



## **Project Report No. 623**

### **Integrated pest management of cabbage stem flea beetle in oilseed rape**

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## 1. Abstract

Cabbage stem flea beetle (CSFB) is currently the most important pest of winter oilseed rape (WOSR) in England. The loss of neonicotinoid seed treatments and the presence of resistance to pyrethroid sprays means that chemical control options are very limited. The development of an integrated pest management (IPM) strategy for this pest is urgently required. In particular, the industry needs non-chemical control options to help rationalise chemical control and prolong the use of existing approved products in areas where they are still effective. The aim of this project was to deliver such a strategy. The specific objectives were:

1. Review information on agronomic factors that affect CSFB adult feeding and larval infestation.
2. Determine the effect of agronomic factors on CSFB adult feeding damage and larval infestation.
3. Understand crop tolerance to adult feeding damage and larval infestation and use this to review thresholds for adults and larvae
4. Assess alternative control options for CSFB
5. Create an IPM strategy for CSFB
6. Transfer knowledge to farmers and agronomists

Most of the factors that potentially have an impact on CSFB pressure are weather-related. This provides potential to predict seasonal risk in pest pressure. The crop growth stage at CSFB migration, stubble management and sow date are factors that growers can use to influence CSFB damage. There was no clear evidence to suggest that WOSR varieties differed in their susceptibility or attractiveness to CSFB. There was no clear evidence that large increases in seed rate reduced CSFB pressure, although small increases in rate may reduce the risk of suboptimal plant populations. Pot and field experiments demonstrated that WOSR shows tolerance to damage from both CSFB adults and larvae, which offers the chance to refine thresholds for the pest. The use of volunteer oilseed rape (vOSR), as a trap crop for adult CSFB, and/or defoliation of the crop in winter, to reduce numbers of CSFB larvae, both show promise as alternative control measures for the pest. Use of vOSR reduced adult CSFB infestation by up to 88%, damage by up to 76%, larval numbers by up to 69% and increased plant population by up to 56%. Defoliation decreased larval numbers by between 23 and 55%, but impacts on crop yield were inconsistent. The project identified 31 factors that could influence CSFB pressure. Of these, 20 decreased risk, seven increased risk and four, on balance, were neutral. Overall, the most promising tools for reducing CSFB risk are adjusted sow dates and the use of vOSR as a trap crop. Other tools also show promise. A potential IPM strategy for the pest is proposed at a range of crop growth stages, from pre-sowing until the following spring.

## 2. Introduction

Cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*) is a primary pest of winter oilseed rape (WOSR). Adults feed on the foliage of emerging crops and can threaten establishment. Their larvae feed within the leaf petioles and stems where they can also have a significant impact on crop yield. Currently chemical control options for this pest are very limited following the loss of neonicotinoid seed treatments and the presence of resistance to pyrethroid sprays. The development of an IPM strategy is urgently required particularly involving non-chemical control options.

### 2.1. Life cycle and biology of CSFB

CSFB breeds only on autumn and winter brassicas. In the UK, France and Hungary, a single generation occurs each year (Williams & Garden, 1961; Bonnemaïson, 1965; Vig, 2003), although two generations have been reported in Germany (Kaufman, 1940 in Ankersmit, 1964). The adult is about 5 mm long and usually shiny greenish- or bluish-black but a bronze form is common (Figure 1). They have long antennae, large hind legs and jump when disturbed. These emerge from the soil-borne pupae in May or June (Williams & Garden, 1961; Bonnemaïson & Jourdheuil, 1954 in Ankersmit, 1964). After a short period of intensive feeding on the pods, stems and leaves of the WOSR crop (which is not thought to affect yield) or wild crucifers they enter an aestivation diapause (Ankersmit, 1964; Saringer, 1984), in which their activity is minimal and their metabolism slows. It is thought that they move to nearby sheltered, humid locations in leaf litter or other crevices to aestivate (Williams & Garden, 1961; Ankersmit, 1964). Aestivation usually occurs in July (Williams & Garden, 1961; Alford, 1979) and is thought to occur in response to high temperatures and drought (Ankersmit, 1964). At around the same time adult CSFB can be seen swarming over machinery and seed stores at harvest (Alford, 1979), though these quickly disperse seeking locations to aestivate. The end of aestivation has been attributed to colder, more humid conditions in late summer (Ankersmit, 1964) or heavy rain followed by a hot spell (Bonnemaïson, 1965) in continental Europe. However, Ankersmit (1964) suggested that this explanation was not satisfactory for maritime climates. Indeed, Alford's (1979) study of CSFB in Cambridgeshire found that the end of aestivation was not linked rainfall or higher humidity, instead ending during a summer drought. Controlled environment experiments found that temperature or day length had no effect on the duration of aestivation (Bonnemaïson & Jourdheuil, 1954 in Ankersmit, 1964; Saringer, 1984), suggesting that, rather than being affected by environmental factors, the period of aestivation is genetically fixed (Saringer, 1984). The period of aestivation is reported to last about 1-3 months (Ebbe-Nyman, 1952 in Ankersmit, 1964; Ankersmit, 1964; Saringer, 1984). In the UK, adults tend to emerge from aestivation from mid- to late August (Alford, 1979), although recent data suggests that the timing of post-aestivation activity is more variable (M. Newbert, pers. comm.).





Figure 1. Adult cabbage stem flea beetle (*Psylliodes chrysocephala*) © Blackthorn Arable.

After emergence in August, they seek out WOSR crops primarily by detecting plant volatiles (see Section 7.1 for more details), although it is possible that they also use visual cues to find crops. Conditions for flight are not well understood but is thought to occur above 16°C in a slight wind and that they are able to fly at least two miles (Bonnemaison, 1965) (see Section 7.4 for more details). Peak adult activity in the UK (based on numbers caught in water traps) is in September (Alford, 1979; Green, 2008). Once in a crop, the adults feed on establishing plants, which can result in the loss of the growing point if plants are attacked sufficiently early or so-called shot-holing of the foliage which can affect crop vigour. Physiological changes also occur in response to shorter daylengths at this time, with the result that activity increases 1-2 hours after sunset (Bonnemaison, 1965) and egg development begins (Ankersmit, 1964). Approximately two weeks after they begin feeding in WOSR the CSFB begin laying eggs (Alford, 1979), although this pre-oviposition period reduces with increasing temperature (Mathiasen *et al.*, 2015a). It has also been noted that flight muscles begin to atrophy as eggs are developed so that CSFB gradually lose the ability to fly (Bonnemaison, 1965).

Eggs are laid in the upper levels of the soil at the base of plants in batches of 2-16 eggs (Williams & Garden, 1961; Ankersmit, 1964; Thioulouse, 1987). Adults can continue to lay eggs for up to eight months (Mathiasen *et al.*, 2015a), pausing only when temperatures drop below 2-4°C (Bonnemaison, 1965; Mathiasen *et al.*, 2015a). It has been estimated that a single adult can lay 696-1000 eggs in their lifetime (Bonnemaison, 1965; Vig, 2003; Mathiasen *et al.*, 2015a), although this is affected by temperature (Mathiasen *et al.*, 2015a). Mortality is thought to be higher in the egg stage than the larval stage (Thioulouse, 1987), with hatching rates of 47-73% (temperature dependent) reported in controlled environment experiments (Mathiasen *et al.*, 2015a). The time until egg hatch decreases with increasing temperature (Bonnemaison, 1965; Alford, 1979; Mathiasen *et al.*, 2015a), taking approximately 175 days at 4°C and 14 days at 20°C (Mathiasen

*et al.*, 2015a). Several day-degree models have been developed to predict egg hatch (Bonnemaison and Jourdheuil, 1954, in Ankersmit, 1964; Alford, 1979; Johnen and Meier, 2000; Mathiasen *et al.*, 2015a). The models are usually initiated by the arrival of adult CSFB but, as eggs need moisture to develop (Bonnemaison and Jourdheuil, 1954, in Ankersmit, 1964), it has been suggested that they would be more accurate if first rainfall following the arrival of adult CSFB was used to initiate the model (Robert, 2012).

Newly hatched larvae enter plants from September to early April but damage is usually first noticed in October (Williams & Garden, 1961). Initially larvae feed in petioles before moving into the stems from late winter/ early spring. Larval feeding affects plant vigour and can result in stunted plants with impaired stem elongation. Damage from larval feeding is generally considered to be worse than that from adult feeding (Green, 2008). The larvae are white with multiple small, dark spots on the back, a black head and tail, and three pairs of dark legs (Figure 2). Initially larvae are 1-3 mm but reach 6 mm when fully grown (Dobson, 1960). Larvae appear to be well adapted to winter conditions, with low mortality recorded even after 4 days of continuous -5°C conditions (Mathiasen *et al.*, 2015b). The developmental rate of larvae, activity periods (in terms of their movement in and out of the plant) and the timing of movements between the leaf petioles and the stem are poorly understood. A better understanding of these will improve risk forecasting and targeting of control treatments. They usually leave the plant to pupate in the soil in late April or May.



Figure 2. CSFB larva.

## 2.2. Current scale of the problem

In 2009, CSFB was estimated to affect around 67% of the total area of OSR (or 438,180 ha, based on the 5-year average harvested area of 654,000 ha) resulting in an average yield loss of 1% in untreated crops (Clarke *et al.*, 2009). In 2013, it was estimated that this level of damage was equivalent to annual yield loss of 15,336 t (based on average yield of 3.5 t/ha) or £5 million (using

the July 2007 to mid-April 2013 average delivered Erith OSR price of £327.13 per tonne) (Nicholls, 2013).

Crop losses to CSFB have since increased dramatically and it is currently the most important pest of WOSR. In 2014/15 it was estimated that by October 2014 2.7% of the crop nationally had been lost to CSFB (Wynn *et al.*, 2014), increasing to 5% by December (Nicholls, 2015). Regional variation in damage was also evident, with counties in the east and south east worst affected. In some regions crop losses by October were estimated at 4.4% of the crop with a further 46% of crop area exhibiting damage that exceeded the treatment thresholds (Wynn *et al.*, 2014). This is in spite of the majority of crops in these areas being treated with repeat applications of pyrethroid sprays, in some counties (e.g. Cambridgeshire, Hertfordshire and Suffolk) three or four applications by the end of September were typical (Wynn *et al.*, 2014).

In 2015/16, 65% of the national crop experienced damage at the cotyledon to two-leaf stage, rising to 69% at the three to four leaf stage (Alves *et al.*, 2015). Generally the severity of damage was less than in 2014/15 with about 1% of the crop lost, although the pest was more widely dispersed than in the previous survey. In 2016/17, 7% of the national crop had been lost to CSFB by the three to four leaf stage, rising to 9% by April (Wynn *et al.*, 2017). In 2018/19, it was estimated that 31% of crops experienced severe damage or crop failure in the autumn (Jones, 2020). Record larval populations were subsequently recorded in spring 2019, meaning that actual crop losses to CSFB in 2019/20 are likely to have been higher. In autumn 2019, almost 50% of crops experienced severe damage or crop failure, with crops in the north and west reporting increased CSFB pressures than previously (Jones, 2020).

Traditionally, control efforts for CSFB have focused on minimising damage from the adults. However, larval pressures have increased significantly in recent years. In 2015/16, larval populations were the highest recorded for at least 14 years and considerably higher than the long-term average (Collins, 2017). In response to increasing CSFB pressure, many farmers have either stopped growing or drastically reduced the area they grow of this important break crop. The UK area of WOSR in 2018/19 was 30% lower than the peak in 2012 (530,000 ha and 756,000 ha respectively), with greatest reductions seen in the east (36%; Defra, 2019). A survey conducted in 2018/19 found that 60% of growers were considering removing OSR from the rotation (Dyer, 2019).

### **2.3. Chemical control options**

On 1 December 2013 a two-year restriction on the use of the neonicotinoids, clothianidin, imidacloprid and thiamethoxam, was enforced by the European Commission. Therefore in 2014 the WOSR crop did not have neonicotinoid seed treatments to protect plants during emergence

and establishment. The restriction remained in place for the 2015 crop but, due to the level of CSFB damage in some parts of England in 2014/15, the National Farmers Union applied for a derogation to use neonicotinoid seed treatments, which was granted for a proportion of the crop in Bedfordshire, Cambridgeshire, Hertfordshire and Suffolk. This only amounted to approximately 5% of the national crop. Monitoring of this derogation at 48 sites found that the seed treatments significantly reduced damage at the cotyledon to two-leaf stage but provided little protection thereafter (White *et al.*, 2017a). Indeed, in some cases high levels of damage were recorded in the presence of a neonicotinoid seed treatment, including three sites where the crop was completely destroyed by the 3-4 leaf stage (White *et al.*, 2017a). No derogations have since been granted for neonicotinoid seed treatments.

Foliar-applied pyrethroids are currently the only chemical control method available in the UK. However, resistance to pyrethroids was detected in CSFB in England in 2014 (Højland *et al.*, 2015), initially in the east and north east but has since spread to most areas of England (S. Foster, pers. comm.). Two forms of resistance are present; knockdown (*kdr*) target-site resistance and an unknown form of metabolic-based resistance (Foster and Williamson, 2015). The metabolic resistance mechanism is thought to confer higher levels of resistance than the *kdr* resistance (Foster and Williamson, 2015). Pyrethroids will provide poor control where resistance frequency is high (IRAG, 2019). A survey in 2019 assessed the resistance in 157 CSFB populations sampled from a wide geographic area of the UK. This found that a median of 60% of the beetles were resistant, with a high proportion of samples having >75-100% of resistant beetles (Farmers Guardian, 2020). There was no statistical difference in the resistance frequency between regions and counties, but variation was present locally, with differences found on almost a farm-by-farm basis. The only fully susceptible population was found in north Scotland (Farmers Guardian, 2020). Both adult and larval CSFB are thought to be resistant (S. Foster, pers. comm.) but this, and other aspects of resistance, are currently the subject of a PhD at Rothamsted.

Foliar insecticide use in WOSR has fluctuated over the last twenty years, increasing from approximately 470,000 spray ha in 2000 (Garthwaite *et al.*, 2003a) to approximately 1,730,000 spray ha in 2012 (Garthwaite *et al.*, 2013) then decreasing to approximately 1,150,000 spray ha in 2018 (Garthwaite *et al.*, 2019). Pyrethroids have accounted for at least 98% of these insecticides in every pesticide usage survey over this period, except for 2018 when at least 90% were pyrethroids (Garthwaite *et al.*, 2003a; Garthwaite *et al.*, 2003b; Garthwaite *et al.*, 2005; Garthwaite *et al.*, 2007; Garthwaite *et al.*, 2010; Garthwaite *et al.*, 2011; Garthwaite *et al.*, 2013; Garthwaite *et al.*, 2015; Garthwaite *et al.*, 2018; Garthwaite *et al.*, 2019). Despite the reduction in insecticide sprays in recent years, the percentage applied for CSFB control has risen sharply from 23% in 2010 (Garthwaite *et al.*, 2011) to 61% in 2018 (Garthwaite *et al.*, 2019). Given this level of usage it is perhaps not surprising that resistance to pyrethroids has appeared and spread so rapidly.

In autumn 2014 and 2015, an emergency authorisation was granted for InSyst (acetamiprid) for use against CSFB. However, there was no further authorisation in subsequent years. Anecdotal information also doubted the efficacy of this product for CSFB control. There is no evidence that insecticides registered for other WOSR pests or available in other crops are effective against CSFB. A new seed treatment is now available via the import of treated seed from EU Member States (Lumiposa, a.i. cyantraniliprole, Corteva Agriscience) and is reported to provide moderate control of CSFB (65% reduction in damage compared to untreated plots) (van Nieuwenhoven, 2017). Despite this welcome addition to the insecticidal armoury for CSFB it is clear that chemical options for this pest are severely limited and that there is an urgent need for alternative control measures that can form part of an IPM strategy. Additionally, given how common resistance is in England, it is likely that the majority of sprays applied for CSFB in 2018 were ineffective. They will however harm natural enemies of CSFB and so rather than reducing pest damage these sprays are likely to reduce the level of control provided by natural enemies (see Section 9.1 for further information on natural enemies).

## **2.4. Aims and objectives**

CSFB is currently the most important pest of WOSR in England although the degree of damage varies markedly across the country. The loss of neonicotinoid seed treatments and the presence of resistance to pyrethroid sprays means that chemical control options are very limited. The development of an IPM strategy for this pest is urgently required, particularly non-chemical control options to help rationalise chemical control and prolong the use of existing approved products in areas where they are still effective. This approach is advocated by the Sustainable Use Directive and is one that that will become increasingly important as the number of insecticides available for pest control declines. Such a strategy could include crop agronomy, the relationship between CSFB infestation and yield, thresholds, pest monitoring, varietal resistance, trap cropping and defoliation as potential components of an IPM package.

This project aims to deliver an IPM strategy for farmers and agronomists to predict the likely risk of CSFB damage and allow rational decisions to be made on the need for control measures. Specific objectives are:

Objective 1: Review existing information on agronomic factors affecting CSFB adult feeding and larval infestation.

Objective 2: Determine the effect of agronomic factors on CSFB adult feeding damage and larval infestation.

Objective 3: Understand crop tolerance to adult feeding damage and larval infestation and use this to revise thresholds for adults and larvae

- Objective 4: Assess alternative control options for CSFB
- Objective 5: Create an IPM strategy for CSFB
- Objective 6: Transfer new knowledge to farmers and agronomists

The project was led by ADAS with collaboration from Fera Science Ltd, Bayer CropScience Ltd, Syngenta UK Ltd and Cotton Farm Consultancy Ltd. Fera provided an extensive long-term data set on CSFB infestation in England as well as expertise on pest monitoring and interpreting monitoring data. Syngenta and Bayer provided further data sets on CSFB damage, control and yield impacts, and Cotton Consultancy were used to provide feedback on the guideline IPM strategy and the practicalities of applying this on farm.

### **3. Reviewing existing information on agronomic factors affecting CSFB adult feeding and larval infestation**

#### **3.1. Introduction**

The aim of this objective was to investigate the influence of a range of agronomic factors over a number of years to determine which have the greatest influence on CSFB infestation (Objective 1). There is anecdotal data to suggest that a number of agronomic factors can limit the level of CSFB damage such as sowing date, soil type and seedbed conditions (Alves *et al.*, 2015). Early sowing is thought to decrease damage presumably because crops are able to establish before the main period of CSFB migration and so are better able to tolerate loss of green leaf area. Soil type and seedbed condition may be important as crops grown in light soil types or sown into ideal seedbeds (containing moisture and with good seed to soil contact) are likely to establish quickly, produce robust growth and so be less susceptible to adult CSFB feeding damage. Farmers and agronomists have also suggested that many other factors could influence CSFB pressure including leaving long stubble, sowing the crop as far away as possible from previous WOSR crops and drilling the crop with machinery that minimises soil disturbance.

There are issues with interpreting the results from surveys and on-farm observations particularly with regard to understanding how cultural control methods affect CSFB pressure. There can be bias involved with site selection for surveys, so that some agronomic factors are overrepresented. For example, agronomists offering sites which have had high CSFB pressure can result in a higher frequency of fields in which WOSR is grown in short rotation than is common practice. Changes in CSFB pressure at any one site over several years can be correlated with several factors and it can be difficult to deduce which is of greatest importance (e.g. a reduction in CSFB damage may have occurred at the same time as a change in WOSR variety which also required a change in seed rate and drilling method). It is also difficult to identify the importance of agronomic factors against a background of temporal and geographic changes in CSFB pressure. For example, the timing of beetle migration is likely to be important in relation to crop emergence. Early sowing may be very effective in a single year when the crops emerge before CSFB migration occurs but may be less effective in another year when emergence and migration coincide. Also early sown crops may flower very early or develop over-large canopies which then require careful management to prevent lodging.

