to have emerged once 313 day degrees are accumulated from 1st January (Table 14). There will be more of a reduced risk of damage by BSF once 384 day degrees have been accumulated from 1st January as 75% of the spring generation are likely to have emerged by this time point. It is more important for growers to use the recommendations on cultural and interference control if they need to sow their crop before 384 day degrees are accumulated from 1st January.

It is important to consider that the current predictions made in Table 14 have not been compared to observed emergence dates of the spring generation of BSF in different years at WCC or in different regions of the UK. The model will be developed further considering the findings of 4.3: Objective 3: Investigate the overwintering biology of bean seed fly and if the model shows errors in predictions for BSF activity in different years and regions.

6. References

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