To evaluate the efficacy of different cultural control methods for CSFB would require significant research input including many replicated plot trials. This would help farmers and agronomists select those options that are likely to be most effective on their farms but would be time consuming and as a result expensive. Instead, this project has adopted a more cost-effective approach to determining those factors which have greatest influence on CSFB pressure. This involved collating

and reviewing large historic datasets containing information on CSFB incidence and damage, along with relevant agronomic factors. To supplement this data, surveys of CSFB damage in crops were done in regions with a history of CSFB pressure and host farmers were asked to complete a questionnaire on crop agronomy for each survey site. This produced a sufficiently large dataset covering a number of years and geographic regions to account for a wide range of levels of CSFB pressure under differing weather conditions and agronomic factors. Novel statistical modelling methods were used to analyse the dataset (see Section 4). These were chosen because of their suitability for analysing datasets with high numbers of potentially confounding explanatory factors (factors that explain the variability in CSFB pressure). Such an approach maximises the likelihood of identifying factors that have greatest influence on CSFB infestation and damage, and subsequently allows research resources to be focussed on the most promising cultural control options. This chapter reviews the current knowledge regarding agronomic risk factors, describes the historic datasets used and the CSFB surveys undertaken in 2016/17 and 2017/18, and ultimately describes any trends present in the dataset.

## **3.2. Review of available literature and information**

### **3.2.1. Field selection**

Selecting fields for WOSR as far apart from previous WOSR as possible has been suggested as a means of reducing CSFB pressure (Kaufman, 1941 in Williams & Garden, 1961), by reducing the number of adults moving into the new crop.

### **3.2.2. Stubble and straw management**

The presence of stubble or straw has been anecdotally reported to reduce CSFB damage, probably by making it more difficult for the pest to find the crop. Robust trial data is sparse. Long straw has been shown to reduce flea beetle damage in mustard (Thomas, 2018). There is also anecdotal evidence that chopped straw may reduce CSFB damage by conserving moisture and improving crop establishment and vigour.

### **3.2.3. Establishment method**

Establishment methods that minimise soil disturbance have been anecdotally reported to result in lower CSFB damage (L. Cotton, pers. comm.). Robust trial data is sparse, however in an unreplicated trial it was found that establishment methods with the lowest soil disturbance had lower adult CSFB damage and higher plant counts during establishment and fewer larvae in the autumn (Grzelak, 2019). It has been suggested that the benefits of low soil disturbance establishment methods is due to the retention of soil moisture, which improves crop establishment, that CSFB are attracted to disturbed soil (for which there is no evidence currently), and because cultivations can harm ground beetles populations (Purvis & Fadl, 1996; Holland & Reynolds, 2003;



House & Parmalee, 1985; Nilsson *et al.*, 2015). The ground beetle, *Trechus quadristriatus*, has been identified as a potentially important predator of CSFB eggs (Warner *et al.*, 2003). However, the impact of cultivation intensity on ground beetles appears to be species-specific, with intensive cultivation methods harmful to large carabids (Symondson *et al.*, 1996; Kennedy *et al.*, 2013) while populations of smaller carabids such as *T. quadristriatus* were lower in less intensively cultivated plots (Kennedy *et al.*, 2013).

#### **3.2.4. Drill depth**

There has been some suggestion that increasing drill depth may reduce CSFB damage by making the germinating seed less accessible to adult CSFB. A similar approach is recommended for slug control in wheat (AHDB, 2013). There is little experimental evidence for this approach in WOSR.

#### **3.2.5. Sow date**

It has been suggested that adjusting sow date can have benefits for reducing CSFB pressure. Williams & Garden (1961) observed that crops sown before mid-July or after August suffered less damage than other sow dates. Bonnemaïson (1965) suggests that sow date should be adjusted depending on local CSFB migration patterns, with late sown crops (early to mid-September) in northern France tending to suffer less damage by emerging after the migration is complete in that region. Other work suggests that late sowing results in lower larval numbers (Conrad *et al.*, 2018). Anecdotal reports in recent years have suggested that early sown crops suffer less damage than later sown crops (L. Cotton, pers. comm.). Robust trial data is sparse but any benefit of early sowing is likely due to establishing a vigorous crop by the time the CSFB arrive, and any benefit of late sowing is likely due to the effects this has on the development of immature stages of the pest.

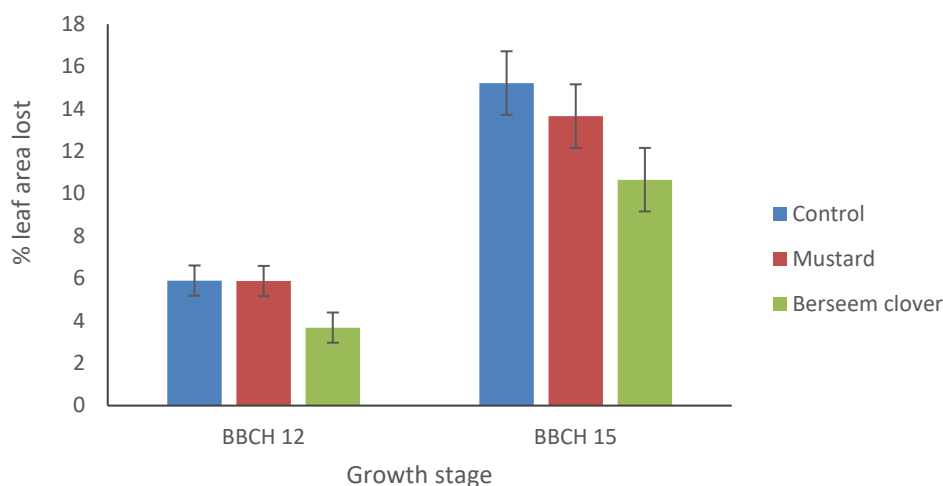
#### **3.2.6. Companion crops**

Several companion crops have been anecdotally reported to benefit WOSR establishment in terms of reducing CSFB pressure. Companion crops include mustards, buckwheat, legumes (e.g. berseem clover and vetch) and fenugreek. They are thought to work either by being more attractive than the WOSR (e.g. mustard), benefiting crop growth through improving soil health (e.g. legumes) (de la Pasture, 2016) or by masking the crop from CSFB (e.g. fenugreek). Breitenmoser *et al.* (2020) investigated two companion crop mixes; berseem clover and niger, and buckwheat, lentil, vetch, grass pea and broad bean. They found that the frequency of plant damage from adult CSFB was significantly lower with both mixes compared to a WOSR crop alone but that larval damage was not affected by the companion crop. The effect of the companion crop was attributed to masking the presence of the WOSR and interfering with CSFB host-location. Other work has found that white mustard is effective at reducing adult and larval CSFB damage, however frosts, which

are unreliable, or herbicides, which require Clearfield varieties be sown, are needed to remove the mustard (NIAB, 2020).

In this project, ADAS monitored CSFB pressure in a 2016/17 companion crop trial managed by Velcourt Ltd. Three companion crop treatments were investigated; WOSR on its own, WOSR with mustard (mustard drilled at approximately 100 seeds per m<sup>2</sup>) and WOSR with berseem clover (berseem clover drilled at approximately 200 seeds per m<sup>2</sup>). Each treatment was drilled as a single block in the same field. There was no replication of the treatments. Five 0.1 m<sup>2</sup> assessment areas were marked out in each block. These assessments areas were spaced approximately equidistantly and no closer than 15 m to the field edge or the boundary with another treatment. Adult feeding damage was assessed by recording the percent leaf area lost in ten plants of each of WOSR, mustard and berseem clover per assessment area. WOSR, mustard and berseem clover plant populations were assessed by counting the number of each species in each assessment area. Adult feeding damage and plant populations were assessed twice during crop establishment. Data on WOSR was analysed using ANOVA.

The results showed that damage to WOSR plants (Figure 3) was significantly lower in WOSR grown with a berseem clover companion crop than WOSR alone or WOSR grown with a mustard companion crop at both the two leaf stage ( $F = 6.4$ ,  $df = 147$ ,  $P = 0.002$ ) and the five leaf stage ( $F = 4.8$ ,  $df = 147$ ,  $P = 0.01$ ). In terms of damage to the mustard plants, 2% and 26% leaf area lost was recorded at the first assessment and the second assessment respectively. In terms of damage to the berseem clover plants, 2% leaf area lost was recorded at both assessments.



*Figure 3. Mean % leaf area lost to adult CSFB in WOSR grown alone (control), or with either mustard or berseem clover companion crops. Error bars indicate standard error of the mean.*

There was no significant difference ( $P > 0.05$ ) in WOSR plant populations between the treatments at either assessment date, although populations were highest in the berseem clover companion crop treatment on both occasions (Figure 4). Plant populations of mustard were 58 per m<sup>2</sup> at both the

first assessment and the second assessment. Plant populations of berseem clover were 82 and 66 per m<sup>2</sup> at the first assessment and the second assessment respectively.

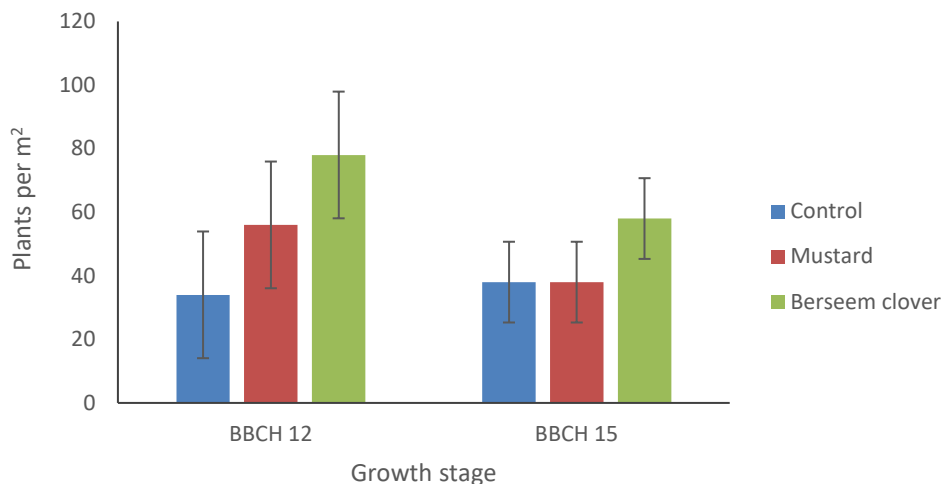


Figure 4. Mean WOSR plant populations (plants per m<sup>2</sup>) in WOSR grown alone (control), or with either mustard or berseem clover companion crops. Error bars indicate standard error of the mean.

Clearly, there is evidence to suggest that companion cropping provides some benefits in managing CSFB populations. However, further work is needed to determine which companion crop is best for which situation. A better understanding of how to manage the companion crop is also crucial as there have been reports of the companion crop overwhelming the WOSR crop (NIAB, 2020; L. Cotton, pers. comm.).

### 3.2.7. Organic amendments

The addition of organic amendments to the crop around establishment have been anecdotally reported to provide benefits in reducing CSFB damage. This has been attributed either to improving crop growth through the provision of nutrients or reducing CSFB infestation by masking the crop or deterring the CSFB. However, trial data to support their benefit is sparse.

### 3.3. Survey of adult CSFB damage

A range of agronomic and environmental factors are thought to influence adult CSFB pressure. Field surveys were done to identify which of these factors (e.g. sowing date, soil type, straw incorporation, and seedbed condition) affect adult feeding damage. It was intended that 75 fields per year would be surveyed in each of two growing seasons (2016/17 and 2017/18), comprising 15, 15 and 45 sites in north east England, the East Midlands, and the east of England respectively. In 2016/17, 85 sites were surveyed comprising 47 in the east of England, 21 in north east England and 17 in the East Midlands. In 2017/18, 74 sites were surveyed, comprising 54 in the east of England and 20 in north east England. Of the farms surveyed in 2017/18, 26% had also been

surveyed in 2016/17. It was decided to not monitor sites in the East Midlands during the 2017/18 assessment period as CSFB pest pressure had been much lower in this area than in the north east and the east of England in 2016/17.

At each site the host farmer was asked to complete an agronomy questionnaire for the surveyed field. The questionnaire collected information on field size, four-year cropping history, OSR variety, seed source, soil type, soil stoniness, straw incorporation/removal, stubble length, establishment technique, row width, sow date, drill depth, seed rate, seedbed quality, rain post sowing, fertiliser use, slug pressure, proximity to the nearest previous WOSR crop, and CSFB pressure in the nearest previous WOSR crop. The survey also collected information on any novel CSFB control strategies which were being trialled or had been trialled in previous years. Questions were kept as simple as possible to minimise the time needed for growers to complete the questionnaire. The answers were provided by host farmers and may have been based on estimates or assessments. Some questions were constrained to multiple choice answers to assist with data analysis. This meant that responses to some questions were somewhat subjective. For example, the amount of rain during the two weeks post drilling could be answered as dry, some rain or lots of rain. The full questionnaire is shown in Appendix 1.

At each site, plant population and CSFB damage assessments were carried out by ADAS staff at approximately the two to five true leaf stage. Plant populations were assessed by counting plants along each side of a 0.5 m rod (and using the row width to calculate plants per m<sup>2</sup>), or by counting all plants within a 0.1 m<sup>2</sup> quadrat, at ten randomly chosen locations per site. The same group of plants were used for the CSFB feeding damage assessments, for which ten plants were randomly selected at each of the ten plant population assessment points per site (giving 100 plants in total) and the % of leaf area lost visually estimated for each plant.

### **3.4. Analysing available data**

#### **3.4.1. Assemblage of datasets**

The 'CSFB dataset' was compiled by collating data generated as part of the project. This comprised data from the adult CSFB damage survey (Section 3.3), CSFB damage assessments done at the RL trials at Benniworth (Lincolnshire) and Cowlinge (Suffolk) in 2016/17 (Section 5.2.1), the seed rate-variety trials at Boxworth (Cambridgeshire) in 2017/18 (Section 5.2.2), the larval impact trials in 2016/17 and 2017/18 at Boxworth (Cambridgeshire) (Section 6.2.2) and the defoliation trial at Boxworth (Cambridgeshire) in 2016/17. These data were supplemented by several datasets provided by project partners. Details regarding the contents of these datasets, in terms of response variables (e.g. adult CSFB feeding damage), explanatory variables (e.g. WOSR variety), the number of sites and the year are given in Table 1.

*Table 1. Datasets provided by project partners. \* LA lost = % leaf area lost to adult CSFB feeding damage, Plants = plant per m<sup>2</sup>, autumn and spring larvae = larvae per plant in autumn and spring respectively. \*\* Data not available for all explanatory variables at all sites.*

Project	Source	Funder	CSFB data *	Sites	Years	Explanatory variables**
National larval surveys	Fera	Defra & AHDB	Autumn larvae, spring larvae	1288	2003/4-15/16	Location, variety, sow date, field area, previous crop
Spring larval surveys	ADAS	AHDB	LA lost, spring larvae	38	2014/15-15/16	Location, variety, sow date, previous crop, WOSR rotation, soil type, stubble mgmt., establishment method, drill depth, seed rate, seedbed quality, rain post drilling, emergence date, fertiliser use, slug pressure, previous CSFB pressure, distance and location of previous WOSR, novel CSFB strategies
Neonicotinoid derogation monitoring	ADAS	Bayer and Syngenta	LA lost, plants, autumn larvae	47	2015/16	Location, variety, sow date, previous crop, WOSR rotation, soil type, stubble mgmt., establishment method, row width, seed rate, seedbed quality, rain post drilling, crop stage at CSFB migration, slug pressure, previous CSFB pressure, distance and location of previous WOSR, pyrethroid resistance
Cruiser trials	ADAS	Syngenta	LA lost, plants, autumn larvae, spring larvae	20	2014/15	Location, variety, sow date, previous crop, stubble mgmt., establishment method, drill depth, seed rate, emergence date

### 3.4.2. CSFB adult feeding and larval infestation data

The full dataset contained 1610 data points spanning 15 years. This comprised 307 adult feeding damage (% leaf area lost) data points spanning four years, 1337 data points spanning 15 years describing numbers of CSFB larvae per plant in the autumn and 684 data points over 14 years describing the numbers of larvae per plant in the spring. For this work, autumn larval assessments were defined as those that occurred from November to January, and spring larval assessments were those that occurred from February to April. Each data point in the dataset on adult CSFB feeding damage or larval population is the average of multiple assessments taken in a plot or field, except for a small minority (3%) that consisted of an estimation of damage.

Where possible these data were taken from plots or fields that had not been treated with an insecticide. However, as much of the dataset came from survey data, many of the crops had received insecticide treatment(s) prior to the assessment of adult CSFB damage or larval population (approximately 70% of sites). The data at these sites, therefore, needed to be adjusted to account for the insecticide use, so creating an “untreated” baseline allowing comparisons to be made across all sites. This was done by estimating the level of control achieved by insecticide treatment and, where relevant, accounting for the reduction in insecticide efficacy due to resistance. Two types of insecticide treatments were encountered in the surveys; foliar pyrethroids and neonicotinoid seed treatments. Other insecticides, e.g. foliar neonicotinoids, were ignored as

they are considered to have low efficacy against CSFB. The presence or absence of pyrethroid resistance for each data point in the dataset and for any trial work used to estimate the efficacy of insecticidal control (see below) was determined either from resistance testing that was done as part of the work from which the data originated or, where there was no testing, by considering where and when the data was collected. Any data on CSFB incidence collected prior to 2014, the year the resistance was first detected in the UK (Højland *et al.*, 2015), was considered not to involve a resistant population. From 2014, resistance was considered to be present if it had been detected in the same county in annual monitoring work (S. Foster, pers. comm.) before the date of the CSFB adult damage or larval assessment. The annual resistance monitoring survey work is not comprehensive but is the best available source of information about the prevalence of pyrethroid resistance in CSFB.

The control of adult CSFB with pyrethroids in the absence of pyrethroid resistance (Table 2) was estimated using data from trials done in 1983/84 (Northwood & Verrier, 1986) and a Syngenta trial done by ADAS in 2014/15 (unpublished data, provided by Syngenta). Data was only available for the efficacy of a single pyrethroid application against adult CSFB in the absence of pyrethroid resistance. The control of adult CSFB with pyrethroids in the presence of pyrethroid resistance (Table 2) was estimated using data from several Syngenta trials in 2014/15 (done by ADAS) and 2015/16 (non-ADAS) (all unpublished data, provided by Syngenta). Any trial sites in the source data used for estimating control efficacy of insecticides that had less than 5% leaf area lost to CSFB adults in the untreated control treatments were disregarded.

The control of larval CSFB with pyrethroids in the absence of pyrethroid resistance (Table 2) was estimated using data from trials carried out in 1982/83 (unpublished ADAS data), 1983/84 (Northwood & Verrier, 1986), 1988/89 (Holliday, 1989) and 2000/01 (Green, 2002). The control of larval CSFB with pyrethroids in the presence of pyrethroid resistance (Table 2) was estimated using data from several Syngenta trials in 2014/15 (done by ADAS) and 2015/16 (non-ADAS), and several Syngenta/Bayer trials in 2015/16 (managed by ADAS) (all unpublished data, provided by Syngenta and Bayer). The effect of neonicotinoid seed treatments on CSFB larvae was estimated using data from several Syngenta trials in 2014/15, several Syngenta/Bayer trials in 2015/16 (all carried out by ADAS, all unpublished data, provided by Syngenta and Bayer) and other trial work in 2015/16 (Conrad *et al.*, 2016). These showed neonicotinoid seed treatments to have a highly variable and inconsistent effect on CSFB larvae (Table 2). All adult CSFB damage data in the dataset occurred at sites in which neonicotinoid seed treatments had not been used, so it was unnecessary to estimate the control efficacy for these insecticides. Any trial sites in the source data used for estimating control efficacy of insecticides that had less than 0.5 larvae per plant in the untreated control treatments were disregarded.

*Table 2. Estimated level of control provided by pyrethroids and neonicotinoids and modifiers used to adjust CSFB adult damage and larval populations. \* Pyrethroids applied prior to 1 November are considered to target adult CSFB. Pyrethroids applied after 31 October are considered to target larval CSFB.*

<b>Dataset data</b>	<b>Insecticide and use*</b>	<b>Pyrethroid resistance status</b>	<b>Control efficacy (%)</b>	<b>Modifier</b>
Adult damage	≥1 foliar pyrethroid	None	74	0.26
Adult damage	1 foliar pyrethroid	Present	13	0.77
Adult damage	2 foliar pyrethroids	Present	38	0.62
Adult damage	≥3 foliar pyrethroids	Present	53	0.47
Larval population	≥1 foliar pyrethroid against adults	None	85	0.15
Larval population	≥1 foliar pyrethroid against larvae	None	79	0.21
Larval population	≥1 foliar pyrethroid against larvae + ≥1 foliar pyrethroid against adults	None	97	0.03
Larval population	≥1 foliar pyrethroid against adults	Present	15	0.85
Larval population	1 foliar pyrethroid against larvae	Present	9	0.91
Larval population	2 foliar pyrethroids against larvae	Present	26	0.74
Larval population	≥1 foliar pyrethroid against larvae + ≥1 foliar pyrethroid against adults	Present	29	0.71
Larval population	Neonicotinoid seed treatment	n/a	0	1

These estimates of insecticide control efficacy were then used to adjust any values (adult feeding damage or larval population) in the CSFB dataset that were taken at sites where insecticides had been applied prior to the assessment. This was done by multiplying the raw value by modifiers calculated from the estimated control efficacy (Table 2). Due to the high estimated control of larvae achieved when at least one pyrethroid was applied to adult CSFB followed by at least one pyrethroid applied to larval CSFB in areas with no pyrethroid resistance (97% control), large adjustments in the raw data occurred at sites where such a spray regime occurred in the absence of resistance. For example, 2 larvae per plant would be adjusted to 67 per plant (using the 0.03 modifier). Therefore, due to the uncertainty in the accuracy of this control estimate and the large effect these adjustments would have on the data, it was decided to exclude sites with this spray regime that did not have pyrethroid resistance (190 sites in total).

### 3.4.3. Agronomic factors

The agronomic factors in the CSFB dataset were those collected in the adult CSFB survey (Section 3.3) and those listed in Table 1. All agronomic factors were selected based on their potential to affect CSFB damage, either directly by affecting the pest itself or indirectly by affecting crop growth. Most agronomic factors are self-explanatory. Data on the proportion of land occupied by WOSR in the region in previous year was calculated for each data point in the dataset by dividing the area of WOSR grown in the region in the previous year (Farming Statistics, 2017) by the region area (Census, 2011).

### 3.4.4. Trends in relationships between CSFB pressure and explanatory variables

This section describes trends in the CSFB dataset between data on CSFB pressure (adult feeding damage or larval populations) and explanatory variables (region, variety, etc.). Data are shown for explanatory variables in which a trend is present or those in which effects on CSFB pressure have been previously reported. For these visual comparisons, factor categories have at least six data points. Factor categories with less than six data points were merged with other relevant categories. Factor categories occasionally differed between response variables due to differences in source data and the need to merge categories to ensure sufficient data points in each category. Due to the complexity of the dataset, statistical analysis was not carried out at this stage. Instead, novel modelling methods were used to statistically analyse the data. This modelling work is described in Section 4.

#### **Adult CSFB feeding damage**

Adult feeding damage was highest in the UK in 2015/16 (mean of 31% leaf area lost). The east and south east tended to have the highest levels of damage (Figure 5).

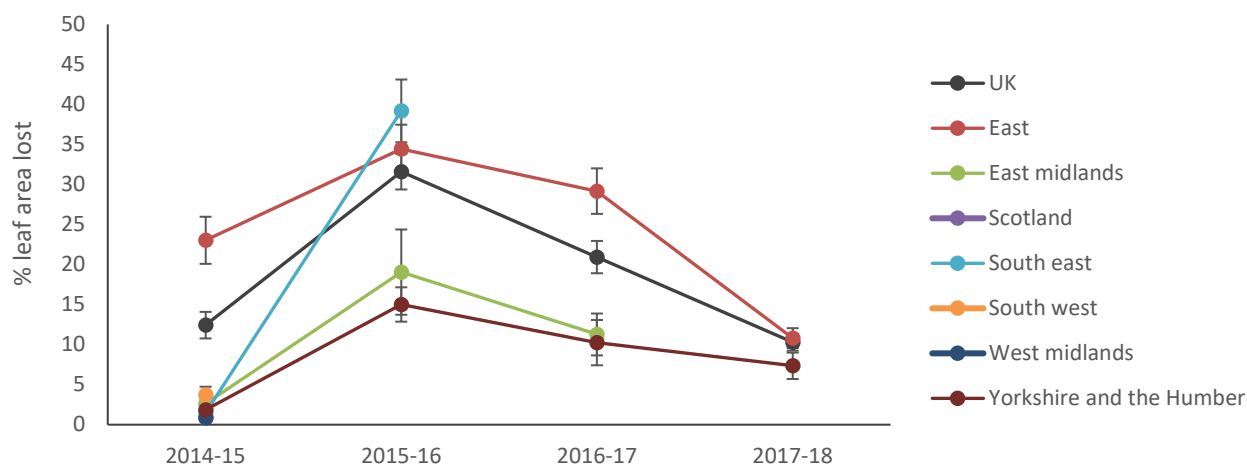
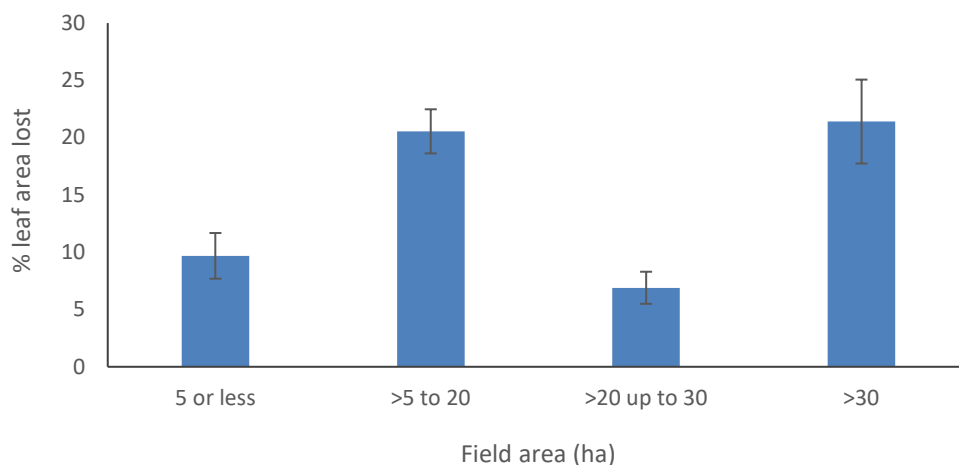


Figure 5. Mean % leaf area lost to adult CSFB between 2014/15 and 2017/18 for the UK, Scotland and English regions. Error bars indicate standard error of the mean. Note data for regions varied with year and data points may overlap.

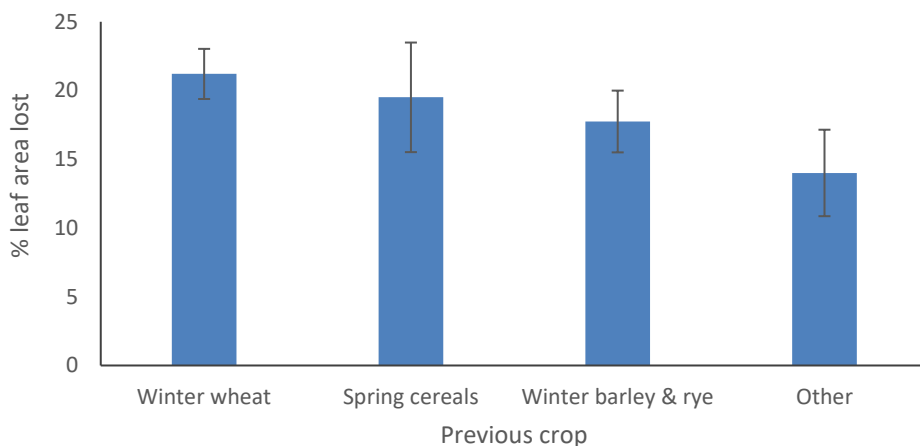


Damage tended to be greater in moderately sized (>5-20 ha = mean of 21% leaf area lost) and large fields (mean of 21% leaf area lost) and lowest in mid-sized fields (>20-30 ha = mean of 7% leaf area lost) (Figure 6).



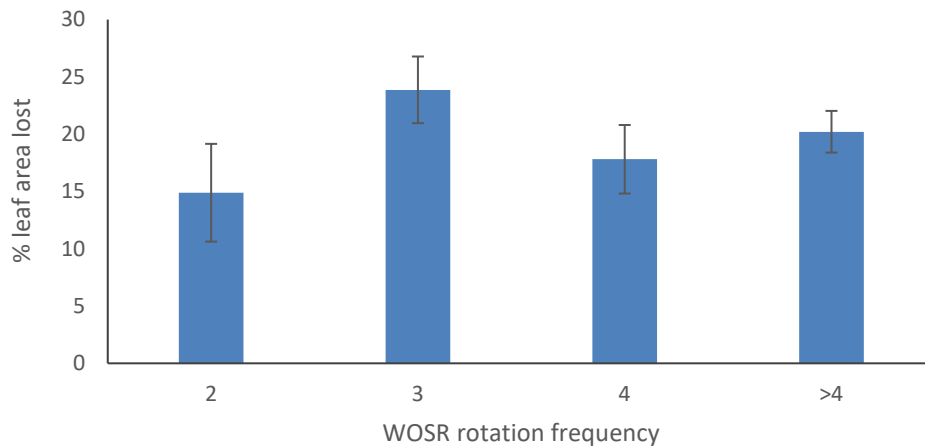
*Figure 6. Mean % leaf area lost to adult CSFB in crops grown in different size fields. Error bars indicate standard error of the mean.*

The previous crop appeared to have little effect on damage, with highest damage seen following winter wheat (mean of 21% leaf area lost) and the lowest following other crops (mean of 14% leaf area lost) (Figure 7). Other crops included spring beans and fallow.



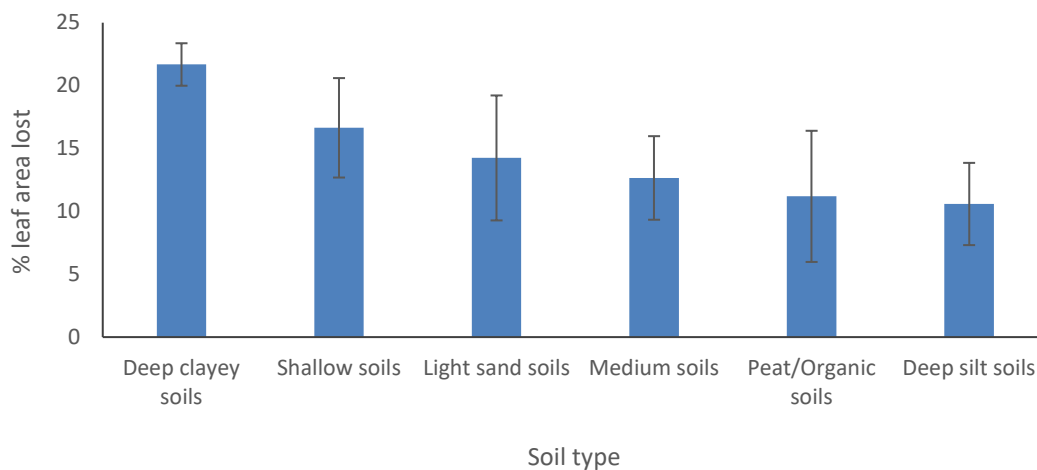
*Figure 7. Mean % leaf area lost to adult CSFB in crops grown after different crops. Error bars indicate standard error of the mean.*

The frequency of WOSR in the rotation appeared to have little effect on damage, with highest damage seen in three-year rotations (mean of 24% leaf area lost) and the lowest in two-year rotations (mean of 15% leaf area lost) (Figure 8).



*Figure 8. Mean % leaf area lost to adult CSFB in crops grown in different WOSR rotation frequencies (i.e. years between growing WOSR in the field). Error bars indicate standard error of the mean.*

There was a slight trend for greater levels of damage in heavy soils (deep clayey soils = mean of 22% leaf area lost) than light soils, e.g. medium soils (mean of 13% leaf area lost) (Figure 9).



*Figure 9. Mean % leaf area lost to adult CSFB in crops grown in different soil types. Error bars indicate standard error of the mean.*

The stoniness of the soil appeared to have a small effect on damage, with highest damage seen in soils with a few stones (mean of 18% leaf area lost) and the least in stony soils (mean of 10% leaf area lost) (Figure 10).

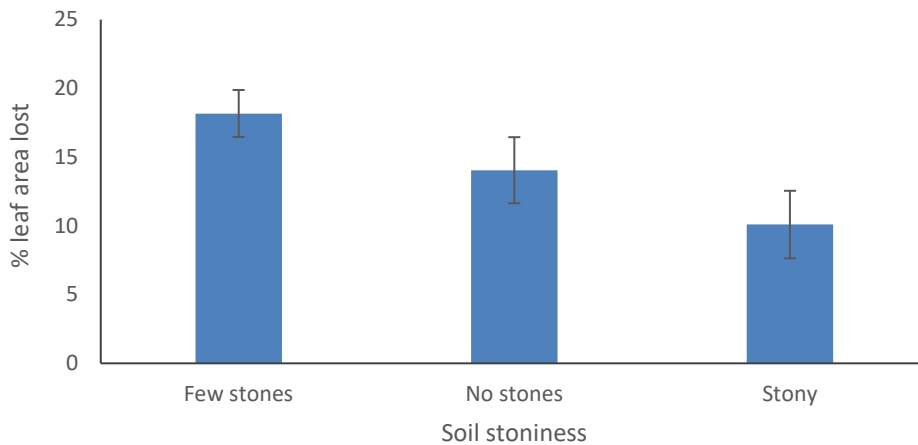


Figure 10. Mean % leaf area lost to adult CSFB in crops grown in soils with different amounts of stones. Error bars indicate standard error of the mean.

Distance to the nearest previous WOSR crop had no clear effect on damage, with the lowest damage seen in crops up to 50 m from the previous WOSR (mean of 18% leaf area lost) and the highest in those between 50 and 500 m from the previous WOSR (mean of 22% leaf area lost) (Figure 11).

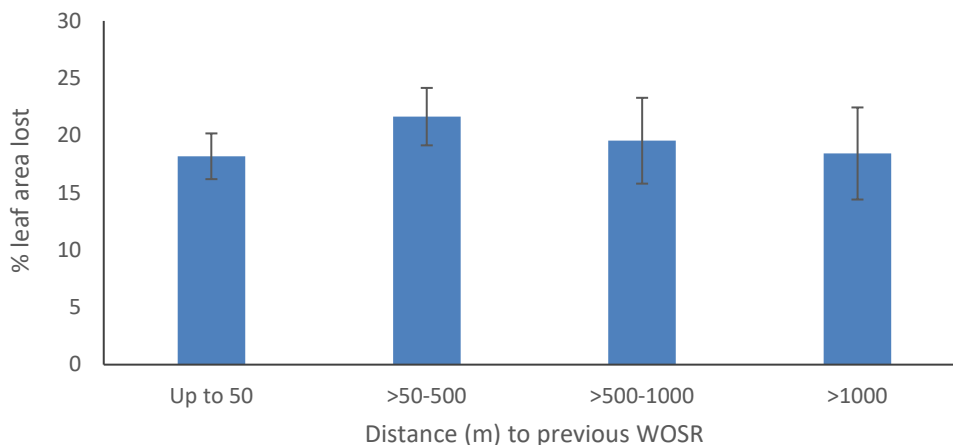


Figure 11. Mean % leaf area lost to adult CSFB in crops grown at different distances from the nearest previous WOSR crop. Error bars indicate standard error of the mean.

There was a slight trend for higher levels of damage in crops in which the adult CSFB pressure in the nearest previous crop was high (mean of 23% leaf area lost) compared to low (mean of 17% leaf area lost) (Figure 12). There was a clearer trend for the impact of larval pressure in the nearest previous crop, with lower damage in crops in which the larval pressure was low in previous WOSR (mean of 8% leaf area lost) and greater damage where larval pressure was moderate in previous WOSR (mean of 16% leaf area lost) (Figure 13).

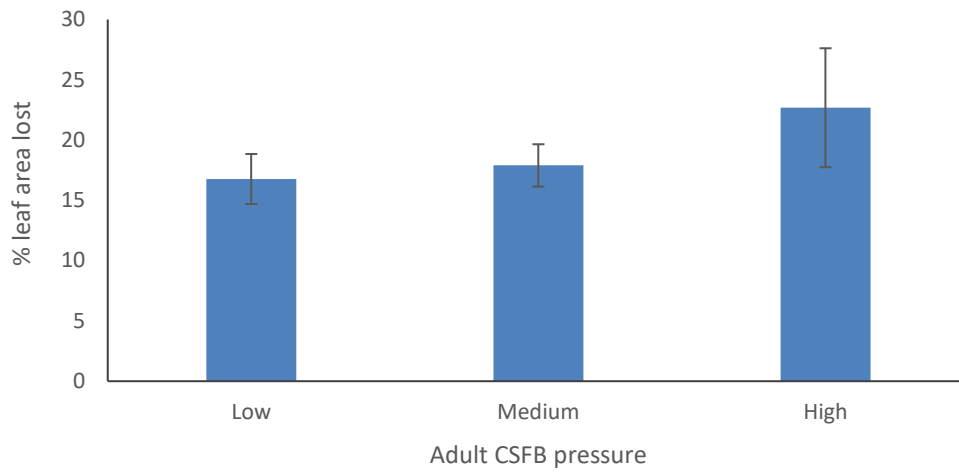


Figure 12. Mean % leaf area lost to adult CSFB in crops in which the nearest previous WOSR crop had different levels of adult CSFB pressure. Error bars indicate standard error of the mean.

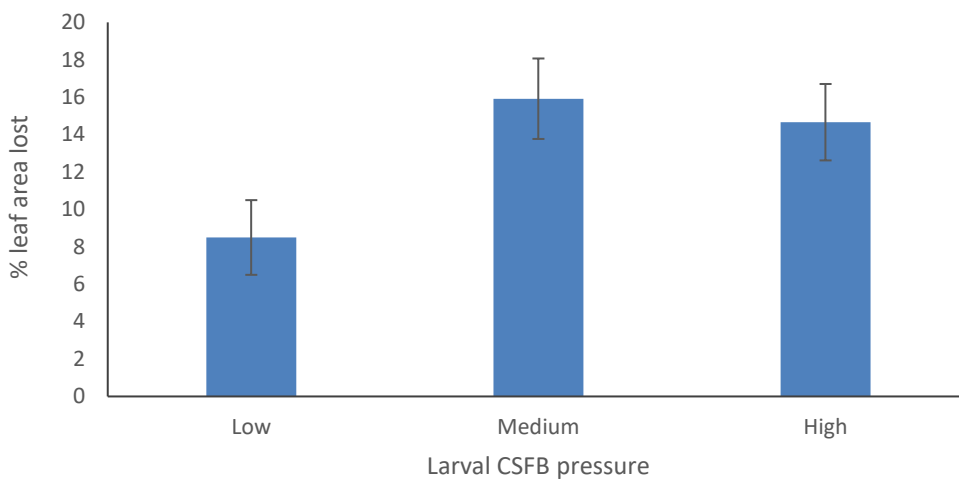
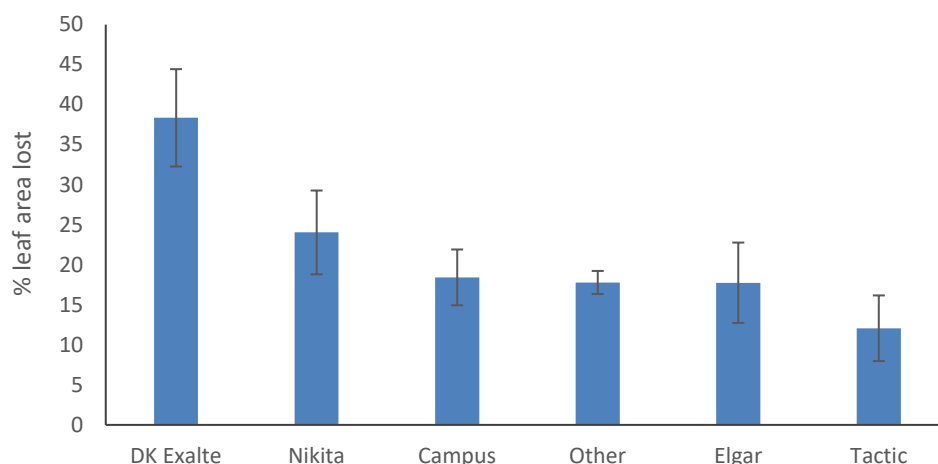
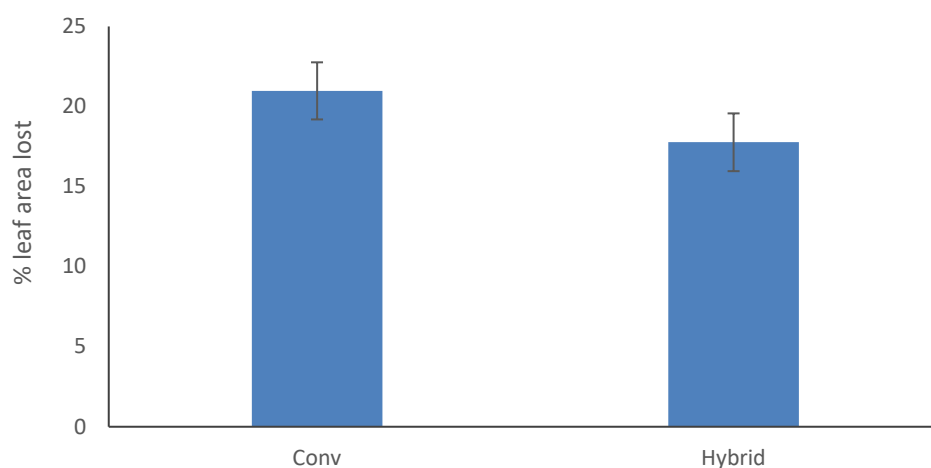


Figure 13. Mean % leaf area lost to adult CSFB in crops in which the nearest previous WOSR crop had different levels of larval CSFB pressure. Error bars indicate standard error of the mean.

Trends between varieties were present, with the greatest level of damage recorded in DK Exalte (mean of 38% leaf area lost) and the least with Tactic (12%) (Figure 14). There was no clear trend between conventional or hybrid varieties (Figure 15).



*Figure 14. Mean % leaf area lost to adult CSFB in varieties appearing in the dataset. Error bars indicate standard error of the mean.*



*Figure 15. Mean % leaf area lost to adult CSFB in conventional or hybrid varieties. Error bars indicate standard error of the mean.*

Adult feeding damage varied with sow date. Greatest levels of damage were recorded in crops sown in early September (mean of 28% and 25% leaf area lost in w/c 1 and 8 September respectively). Lowest levels of damage were observed in crops sown in early and late August (mean of 15% and 14% leaf area lost before 11 August and in w/c 25 August respectively) and late September (mean of 16% leaf area lost after 14 September) (Figure 16).

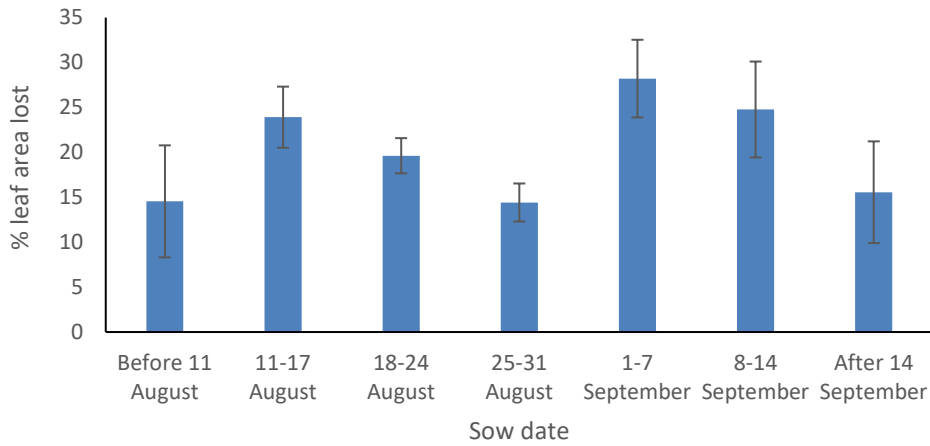


Figure 16. Mean % leaf area lost to adult CSFB in crops sown in different periods. Error bars indicate standard error of the mean.

Crop emergence also appeared important, with damage tending to be greatest in crops emerging in the second half of August (mean of 19% leaf area lost) or first half of September (mean of 16% leaf area lost) and lowest in crops emerging in the first half of August (mean of 9% leaf area lost) (Figure 17).

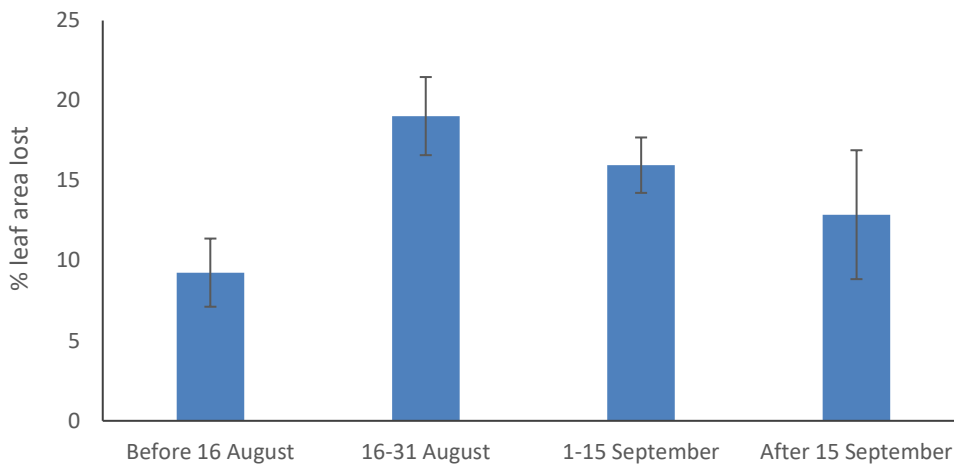


Figure 17. Mean % leaf area lost to adult CSFB in crops emerging in different periods. Error bars indicate standard error of the mean.

Greater levels of damage were associated where no pre-emergence herbicides were applied (mean of 15% leaf area lost) compared to where they were applied (mean of 8% leaf area lost) (Figure 18).

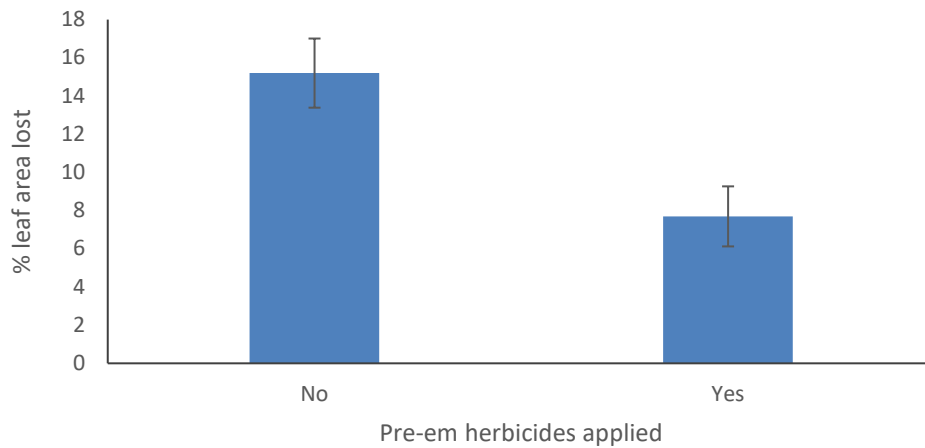


Figure 18. Mean % leaf area lost to adult CSFB in crops in which pre-emergence herbicides had or had not been applied. Error bars indicate standard error of the mean.

Leaving long stubble appeared to be associated with increased damage (>15 cm stubble = mean of 22% leaf area lost) compared to not leaving stubble (mean of 10% leaf area lost) (Figure 19).

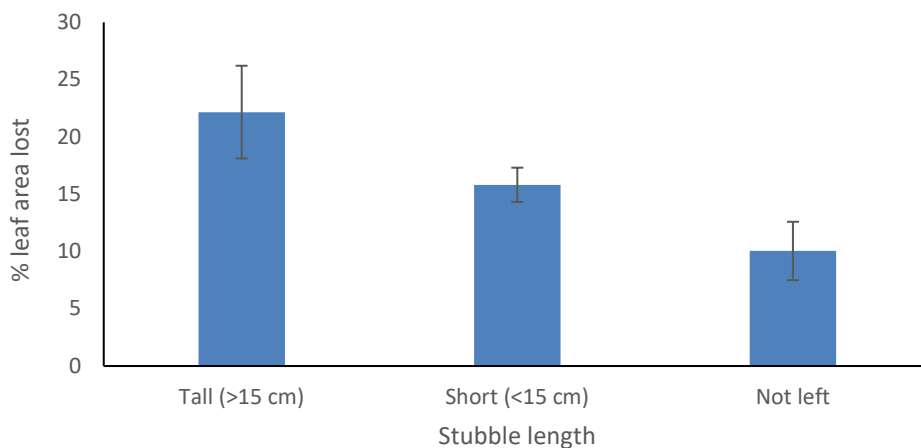


Figure 19. Mean % leaf area lost to adult CSFB in crops with different stubble lengths. Error bars indicate standard error of the mean.

Lower levels of damage were associated with establishment methods involving minimal soil disturbance (e.g. direct drill = mean of 13% leaf area lost) compared to more intensive methods (e.g. subcast = mean of 25% leaf area lost) (Figure 20).

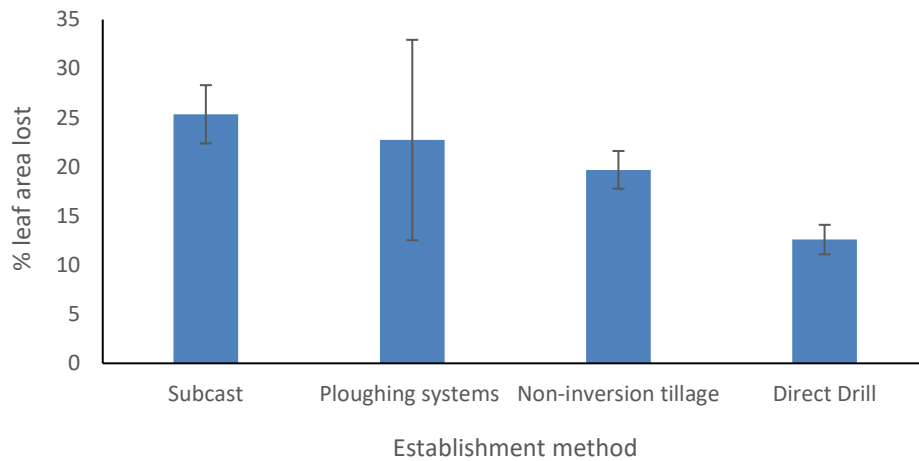


Figure 20. Mean % leaf area lost to adult CSFB in crops with different establishment methods. Error bars indicate standard error of the mean.

Deep drilling of seed appeared to be associated with higher levels of damage (mean of 37% leaf area lost) than shallow drilling (e.g. <2 cm = mean of 15% leaf area lost) (Figure 21).

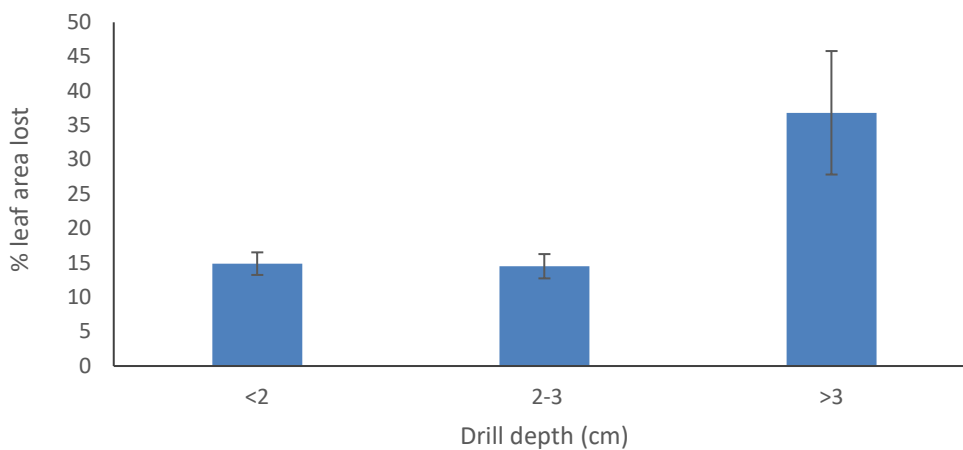


Figure 21. Mean % leaf area lost to adult CSFB in crops with different drill depths. Error bars indicate standard error of the mean.

The effect of seed rate on damage was inconsistent, with greatest damage at high seed rates (e.g. >80-100 seeds per m<sup>2</sup> = mean of 27% leaf area lost) and lower levels at low or very high seed rates (e.g. > 100 seeds per m<sup>2</sup> = mean of 15% leaf area lost) (Figure 22).



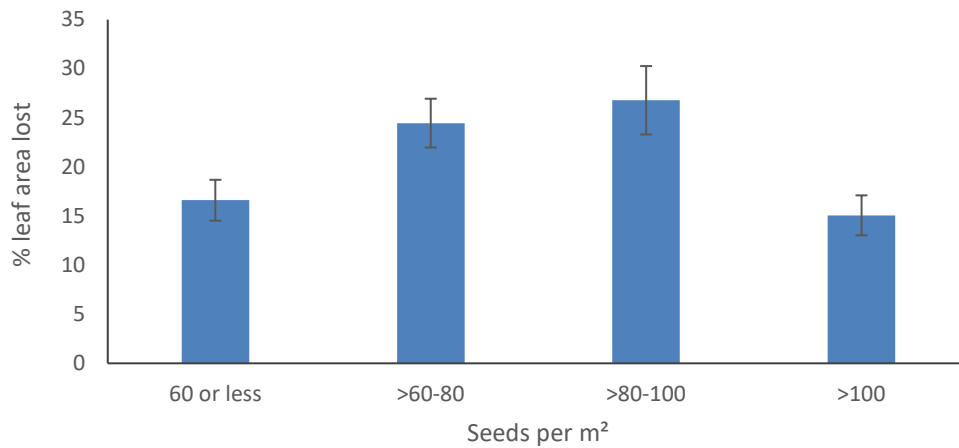


Figure 22. Mean % leaf area lost to adult CSFB in crops with seed rates. Error bars indicate standard error of the mean.

Dry seedbeds were associated with higher levels of damage (mean of 27% leaf area lost) than moist seedbeds (mean of 16% leaf area lost) (Figure 23). A low level of rainfall after sowing was also associated with higher levels of damage (mean of 27% leaf area lost) than where significant rainfall was recorded (mean of 15% leaf area lost) (Figure 24).

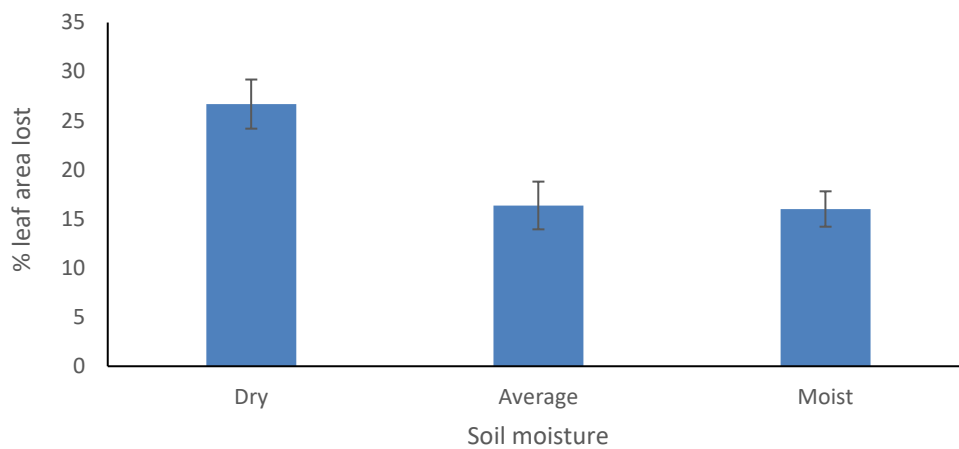
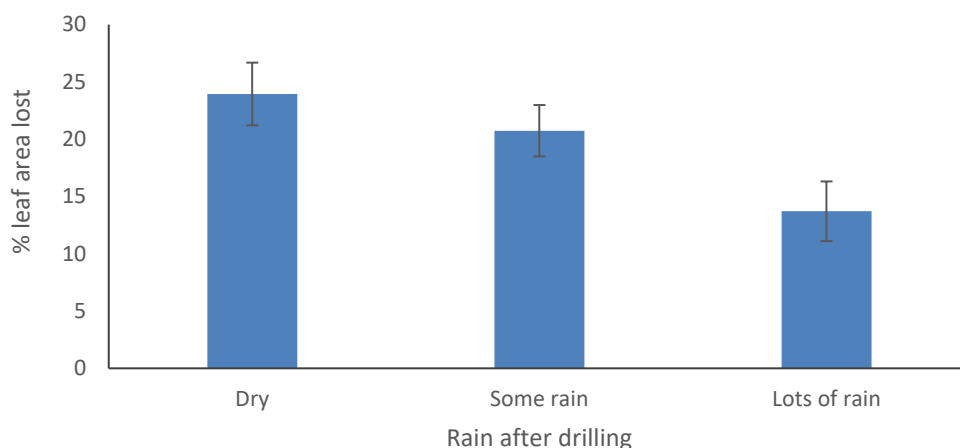
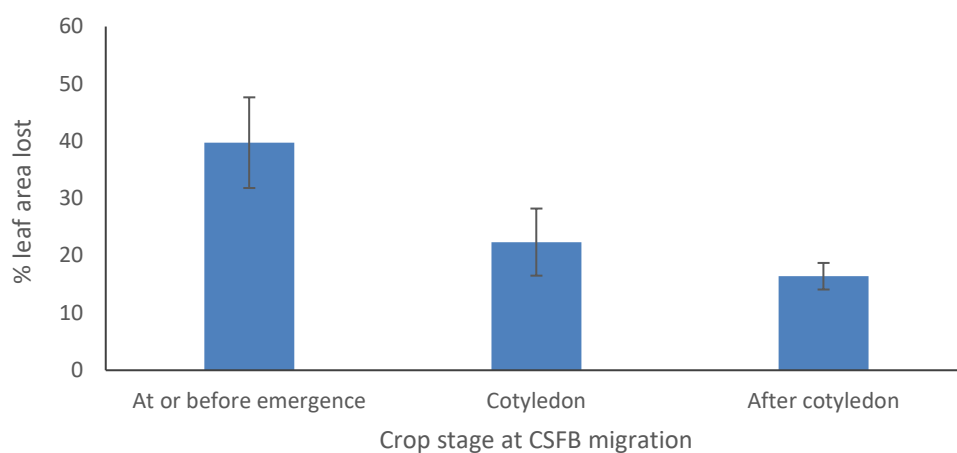


Figure 23. Mean % leaf area lost to adult CSFB in crops sown into seedbeds with different soil moisture levels. Error bars indicate standard error of the mean.



**Figure 24.** Mean % leaf area lost to adult CSFB in crops with different levels of rainfall after sowing. Error bars indicate standard error of the mean.

A clear trend was evident between feeding damage and the crop stage at the time of first CSFB migration, with high levels of damage occurring when CSFB migration occurred at or before emergence (mean of 40% leaf area lost) and lower levels at later crop stages (e.g. after cotyledon stage = mean of 16% leaf area lost) (Figure 25).



**Figure 25.** Mean % leaf area lost to adult CSFB in crops at different growth stages at the point of the first CSFB migration. Error bars indicate standard error of the mean.

High levels of slug pressure were associated with high levels of CSFB damage (mean of 28% leaf area lost) compared to lower levels of slug pressure (e.g. moderate slug pressure = mean of 16% leaf area lost) (Figure 26).

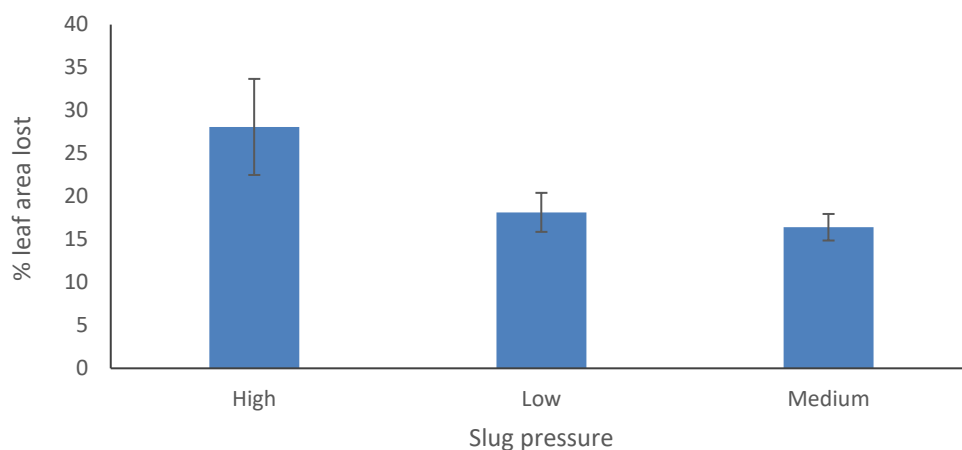


Figure 26. Mean % leaf area lost to adult CSFB in crops with different levels of slug pressure. Error bars indicate standard error of the mean.

There was a strong trend for high levels of damage in crops in which pyrethroid resistance had been reported in the region by the time of the assessment in comparison with crops with no resistance (mean of 20% and 10% leaf area lost where resistance had and had not been detected respectively) (Figure 27).

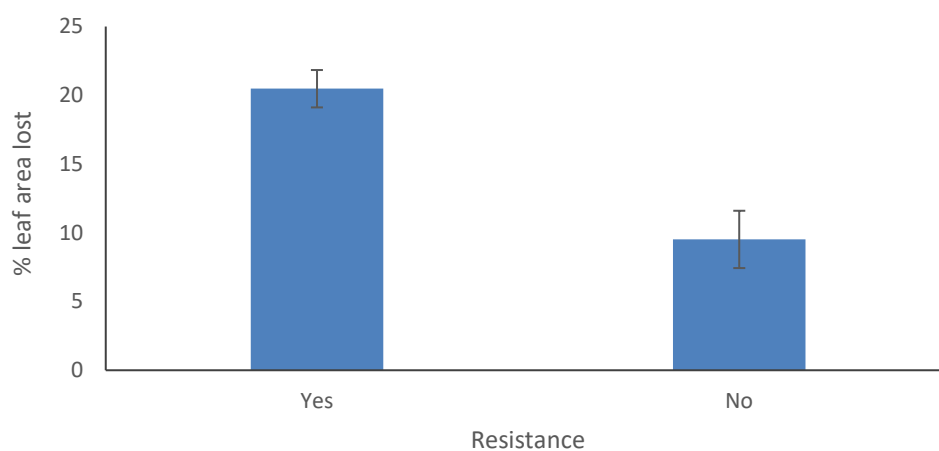


Figure 27. Mean % leaf area lost to adult CSFB in crops depending on whether pyrethroid resistance has been reported in CSFB in the region. Error bars indicate standard error of the mean.

### Autumn larval populations

Larval pressures were generally low until 2014/15 (Figure 28). Between 2003/4 and 2014/15, the average autumn population across the UK was 0.5 larvae per plant. From 2014/15, populations increased rapidly, with a peak UK population of >8 larvae per plant in 2016/17. The highest pressures in recent years have tended to be in the east and south east.

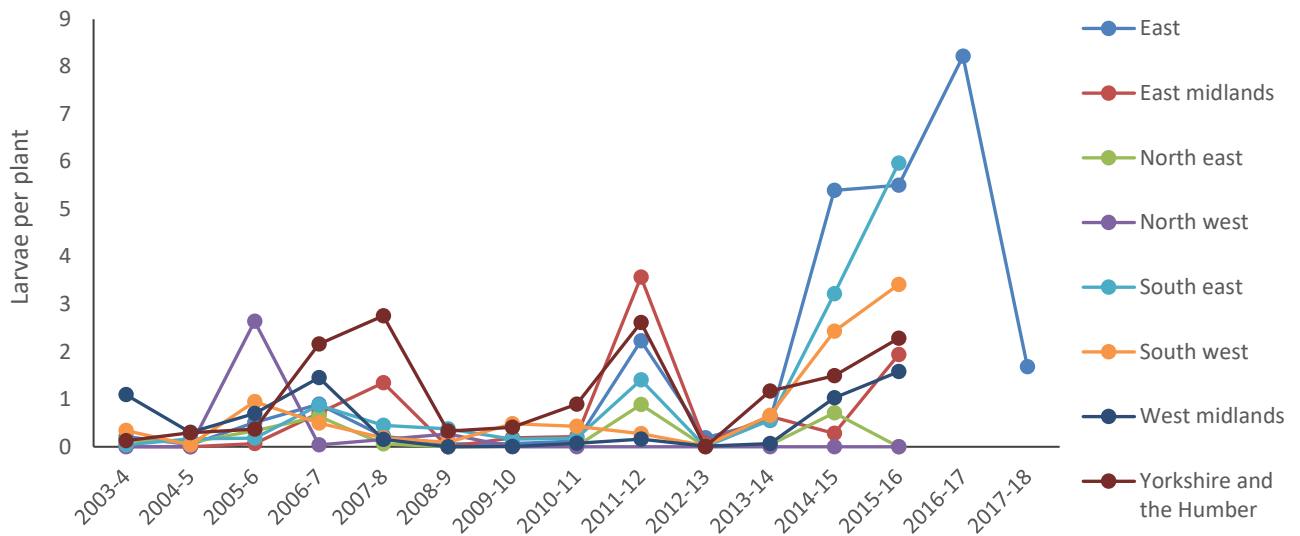


Figure 28. Mean CSFB larvae per plant in the autumn between 2003/04 and 2017/18 for the English regions.

There was a strong trend for larval populations to be greatest in large fields (>30 ha = mean of 1.4 larvae per plant) and lowest in small fields (<5 ha = mean of 0.7 larvae per plant) (Figure 29).

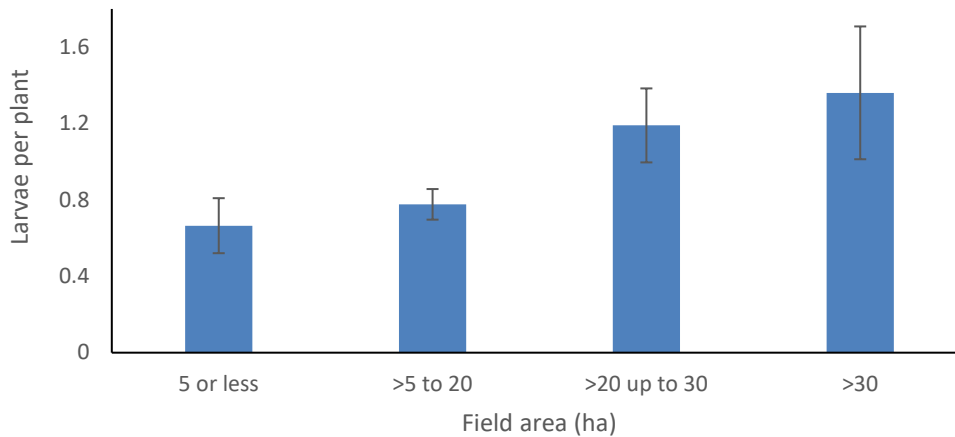


Figure 29. Mean CSFB larvae per plant in the autumn in crops grown in different size fields. Error bars indicate standard error of the mean.

Long WOSR rotations appeared to be associated with lower larval pressures, with a mean of 4.4 larvae per plant in WOSR frequencies of 1 in 3, 5.6 per plant in WOSR frequencies of 1 in 4 and dropping to 3 per plant in WOSR frequencies of more than 1 in 4 (Figure 30).

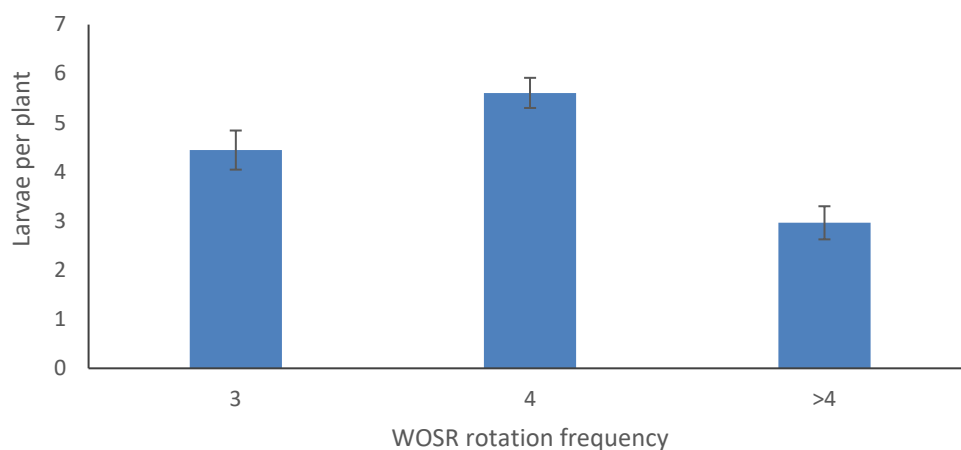


Figure 30. Mean CSFB larvae per plant in the autumn in crops grown in different WOSR rotation frequencies (i.e. years between growing WOSR in the field). Error bars indicate standard error of the mean.

Close proximity to the nearest previous WOSR crop had no clear effect on larval populations, with the lowest pressure seen in crops up to 50 m from the nearest previous WOSR (mean of 3 larvae per plant) and the highest in those more than 50 m from the nearest previous WOSR (mean of 5 larvae per plant) (Figure 31).

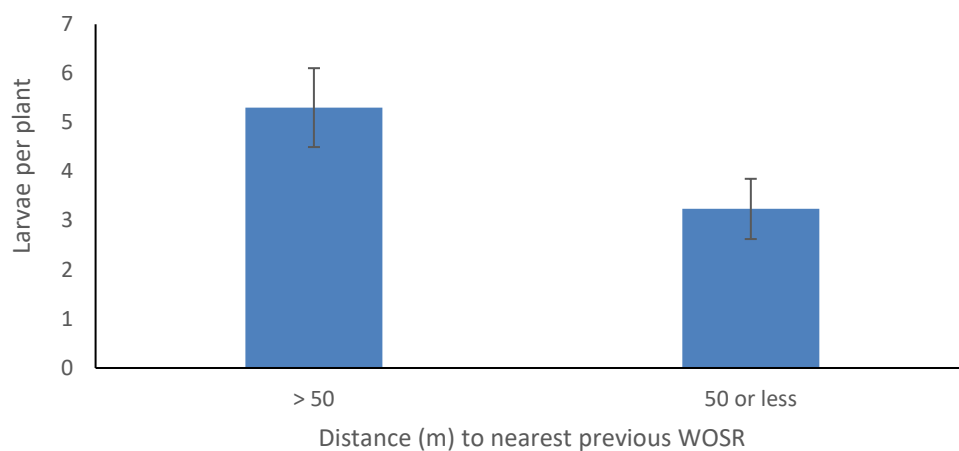


Figure 31. Mean CSFB larvae per plant in the autumn in crops grown at different distances from the nearest previous WOSR crop. Error bars indicate standard error of the mean.

Excalibur had the greatest autumn larval pressure (mean of 0.9 larvae per plant) and Winner the least (0.3 per plant) (Figure 32). Higher larval pressures were also associated with non-HEAR varieties (mean of 1.1 larvae per plant compared to 0.6 larvae per plant in HEAR varieties) (Figure 33). There was no clear trend between conventional and hybrid varieties (Figure 34).

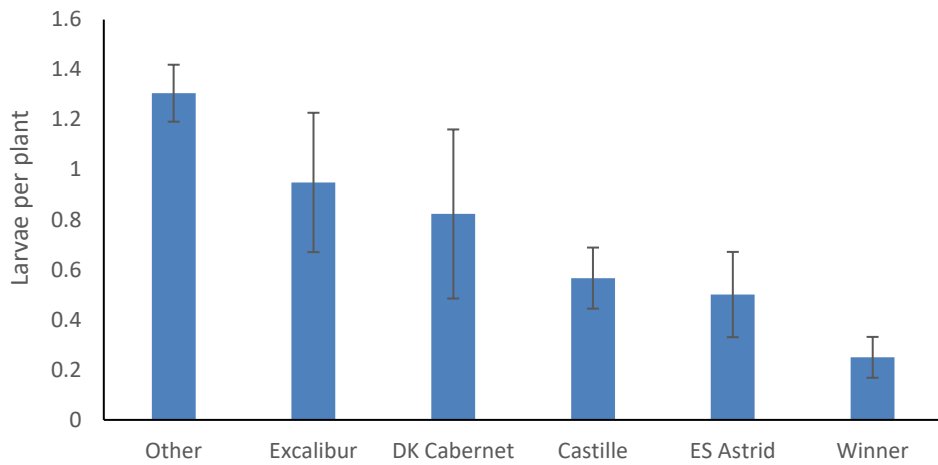


Figure 32. Mean CSFB larvae per plant in the autumn in varieties appearing in the dataset. Error bars indicate standard error of the mean.

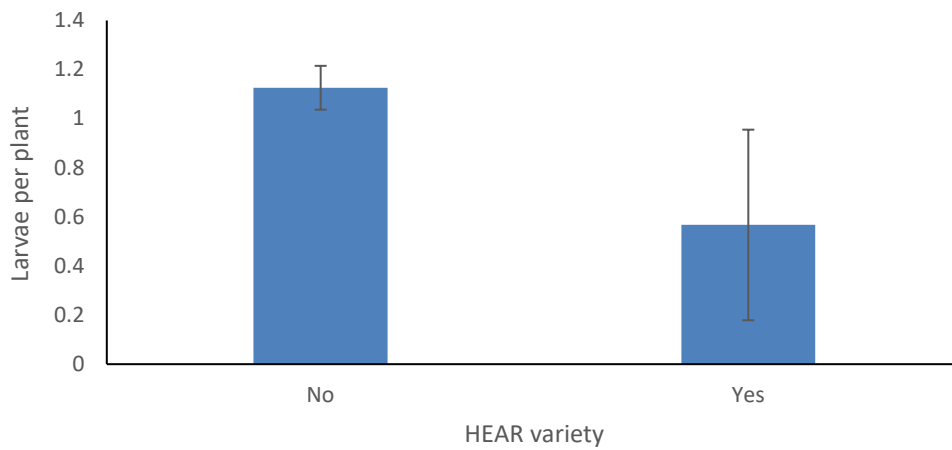


Figure 33. Mean CSFB larvae per plant in the autumn in HEAR or non-HEAR varieties. Error bars indicate standard error of the mean.

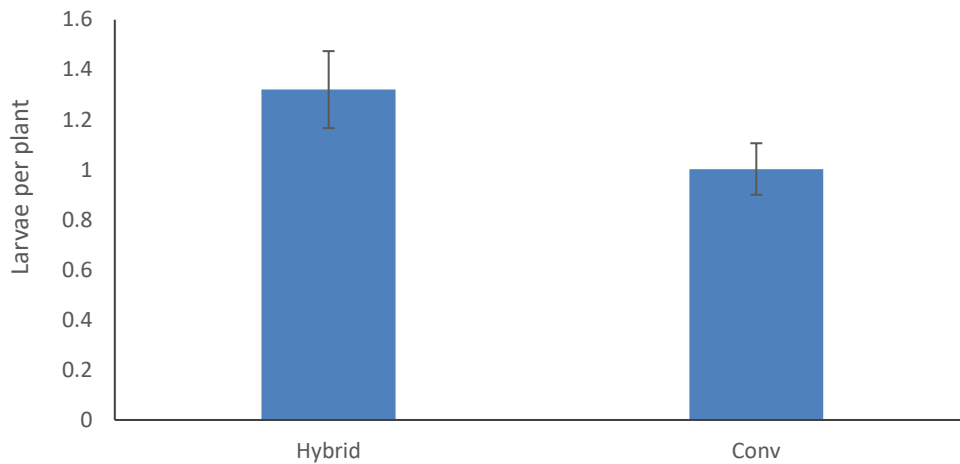


Figure 34. Mean CSFB larvae per plant in the autumn in conventional or hybrid varieties. Error bars indicate standard error of the mean.

There was a strong relationship between sow date and larval pressure, with the highest larval populations in crops sown before 11 August (mean of 2.6 larvae per plant) and the lowest in those sown between 15-21 September (mean of 0.04 larvae per plant) (Figure 35).

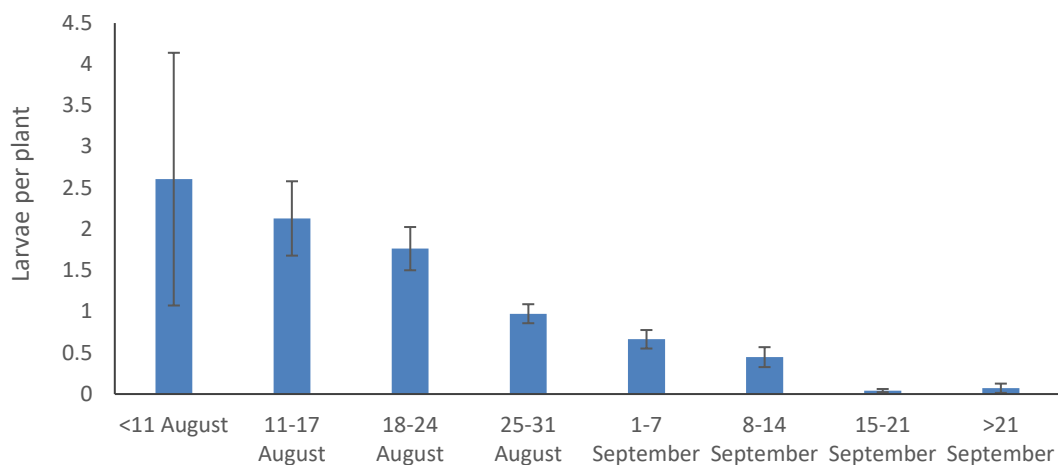


Figure 35. Mean CSFB larvae per plant in the autumn in crops sown in different periods. Error bars indicate standard error of the mean.

The proportion of land grown with WOSR in the region in the previous year was also associated with autumn larval pressure, with highest larval populations in areas with highest proportions of WOSR (Figure 36).

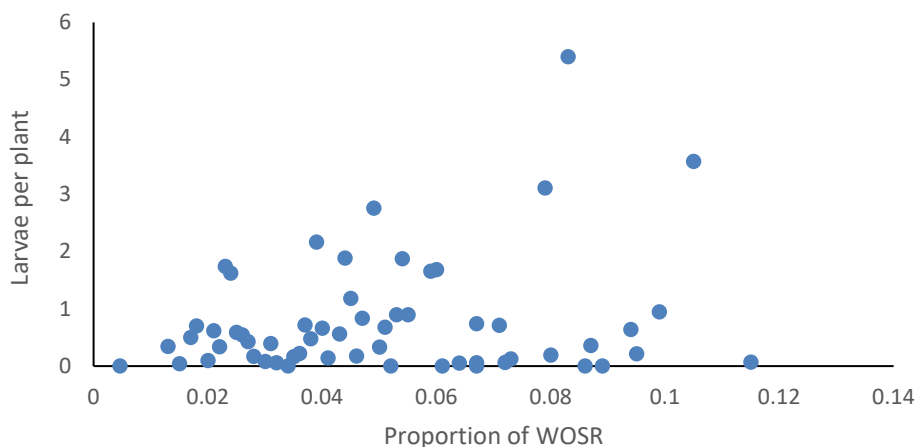


Figure 36. Mean CSFB larvae per plant in the autumn in crops with different proportions of WOSR grown in the region the previous year.

### Spring larval populations

Spring larval pressures have increased markedly in most regions since 2013/14, with the exception of Yorkshire and the Humber where they have tended to be high for some time (Figure 37).

Between 2003/4 and 2013/14, the mean spring larval population across the UK was 0.7 larvae per

plant. Peak mean UK larval population in the spring occurred in 2016/17 (mean of 10.9 larvae per plant). The highest pressures in recent years have tended to be in the east and south east.

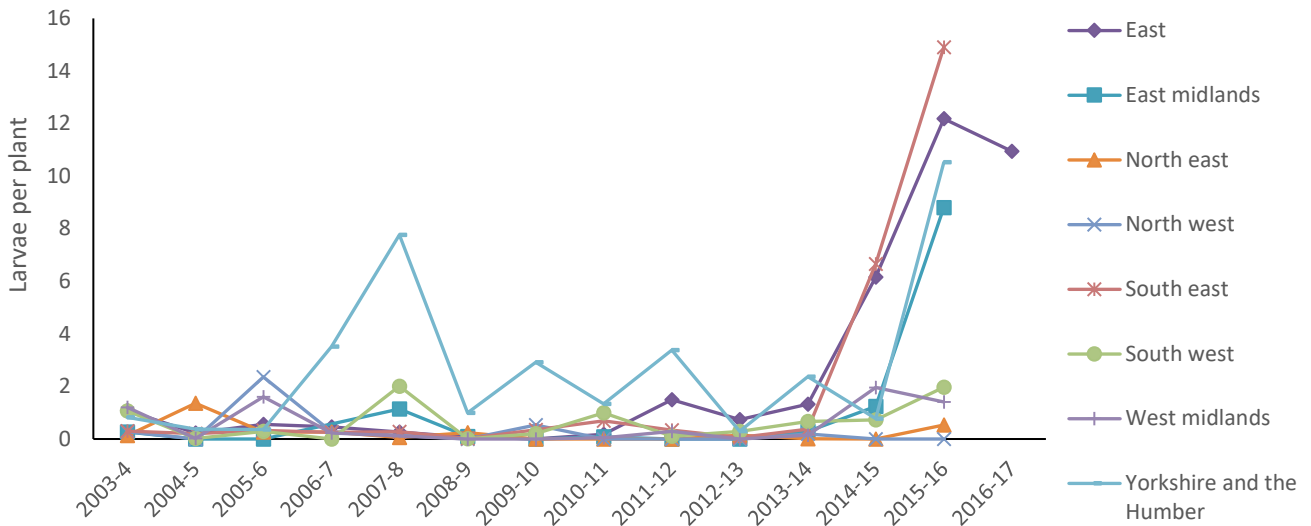


Figure 37. Mean CSFB larvae per plant in the spring between 2003/04 and 2016/17 for the English regions.

There was a strong trend for spring larval populations to be greatest in large fields (>30 ha = mean of 2.4 larvae per plant) and lowest in small fields (<5 ha = mean of 0.6 larvae per plant) (Figure 38).

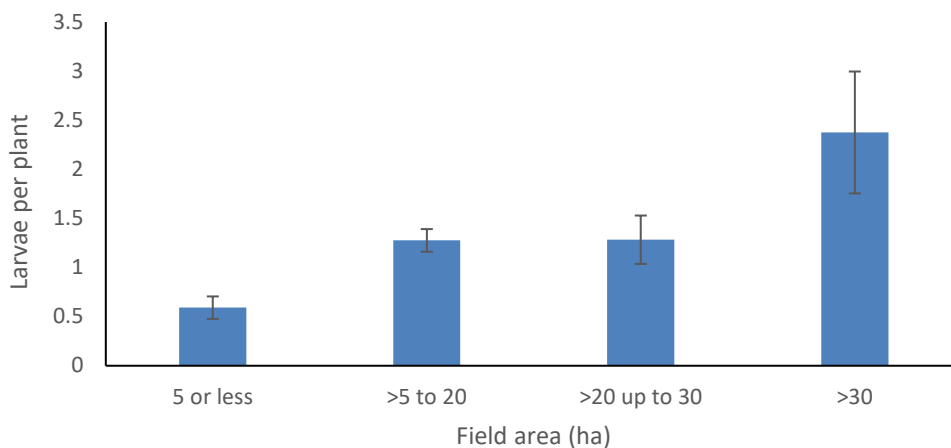


Figure 38. Mean CSFB larvae per plant in the spring in crops grown in different size fields. Error bars indicate standard error of the mean.

There was no clear trend in larval numbers with WOSR rotation frequency. Long WOSR rotations appeared to be associated with slightly lower larval pressures, with a mean of 11 larvae per plant



in WOSR frequencies of more than 1 in 4, and a mean of 17.5 larvae per plant in WOSR frequencies of 1 in 3 (Figure 39). However, the lowest larval populations (7.3 per plant) were associated with a WSOR frequency of 1 in 2 years, although this could be due to the low sample size (9 sites).

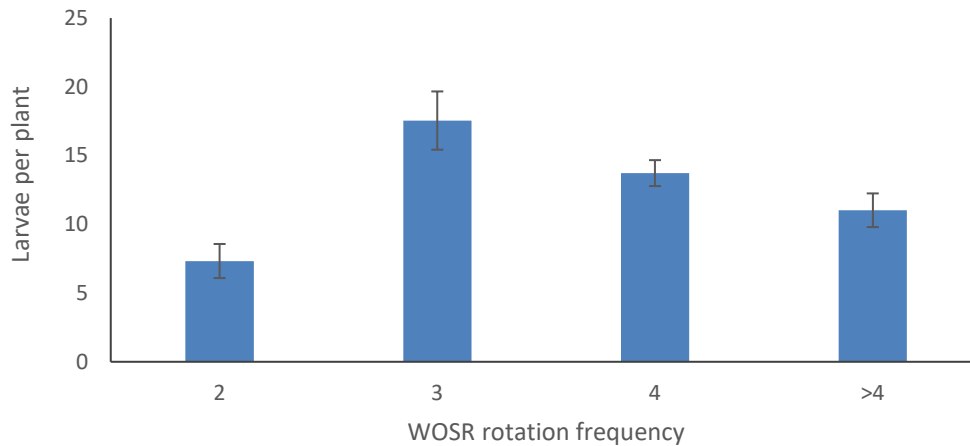


Figure 39. Mean CSFB larvae per plant in the spring in crops grown in different WOSR rotation frequencies. Error bars indicate standard error of the mean.

DK Cabernet had the greatest spring larval pressure (mean of 1.4 larvae per plant) and ES Astrid the least (0.3 per plant) (Figure 40). Higher larval pressures were also associated with non-HEAR varieties (mean of 2.1 larvae per plant) than HEAR varieties (mean of 0.8 larvae per plant) (Figure 41), and with hybrid varieties (mean of 3.1 larvae per plant) in comparison with conventional varieties (mean of 1.7 larvae per plant) (Figure 42).

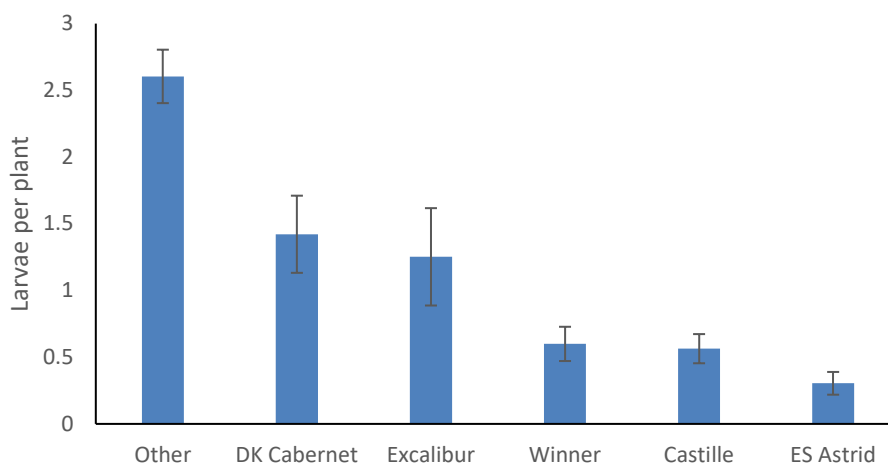


Figure 40. Mean CSFB larvae per plant in the spring in varieties appearing in the dataset. Error bars indicate standard error of the mean.

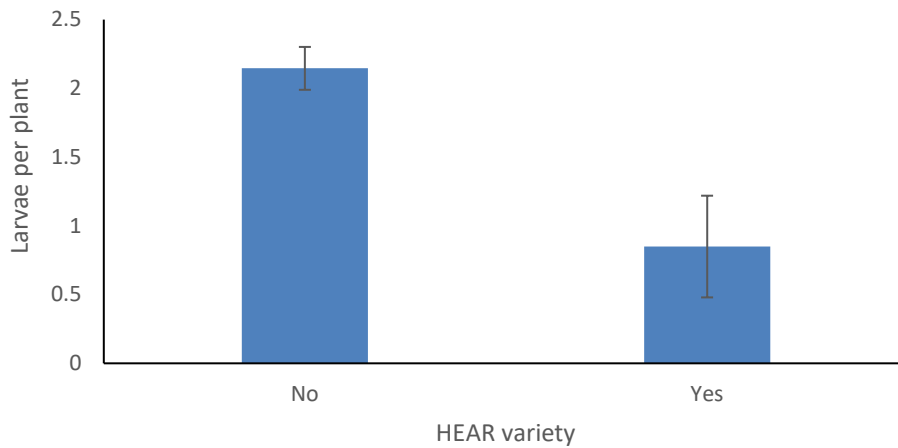


Figure 41. Mean CSFB larvae per plant in the spring in HEAR or non-HEAR varieties. Error bars indicate standard error of the mean.

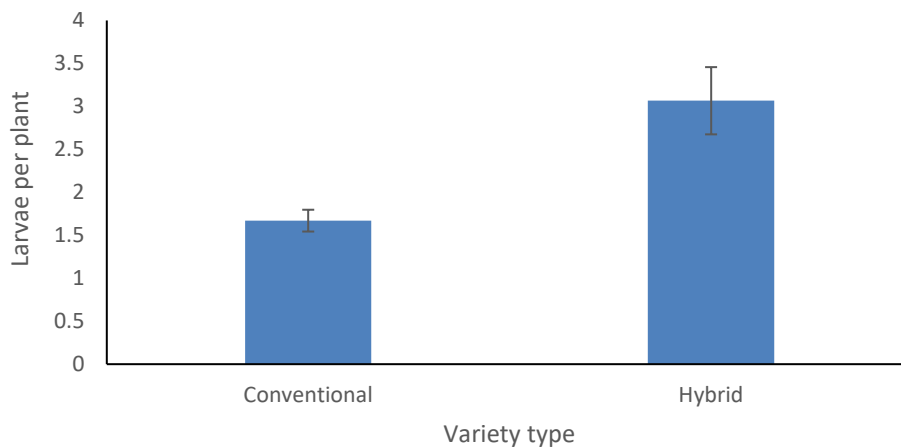


Figure 42. Mean CSFB larvae per plant in the spring in conventional or hybrid varieties. Error bars indicate standard error of the mean.

There was a strong relationship between sow date and spring larval pressure, with the highest larval populations in crops sown 11-17 August (mean of 4.9 larvae per plant) and the lowest in those sown after 21 September (mean of 0.03 larvae per plant) (Figure 43).

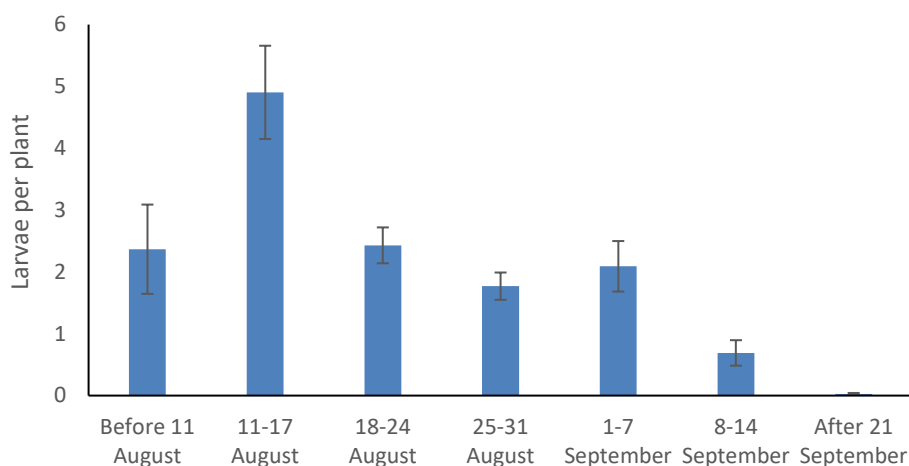


Figure 43. Mean CSFB larvae per plant in the spring in crops sown in different periods. Error bars indicate standard error of the mean.

### 3.4.5. Novel control strategies identified in the surveys

A total of 12 sites were using novel CSFB control strategies. The majority of these (eight sites) were using companion crops, seven of which used berseem clover. At the eighth site the companion crop was not specified. Two sites used trap crops to reduce adult CSFB pressure, with radish drilled in an adjacent field at one site and OSR volunteers left in the adjacent field at another site (the use of OSR volunteers as a trap crop is investigated in detail in Section 7). Two sites applied pyrethroids at night every three nights to protect establishing crops. These novel control strategies were used at too few sites to draw any conclusions on their efficacy.

## 3.5. Discussion

Clearly it is possible to identify trends in the dataset for a number of agronomic factors that appear to influence CSFB pressure from this dataset. For adult CSFB, lower damage was seen in relation to several factors, including low CSFB pressure in the nearest previous WOSR crop, crops sown in the first half of August and second half of September, where no stubble was left, direct drilled crops, shallow sown seed, moisture around sowing (be it soil moisture or rainfall) and where no resistance had been recorded in the area. For larval populations (autumn and spring), lower numbers were recorded in relation to several factors, including small fields, certain varieties (e.g. ES Astrid), HEAR varieties, certain regions (e.g. the north west) and late sown crops.

For some trends the explanatory variable is likely to be having a direct effect on the response variable in the field. For example, it would be expected that CSFB pressure would be greatest in those regions in which pyrethroid resistance has been detected compared to those without it as resistance will reduce the efficacy of pyrethroid sprays (the only chemical control option available at many of the sites featured in this dataset). Other relationships between explanatory and response variables may be spurious, either because they are correlated with other explanatory

variables that may be the true causal factor or have arisen simply by chance. For example, the finding that the lowest spring larval numbers were found in tight WOSR rotations is unlikely to mean that growing WOSR more often will reduce CSFB pressures, rather that this is either a chance finding due to the low number of sites in the dataset at the lowest WOSR frequency or that this is correlated with other factors (e.g. that these crops were grown in low pressure areas or in low pressure years). The number of relationships and the difficulty in determining their veracity illustrates the need to use complex statistical methods to better identify the true relationships between agronomic factors and CSFB pressure. Such modelling approaches have been utilised for this dataset and are described in Section 4. As this modelling is likely to identify only a subset of the explanatory variables for which relationships with CSFB pressure have been described, discussion of the mechanistic causes involved in these relationships will be discussed in Section 4.4.

## **4. Determining the effect of agronomic factors on CSFB pressure**

### **4.1. Introduction**

CSFB survey and other data were collated to produce large datasets on adult CSFB damage and larval populations in autumn and spring (see Section 3). The datasets showed trends in CSFB pressure associated with a range of agronomic factors (Sections 3.4.4 and 3.5). In some cases these trends agreed with anecdotal reports of factors affecting CSFB pressure, for example the effect of late sowing on reducing CSFB pressure. While in others they disagreed with other work, e.g. the effect of leaving stubble. Datasets such as that compiled in Section 3 often have several characteristics that make statistical analysis difficult, including missing data points, correlation between explanatory variables and large numbers of explanatory variables. Such datasets are often analysed using stepwise regression (e.g. White *et al.*, 2017b), non-parametric tests (e.g. Foster *et al.*, 2004) or non-statistically (Wynn *et al.*, 2017). However, these approaches can present issues in some cases, including poor ability to account for interactions and correlations between explanatory variables, problems associated with the order in which variables are entered into the analysis, and the method in which variables are selected by the analysis for inclusion in the model (Mitzi 2007; Mundry & Nunn, 2008; Smith, 2018). Ultimately, this can produce models or analyses that do not account for the greatest variance possible and perform poorly. Indeed, it is widely accepted that the larger the number of explanatory variables the better for developing models to predict a response variable, but stepwise regression becomes less effective as the number of potential explanatory variables increase (Smith, 2018). Other techniques such as decision tree or ensemble learning methods offer viable alternatives (Breiman *et al.*, 2001; Tso & Yau, 2007). Therefore, it was decided to use novel statistical techniques to model the CSFB dataset, combining useful aspects of ensemble learning and regression analysis methods. This chapter describes the risk factors for CSFB identified through the analysis (Objective 2).

### **4.2. Materials and methods**

An exploratory analysis was used to identify potential agronomic factors and weather patterns affecting CSFB adult feeding and autumn and spring larval infestation. A dataset was compiled from existing information (see Section 3.4). Due to the large number of potential explanatory factors, a two-stage process was used for the exploratory analysis. First, a random forest analysis was used to assess the relative importance of all of the potential explanatory factors. A subset of these with the highest relative importance was then analysed using stepwise regression, to select those factors that explained the greatest proportion of the variability in the data, and to give an indication of how each factor was associated with high or low levels of CSFB adult feeding damage and larval infestation.

#### 4.2.1. Data sources

##### ***CSFB adult feeding and larval infestation data***

The assemblage of CSFB data is described in Section 3.4.1. For mean larval numbers per plant (autumn and spring), the analysis was done using the logged variable (0.01 added to all values before logging calculating the natural log), whilst a logit transformation was used for the adult CSFB damage data (using a 0.01% adjustment for 0% and 100% leaf area lost).

##### ***Agronomic data***

The source of the agronomic data in the dataset is described in Section 3.4. Variables which had less than 50 data points were excluded from further analysis. The agronomic variables analysed are shown in Table 3. An agronomic factor based on whether the crop was sown before 2014 was included to capture any impact of the restriction on neonicotinoid seed treatments (introduced on 1 December 2013) or pyrethroid resistance, which was detected for the first time in the UK in 2014 (Højland *et al.*, 2015) and has since spread to most areas of England (S. Foster, pers. comm.).

Factor categories were selected based on the number of data points available. Factor categories with less than 10 data points were merged with other relevant categories. For example, dozens of soil types were present in the dataset and these were amalgamated based on RB209 categories (AHDB, 2020a). This included a 'Deep clayey' soil category, which included deep silty clay, Hanslope clay, clay loam, etc. RB209 categories with less than 10 data points were merged with other categories to create an 'Other' soil category. Factor categories occasionally differed between response variables due to differences in source data and the need to merge categories to ensure sufficient data points in each category. For example, region appeared in the dataset for adult damage and larval populations but due to the smaller number of data points in the adult damage dataset some regions had been amalgamated into an 'Other' category, whereas all regions were present as separate categories in the larval population datasets.

The methods used to analyse the datasets assume all data points were independent. However, some data points were sourced from very similar geographical locations, including some from the same site and year (for example, data collected as part of trials where different agronomic factors had been investigated). These made up a relatively small proportion of the dataset (the number of data points affected is shown in Table 3). The implications of this are discussed further in the Discussion (Section 4.4).

Table 3. Number of data points and unique site/years for each agronomic explanatory variable used in the modelling analysis. LA lost = adult feeding damage response variable. \*Some explanatory variables were not analysed for all response variables (indicated by N/A) due to lack of data. N.B. 'Unknown' or 'missing' categories not included in counts of data points.

Variable*	Data categories	No. data points			No. data points with unique site/year		
		LA lost	Autumn larvae	Spring larvae	LA lost	Autumn larvae	Spring larvae
Year of sowing prior to 2014	Yes, No	N/A (all data 2014 onwards)	1154	585	N/A	1127	585
Region (LA lost)	East, Yorkshire and Humber, Other	299	N/A	N/A	245	N/A	N/A
Region (autumn larvae)	East, east Midlands, north east, north west, south east, south west, west Midlands, Yorkshire and Humber	N/A	1154	N/A	N/A	1127	N/A
Region (spring larvae)	East, east Midlands, north east, north west and west Midlands, south east, south west, Yorkshire and Humber	N/A	N/A	585	N/A	N/A	585
Variety (LA lost)	Campus, DK Exalte, Elgar, Nikita, Tactic, Other	299	N/A	N/A	245	N/A	N/A
Variety (autumn and spring larvae)	Castille, DK Cabernet, ES Astrid, Excalibur, Winner, Other	N/A	1154	585	N/A	1127	585
Variety type	Conventional, hybrid, unknown	299	1104	558	245	1077	558
HEAR variety	Yes, no, unknown	N/A	1083	549	N/A	1056	549
Sow date (LA lost)	<18/8, 18-24/8, 25-31/8, 1-7/9, >7/9	299	N/A	N/A	245	N/A	N/A
Sow date 1 (autumn larvae)	<11/8, 11-17/8, 18-24/8, 25-31/8, 1-7/9, 8-14/9, 15-21/9, >21/9	N/A	1154	N/A	N/A	1127	N/A
Sow date 2 (autumn larvae)	<16/8, 16-31/8, 1-15/9, >15/9	N/A	1154	N/A	N/A	1127	N/A
Sow date (spring larvae)	<18/8, 18-24/8, 25-31/8, 1-7/9, >7/9	N/A	N/A	585	N/A	N/A	585
Field area	≤5 ha, >5 to 20 ha, >20 to 30 ha, >30ha, missing	203	1154	585	149	1127	585
Previous crop (LA lost)	Spring cereals, winter wheat, winter barley and rye, other	299	N/A	N/A	245	N/A	N/A
Previous crop (autumn larvae)	Fallow, winter wheat, other cereal, other	N/A	1154	N/A	N/A	1127	N/A
Previous crop (spring larvae)	Winter wheat, other cereal, other	N/A	N/A	585	N/A	N/A	585
Proportion of land occupied by OSR in region in previous year	Continuous variable	299	1154	585	245	1127	585
OSR rotation	2-3, 4, >4 year	299	N/A	N/A	245	N/A	N/A
Soil type	Medium, deep clayey, other, missing	252	N/A	N/A	198	N/A	N/A
Soil stoniness	No stones, few stones, stony, missing	205	N/A	N/A	155	N/A	N/A
Pre-emergence herbicide	Yes, no, missing	112	N/A	N/A	60	N/A	N/A
Straw management	Chopped, baled and removed, missing	246	N/A	N/A	204	N/A	N/A

Variable*	Data categories	No. data points			No. data points with unique site/year		
		LA lost	Autumn larvae	Spring larvae	LA lost	Autumn larvae	Spring larvae
Establishment technique	Direct drill and roll, non-inversion tillage, subcast, plough, unknown	277	N/A	N/A	223	N/A	N/A
Stubble length	Not left, short (<15cm), tall (>15cm), missing	203	N/A	N/A	149	N/A	N/A
Seed home-saved	Yes, no, missing	250	N/A	N/A	196	N/A	N/A
Row width	<15 cm, 15 to 30 cm, >30 cm, missing	247	N/A	N/A	193	N/A	N/A
Drilling depth	<2 cm, ≥2cm, missing	200	N/A	N/A	146	N/A	N/A
Seeds per m <sup>2</sup>	≤60, >60 to 80, >80 to 100, >100 cm, missing	282	N/A	N/A	243	N/A	N/A
Seedbed cloddiness	Cloddy, average, ideal, missing	254	N/A	N/A	200	N/A	N/A
Seedbed condition	Clean, average, trashy, missing	235	N/A	N/A	181	N/A	N/A
Seedbed moisture	Dry, average, moist, missing	253	N/A	N/A	199	N/A	N/A
Rain post drilling	Dry, some rain, lots of rain, missing	205	N/A	N/A	176	N/A	N/A
Emergence date	< 1 September or ≥ 1 September	170	N/A	N/A	130	N/A	N/A
Crop stage at adult CSFB migration	At or before emergence, cotyledon, after cotyledon, missing	94	N/A	N/A	42	N/A	N/A
Fertiliser before drilling	Yes, no, missing	206	N/A	N/A	152	N/A	N/A
Nutrient before drilling	No fertiliser, P, N, P and N, unknown/ n/a	206	N/A	N/A	152	N/A	N/A
Fertiliser at drilling	Yes, no, missing	207	N/A	N/A	153	N/A	N/A
Fertiliser after drilling	Yes, no, missing	199	N/A	N/A	145	N/A	N/A
Fertiliser application method at drilling	Broadcast, placed with seed, no fertiliser, unknown/ n/a	205	N/A	N/A	151	N/A	N/A
Slug pressure	Low, medium, high, missing	233	N/A	N/A	179	N/A	N/A
Distance from nearest, previous OSR 1	≤50, 51-500, 501-1000, >1000 m, missing	252	N/A	N/A	198	N/A	N/A
Distance from nearest, previous OSR 2	≤50, >50 m, missing	269	N/A	N/A	215	N/A	N/A
Direction from nearest, previous OSR	NE quadrant, SW quadrant, other, missing	241	N/A	N/A	187	N/A	N/A
Adult CSFB pressure in nearest WOSR in previous year	Low, medium, high, don't know, missing	220	N/A	N/A	168	N/A	N/A
Larval CSFB pressure in nearest WOSR in previous year	Low, medium, high, don't know, missing	220	N/A	N/A	168	N/A	N/A
Pyrethroid resistance reported in area by assessment date	Yes, no	299	1154	585	245	1127	585



### **Weather data**

Weather conditions, including temperature (Alford, 1979; Mathiasen *et al.*, 2015a; Mathiasen *et al.*, 2015b) and moisture (Bonnemaïson and Jourdeuil, 1954, in Ankersmit, 1964; Robert, 2012), have been shown to affect CSFB life cycles, survival and behaviour. Therefore, data on temperature and rainfall were included in the analysis as candidate explanatory factors.

Daily weather data were sourced from MetMake within the IRRIGUIDE tool (Silgram *et al.*, 2007), interpolated by the MetMake tool for each site (using the grid reference) from reported weather data recorded at Met Office weather stations. Altitudes for each site were required as input data for MetMake. Altitude data were sourced from OSTerrain50GB (Open Data). The grid references for each site were converted to six figure Eastings and Northings and the locations converted to a point shape file. Height values for each location were extracted using a Spatial Analyst tool in ArcGIS ('Extract Multi Values to Points').

Summary variables were calculated from daily weather data. Weather data were summarised as total rainfall, average air temperature and sum of day degrees above 3.2°C for monthly periods, and the quarters June-August, September to November, December to February and March-May, from the winter prior to sowing of the crop, to the following spring. The 3.2°C threshold was selected for the sum of day degrees calculation as Alford (1979) suggested that egg development would only occur above this temperature. A different threshold to Alford's was identified by Mathiasen *et al.* (2015a) (5.1°C) but Alford's was chosen as this was based on UK CSFB populations whereas Mathiasen's used Danish populations. Therefore, the sum of day degree explanatory factors would capture conditions above those amenable to egg hatch. Weather in the winter following sowing was considered as an explanatory factor for spring larval numbers only.

### **Validation data**

To validate the model a dataset was compiled, consisting of data on adult CSFB feeding damage and larval populations (autumn and spring) from untreated plots in trials managed by ADAS, and Fera larval survey data. Data were adjusted where insecticides had been applied at Fera survey sites (as described in Section 3.4.2). Data were taken from trials done as part of this project (larval impact trials in 2016/17 and 2018/19, a larval impact trial at Boxworth in 2018/19, volunteer OSR trap crop trials in 2018/19, a companion crop trial in 2017/18, variety and seed rate trials at High Mowthorpe in 2017/18 and 2018/19, variety and seed rate trials at Boxworth in 2018/19, and a defoliation trial at Boxworth in 2018/19), trials in previous AHDB-funded projects, Fera larval surveys in 2016/17 and untreated plots in commercially-funded trials (data kindly provided by BASF, Corteva, DSV, DuPont, Gowan, Interagro and Syngenta). Explanatory variable data included only those variables selected from the final stepwise regression analysis for each response variable. For adult CSFB damage, the validation dataset consisted of 58 data points

spanning 2015/16 to 2019/20, with the majority recorded in 2017/18 and 2018/19 (22% and 59% respectively). Not all explanatory variables identified by the model were available in this dataset so some ('Crop stage at migration' and 'Rain post-sowing) were estimated based on sow date and weather data respectively. Information on 'Stubble length' was also not available and so was considered as 'missing'. For larval CSFB populations in the autumn, the validation dataset consisted of 128 data points spanning 2015/16 to 2019/20, with the majority recorded in 2016/17 and 2018/19 (55% and 28% respectively). For larval CSFB populations in the spring, the validation dataset consisted of 99 data points spanning 2015/16-2016/17 and 2018/19-2019/20, with the majority recorded in 2016/17 and 2018/19 (38% and 41% respectively).

#### **4.2.2. Random Forest analysis**

##### ***Overview of Random Forest methods***

Random Forests (Breiman, 2001; Breiman & Cutler, 2008) is an ensemble machine learning approach that can be used to assess the relative importance of multiple candidate explanatory variables. That is to say it ranks the explanatory variables in terms of how well they account for the variability within the experimental dataset. The approach is appropriate for handling datasets with large numbers of candidate explanatory variables of mixed types (categorical and numerical), assessing the correlation between those variables, and taking account of missing values (Hapfelmeier & Ulm, 2013), all of which were features of the CSFB adult feeding and larval infestation datasets. Random Forests were used here for a regression analysis, as the response variables (adult feeding damage or larval numbers) were numerical.

Random Forests use decision tree, 'CART' (classification and regression tree) methods (Breiman *et al.*, 1984). In a regression decision tree, a single decision tree splits the dataset into multiple groups, where each split, or 'node', is decided based on the value of a single variable. For example, for a split based on the variable 'HEAR' (High erucic acid rape), with the categories 'Yes' or 'No', any data points with a 'HEAR' value of 'Yes' would be split into one group, and those with a value of 'No' would be split into a second group. A split based on a numerical variable splits the data into two groups based on a threshold value of the variable, for example 'Total rainfall in August < 30 mm' or '≥ 30 mm'. The value predicted by the regression tree for each group is the average value of all data points in the group. Within each group, the decision tree algorithm then considers, separately, a potential further split of the data based on each potential explanatory variable. When this process is repeated multiple times, it leads to a branching flow-chart that resembles the structure of a tree. It is possible to adjust the settings of the decision tree algorithm to place restrictions on the minimum size of the final sub-groups (or 'leaves' of the tree). An example regression decision tree is shown in Figure 44. In this example, the child's age and their preference for broccoli is used to predict the child's height.

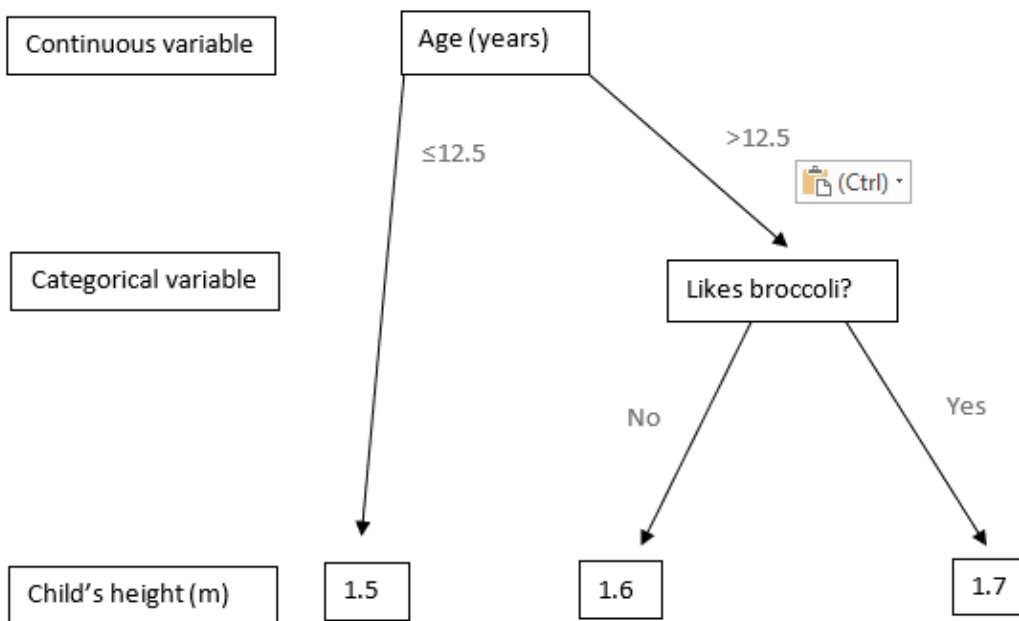


Figure 44. An example of a very simple regression decision tree, illustrating how a response variable (child height) can be predicted by a continuous variable (age) and a categorical variable (dietary preference).

The basic decision tree algorithm is known as a ‘greedy algorithm’, because at each node, it chooses the split that explains the maximum proportion of the variability for the particular subgroup under consideration, rather than optimising the overall tree to explain the maximum proportion of the variability for the entire dataset. The Random Forests approach aims to overcome this ‘greedy’ behaviour, by using multiple decision trees and a bootstrapping approach. Each decision tree is trained on a different, random subset of the data, and the performance of each decision tree is tested against the remainder of the data that was not used to fit the decision tree (known as ‘OOB’ data, that has been left ‘out of the bag’ of data used to fit the decision tree). Each decision tree in the Random Forest then ‘votes’ on the importance of each candidate explanatory variable. The variables that gain the highest variable importance weighting are those chosen by a large number of decision trees. The variable importance is a measure of how useful the variable is, on average, for explaining variability in the response variable. It is informative to compare the relative magnitude of variable importance for potential explanatory variables: the variables with the highest variable importance are ranked as being the most useful. The approach used for the Random Forest analyses in this work is described in the following section.

### **Random Forest analyses**

The Random Forest analysis was done in R studio. As a number of the potential explanatory variables were correlated with one another, the conditional variable importance (Strobl *et al.*, 2008) was used for this analysis using the R package ‘party’, rather than using the marginal approach used in the original ‘Random Forests’ algorithm. This measures the relative importance of the variables, compared to one another and is recommended in cases where potential explanatory

variables are correlated with one another. A three-step process was used to assess which variables were sufficiently informative to merit inclusion in the stepwise regression.

Firstly, variables were selected from the full list of explanatory variables using a permutation test, carried out using R code published by Alexander Hapfelmeier (Hapfelmeier & Ulm, 2013). The permutation test used a total of 400 permutations ('nperm = 400') of Random Forests of 500 conditional regression trees ('ntree = 500') were used, and alpha values calculated using a Bonferonni-Adjustment. An alpha value of 0.05 was used as the threshold for significance in the permutation test. Variables giving significant alpha values for importance in tests of the transformed variables were selected for further analysis.

The second stage of the analysis addressed the fact that some weather variables selected through the first stage of permutation tests partially contained the same information (e.g. total rainfall in December and total rainfall in the three-month period December to February). In these cases, the most informative weather variable of those with overlapping information was selected for further analysis. This was based on the average conditional importance score from three conditional forests of 1000 trees (fitted using different random seeds in R), using only those variables selected through the first stage of permutation tests for the transformed response variables.

With the shortlist of potential weather variables reduced (by the second stage of the process), the mean conditional importance of these and all other explanatory variables was recalculated (as the exclusion of some weather variables with overlapping information was expected to affect the conditional importance of the remaining variables). Three conditional forests of 1000 trees (fitted using different random seeds in R) were again fitted, using the selected weather variables and other variables selected through the permutation test, and the mean conditional importance of these variables was calculated.

Finally, the variables selected through the first two stages of analysis were carried forward for further analysis, as described in the section below ('Threshold values, t-test and linear regression analyses').

### ***Threshold values, t-test and linear regression analyses***

The regression decision trees within the Random Forests algorithm place data points into groups by splitting explanatory variables based on categories or a threshold value of a numerical variable. High variable importance for a numerical variable does not therefore necessarily indicate a linear relationship. Therefore, continuous variables were categorised based on threshold values prior to the next step in the analysis; stepwise regression. The threshold values were determined by running ten individual decision trees in R, using only the variables selected in the first two stages

described in the section above ('Random Forest analyses'). Threshold values were set as the average of the splitting points indicated by the individual decision trees. For continuous variables which were clearly split at more than one distinct threshold value in the decision trees, multiple thresholds were used (a maximum of two thresholds was set for this analysis). Categories within categorical variables that were always grouped together in the decision trees were merged into a single category.

A t-test (using the categories defined by the threshold values) and linear regression (for continuous variables) analysis was carried out in Genstat 18<sup>th</sup> edition for each individual variable. Where the variable had more than two factor levels, a general linear model analysis was used instead of a t-test. Any variables with a non-significant result in both the individual t-test and linear regression (where relevant) were not used in the stepwise regression. For continuous variables, only one type of variable (either continuous, or the factor levels defined by the threshold value/s) was carried forward to the stepwise regression, depending on which type explained the greatest percentage of the variance in the data.

#### **4.2.3. Stepwise regression and general linear regression**

##### ***Stepwise regression analysis***

A stepwise regression was used to select a subset of factors that explained the greatest proportion of the variability in the data, and to give an indication of how each factor was associated with high or low levels of CSFB adult feeding and larval infestation.

A forward stepwise all-subsets regression analysis was done in Genstat 18<sup>th</sup> edition using all variables selected through the Random Forest analysis, and transformed values of the response variables. The accumulated analysis of variance was output, and the subset of variables selected by the all-subsets regression analysis was carried forward to a final general linear regression. The subset with the maximum number of variables that all had an F probability <0.05 were carried forward to a final general linear regression.

##### ***General linear regression***

A general linear regression analysis was done in Genstat (18<sup>th</sup> edition) using only the variables selected by the stepwise regression analysis step. The fitted coefficients were extracted to give an indication of how each factor was associated with high or low levels of CSFB adult feeding damage and larval infestation.

#### **4.2.4. Validation**

The datasets assembled for validation were used to test the general linear regression analyses built using the stepwise regression. The regression model was used to predict a value for each

data point based on the explanatory variables, and the predicted value compared to the observed values. T-test (factors) or regression analyses (continuous/numerical variables) were also done in Genstat (18<sup>th</sup> edition) for the site averaged data.

### **4.3. Results**

#### **4.3.1. Random forest analysis**

##### ***Adult CSFB damage (% leaf area lost)***

Explanatory variables selected by the random forest analysis and identified as significant in the permutation tests are given in Table 4 for both the untransformed and transformed response variable. The conditional variable importance and threshold values are also given in Table 4 for those explanatory variables selected for stepwise regression analyses. The explanatory variable with the greatest conditional importance was 'Crop stage at migration', followed by 'Region' and 'Soil stoniness' (Figure 45). The pseudo R-squared value for the conditional random forest analysis (based on the variables selected for stepwise regression) was 29.6%.

Table 4. Results of Random Forests analysis and permutation tests for the CSFB adult feeding damage dataset. ORV = original response variable (% leaf area lost). TRV = transformed (logit) response variable.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Crop stage at migration	NS	Y	0.146	At or before emergence / Other (Missing, Cotyledon, After cotyledon)
Region	Y	Y	0.063	East / Other
Soil stoniness	Y	Y	0.060	Few stones / Other (Missing, No stones, Stony).
Average temperature in August	Y	Y	0.050	>16.9°C
Total rainfall in July before crop sown	Y	Y	0.045	>29mm
Average temperature in July before crop sown	Y	Y	0.039	>17.2°C
Average temperature in February before crop sown	Y	Y	0.037	>4.6°C
Rain post drilling	NS	Y	0.025	Dry, Some rain / Other (Lots of rain, Missing)
Proportion of land occupied by OSR in region in previous year	Y	Y	0.022	>0.06
Field area	Y	Y	0.019	20-30 ha, Other (Missing, <5ha, 5-20ha, >30ha).
Sum of day degrees >3.2°C in May before crop sown	NS	Y	0.017	>275
Total rainfall in August	Y	Y	0.013	>38mm
Total rainfall in December (year prior to sowing)	NS	Y	0.012	>71mm
Average temperature in January before crop sown	NS	Y	0.012	>5.2°C
Sum of day degrees >3.2°C in December before crop sown	NS	Y	0.011	>187
Stubble length	Y	Y	0.008	Not left / <i>Short (&lt;15 cm)</i> / Tall (>15 cm), Missing
Nutrient before drilling	NS	Y	0.008	P or Unknown / Other (P and N, N, No Fertiliser)

Table 4. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Drilling depth	NS	Y	0.004	Less than 2cm / Other (Greater than 2cm, Missing)
Sum of day degrees >3.2°C in December to February before crop sown	NS	Y	< C.I. temperature variables for individual months	Not used
Sum of day degrees >3.2°C in February before crop sown	Y	Y	< C.I. average temperature in February	Not used
Sum of day degrees >3.2°C in August	Y	Y	< C.I. average temperature in August	Not used
Pyrethroid resistance reported in area by assessment date	Y	NS	N/A	Not used
Seedbed condition	Y	NS	N/A	Not used
Larval CSFB pressure in nearest WOSR in previous year	Y	NS	N/A	Not used
Pre-emergence herbicide	Y	NS	N/A	Not used
Fertiliser before drilling	Y	NS	N/A	Not used
Average temperature in March before crop sown	Y	NS	N/A	Not used
Average temperature in June before crop sown	Y	NS	N/A	Not used
Sum of day degrees >3.2°C in March before crop sown	Y	NS	N/A	Not used
Sum of day degrees >3.2°C in June before crop sown	Y	NS	N/A	Not used
Sum of day degrees >3.2°C in July (year of sowing)	Y	NS	N/A	Not used
Total rainfall in February before crop sown	Y	NS	N/A	Not used
Total rainfall in April before crop sown	Y	NS	N/A	Not used
Total rainfall in June before crop sown	Y	NS	N/A	Not used
Sow date (<18 August, then weekly, >7 September)	NS	NS	N/A	Not used
OSR rotation	NS	NS	N/A	Not used
Soil type	NS	NS	N/A	Not used
Variety	NS	NS	N/A	Not used
Variety type	NS	NS	N/A	Not used
Establishment technique	NS	NS	N/A	Not used



Table 4. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Row width	NS	NS	N/A	Not used
Seeds per m <sup>2</sup>	NS	NS	N/A	Not used
Seedbed moisture	NS	NS	N/A	Not used
Fertiliser after drilling	NS	NS	N/A	Not used
Fertiliser at drilling	NS	NS	N/A	Not used
Fertiliser application method at drilling	NS	NS	N/A	Not used
Slug pressure	NS	NS	N/A	Not used
Straw management	NS	NS	N/A	Not used
Seed source	NS	NS	N/A	Not used
Seedbed cloddiness	NS	NS	N/A	Not used
Emergence date 1 (<18 August, then weekly or >14 September)	NS	NS	N/A	Not used
Emergence date 2 (< 1 September or ≥ 1 September)	NS	NS	N/A	Not used
Distance from nearest, previous OSR 1 (≤50, 51-500, 501-1000 or >1000 m)	NS	NS	N/A	Not used
Distance from nearest, previous OSR 2 (≤50 or >50 m)	NS	NS	N/A	Not used
Direction from nearest, previous OSR	NS	NS	N/A	Not used
Adult CSFB pressure in nearest WOSR in previous year	NS	NS	N/A	Not used
Previous crop	NS	NS	N/A	Not used
Average temperature in December (year prior to sowing)	NS	NS	N/A	Not used
Average temperature in January before crop sown	NS	NS	N/A	Not used
Average temperature in previous December to February	NS	NS	N/A	Not used
Average temperature in March to May before crop sown	NS	NS	N/A	Not used
Average temperature in April before crop sown	NS	NS	N/A	Not used
Average temperature in May before crop sown	NS	NS	N/A	Not used

Table 4. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Average temperature in June to August (year of sowing)	NS	NS	N/A	Not used
Average temperature in September (year of sowing)	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in January before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in March to May before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in April before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in June to August (year of sowing)	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in September	NS	NS	N/A	Not used
Total rainfall in June to August (year of sowing)	NS	NS	N/A	Not used
Total rainfall in September	NS	NS	N/A	Not used
Total rainfall in previous December to February	NS	NS	N/A	Not used
Total rainfall in January before crop sown	NS	NS	N/A	Not used
Total rainfall in March before crop sown	NS	NS	N/A	Not used
Total rainfall in March to May before crop sown	NS	NS	N/A	Not used
Total rainfall in May before crop sown	NS	NS	N/A	Not used

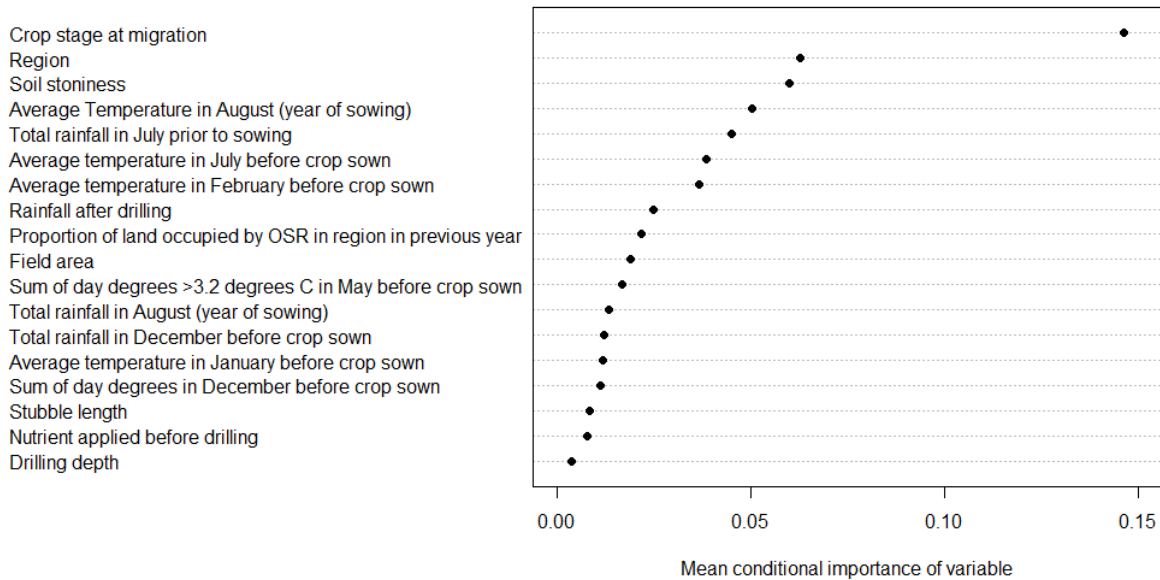


Figure 45. Conditional variable importance of explanatory variables selected through permutation test, logit-transformed % leaf area lost to CSFB adults. The position of the dot relative to the x axis indicates the importance of the variable, with values further from zero being more important.

**Larval CSFB populations in the autumn**

Explanatory variables selected by the random forest analysis and identified as significant in the permutation tests are given in Table 3 for both the untransformed and transformed response variable. The conditional variable importance and threshold values are also given in Table 5 for those explanatory variables selected for stepwise regression analyses. The explanatory variable with the greatest conditional importance was ‘Sow date 2’, followed by ‘Region’ and the average temperature in the winter prior to sowing (Figure 46). The pseudo R-squared value for the conditional random forest analysis (based on the variables selected for stepwise regression) was 39.9%.

Table 5. Results of Random Forests analysis and permutation tests for the CSFB autumn larvae dataset. ORV = original response variable (larvae per plant). TRV = transformed (log) response variable.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/Threshold Value
	ORV	TRV		
Sow date 2 (<16 August, then fortnightly, >15 September)	NS	Y	0.00931	August / September
Region	Y	Y	0.00107	East / Other
Average temperature in previous December to February before crop sown	NS	Y	0.00102	> 4.5°C
Sum of day degrees >3.2°C in October	NS	Y	0.00082	> 260
Total rainfall in August	NS	Y	0.00073	> 44 mm
Total rainfall in April before crop sown	NS	Y	0.00071	> 13 mm
Total rainfall in July before crop sown	Y	Y	0.00067	> 45 mm
HEAR variety	NS	Y	0.00028	Yes / No & Unknown
Pyrethroid resistance reported in area by assessment date	Y	Y	0.00025	Yes / No
Total rainfall in October	NS	Y	0.00023	> 73 mm
Total rainfall in previous December (year prior to sowing)	NS	Y	0.00023	> 50 mm
Proportion of land occupied by OSR in region in previous year	Y	Y	0.00017	> 0.06
Total rainfall in March before crop sown	Y	Y	0.00017	> 30 mm
Sum of day degrees >3.2°C in November	Y	Y	0.00014	> 125
Average temperature in September	NS	Y	0.00007	> 15°C
Year of sowing prior to 2014	Y	Y	< 0.00001	Yes (i.e. 2013 or earlier) / No
Sum of day degrees >3.2°C in August	Y	Y	< 0.00001	> 410
Total rainfall in May before crop sown	Y	Y	< 0.00001	> 70 mm
Total rainfall in June before crop sown	Y	Y	< 0.00001	> 30 mm
Average temperature in July before crop sown	Y	Y	< 0.00001	> 16°C

Table 5. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/Threshold Value
	ORV	TRV		
Average temperature in previous December (year prior to sowing)	Y	Y	< C.I. winter average temperature	Not used
Average temperature in August	Y	Y	< C.I. sum of day degrees >3.2°C in August	Not used
Average temperature in September to November	Y	Y	< C.I. temperature variables for individual months	Not used
Average temperature in November	Y	Y	< C.I. sum of day degrees >3.2°C in November	Not used
Sum of day degrees >3.2°C in July before crop sown	Y	Y	< C.I. average temperature in July	Not used
Sum of day degrees >3.2°C in September	Y	Y	< C.I. average temperature in September	Not used
Sow date 1 (<11 August, then weekly, > September)	NS	Y	< C.I. Sow date 2	Not used
Average temperature in previous January before crop sown	NS	Y	< C.I. winter average temperature	Not used
Average temperature in previous February before crop sown	NS	Y	< C.I. winter average temperature	Not used
Average temperature in June to August before crop sown	NS	Y	< C.I. temperature variables for individual summer months	Not used
Average temperature in October	NS	Y	< C.I. sum of day degrees >3.2°C in October	Not used
Total rainfall in March to May before crop sown	NS	Y	< C.I. rainfall variables for individual months	Not used
Sum of day degrees >3.2°C in previous December (year prior to sowing)	Y	NS	N/A	Not used
Sum of day degrees >3.2°C in previous January before crop sown	Y	NS	N/A	Not used
Sum of day degrees >3.2°C in previous February before crop sown	Y	NS	N/A	Not used

Table 5. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/Threshold Value
	ORV	TRV		
Sum of day degrees >3.2°C in September to November	Y	NS	N/A	Not used
Variety	NS	NS	N/A	Not used
Variety type	NS	NS	N/A	Not used
Field area	NS	NS	N/A	Not used
Previous crop	NS	NS	N/A	Not used
Average temperature in March to May before crop sown	NS	NS	N/A	Not used
Average temperature in March before crop sown	NS	NS	N/A	Not used
Average temperature in April before crop sown	NS	NS	N/A	Not used
Average temperature in May before crop sown	NS	NS	N/A	Not used
Average temperature in June before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in previous December to February before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in March to May before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in March before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in April before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in May before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in June to Aug before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in June before crop sown	NS	NS	N/A	Not used

Table 5. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/Threshold Value
	ORV	TRV		
Total rainfall in previous December to February before crop sown	NS	NS	N/A	Not used
Total rainfall in previous January before crop sown	NS	NS	N/A	Not used
Total rainfall in previous February before crop sown	NS	NS	N/A	Not used
Total rainfall in June to August before crop sown	NS	NS	N/A	Not used
Total rainfall in September to November	NS	NS	N/A	Not used
Total rainfall in September	NS	NS	N/A	Not used
Total rainfall in November	NS	NS	N/A	Not used

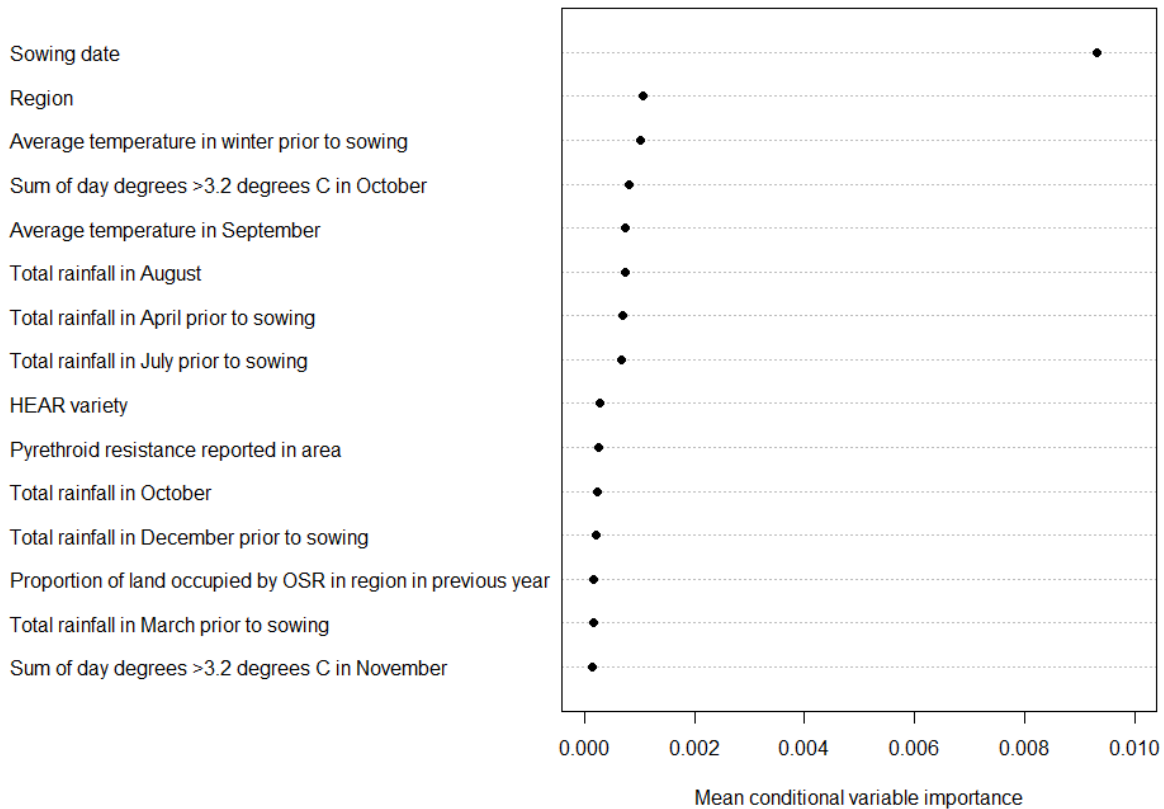


Figure 46. Conditional variable importance of explanatory variables selected through permutation test, log-transformed mean CSFB larval count per plant in autumn. The position of the dot relative to the x axis indicates the importance of the variable, with values further from zero being more important.

### **Larval CSFB populations in the spring**

Explanatory variables selected by the random forest analysis and identified as significant in the permutation tests are given in Table 4 for both the untransformed and transformed response variable. The conditional variable importance and threshold values are also given in Table 6 for those explanatory variables selected for stepwise regression analyses. The explanatory variable with the greatest conditional importance was 'Region', followed by 'Sow date' and the total rainfall in August (Figure 47). The pseudo R-squared value for the conditional random forest analysis (based on the variables selected for stepwise regression) was 38.7%.



Table 6. Results of Random Forests analysis and permutation tests for CSFB spring larvae dataset. ORV = original response variable (larvae per plant). TRV = transformed (log) response variable.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Region	NS	Y	0.02497	Yorkshire and The Humber / North East, North West and West Midlands / East, East Midlands, South East and South West
Sow date (<18 August, then weekly, >7 September)	NS	Y	0.01508	August / September
Total rainfall in October	NS	Y	0.00481	>74mm
Sum of day degrees >3.2°C in March before crop sown	NS	Y	0.00365	>100
Total rainfall in March to May before crop sown	NS	Y	0.00317	>148mm
Pyrethroid resistance reported in area by assessment date	Y	Y	0.00270	Yes / No
Sum of day degrees >3.2°C in November	Y	Y	0.00269	>152
Year of sowing prior to 2014	Y	Y	0.00269	Yes (i.e. 2013 or earlier) / No
Sum of day degrees >3.2°C in September	Y	Y	0.00263	>309
Average temperature in July before crop sown	NS	Y	0.00241	>17.7°C
Average temperature in October	NS	Y	0.00241	>10.9°C
Average temperature in January before crop sown	NS	Y	0.00226	>4.8°C
Proportion of land occupied by OSR in region in previous year	NS	Y	0.00224	>0.07
Total rainfall in September	NS	Y	0.00198	>70mm
Sum of day degrees >3.2°C in December	Y	Y	0.00183	>129
Average temperature in January	NS	Y	0.00134	>3.4°C
Sum of day degrees >3.2°C in August	NS	Y	0.00126	>400
Sum of day degrees >3.2°C in February	NS	Y	0.00114	>72
Total rainfall in January	NS	Y	0.00098	>73mm
Sum of day degrees >3.2°C in previous February before crop sown	NS	Y	0.00084	>43
Average temperature in previous December before crop sown	NS	Y	<0.00001	>4.7°C
Total rainfall in June to August before crop sown	NS	Y	<0.00001	>160mm

Table 6. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Average temperature in March before crop sown	NS	Y	< C.I. sum of day degrees >3.2°C in March	Not used
Sum of day degrees >3.2°C in July before crop sown	NS	Y	< C.I. average temperature in July	Not used
Average temperature in August	NS	Y	< C.I. sum of day degrees >3.2°C in August	Not used
Average temperature in September to November	NS	Y	< C.I. temperature variables for individual months	Not used
Sum of day degrees >3.2°C in September to November	NS	Y	< C.I. temperature variables for individual months	Not used
Average temperature in September	NS	Y	< C.I. sum of day degrees >3.2°C in September	Not used
Sum of day degrees >3.2°C in October	NS	Y	< C.I. average temperature in October	Not used
Average temperature in November	NS	Y	< C.I. sum of day degrees >3.2°C in November	Not used
Average temperature in December to February	Y	Y	< C.I. temperature variables for individual months	Not used
Sum of day degrees >3.2°C in December to February	Y	Y	< C.I. temperature variables for individual months	Not used
Average temperature in December	Y	Y	< C.I. sum of day degrees >3.2°C in December	Not used
Sum of day degrees >3.2°C in January	NS	Y	< C.I. average temperature in January	Not used
Total rainfall in March before crop sown	NS	Y	< C.I. spring average rainfall	Not used
Total rainfall in April before crop sown	NS	Y	< C.I. spring average rainfall	Not used
Total rainfall in June before crop sown	NS	Y	< C.I. summer average rainfall	Not used
Variety	NS	NS	N/A	Not used
Variety type	NS	NS	N/A	Not used
HEAR variety	NS	NS	N/A	Not used
Field area	NS	NS	N/A	Not used
Previous crop	NS	NS	N/A	Not used

Table 6. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Average temperature in previous December to February before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in previous December to February before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in previous December before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in previous January before crop sown	NS	NS	N/A	Not used
Average temperature in previous February before crop sown	NS	NS	N/A	Not used
Average temperature in March to May before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in March to May before crop sown	NS	NS	N/A	Not used
Average temperature in April before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in April before crop sown	NS	NS	N/A	Not used
Average temperature in May before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in May before crop sown	NS	NS	N/A	Not used
Average temperature in June to August before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in June to August before crop sown	NS	NS	N/A	Not used
Average temperature in June before crop sown	NS	NS	N/A	Not used
Sum of day degrees >3.2°C in June before crop sown	NS	NS	N/A	Not used
Average temperature in February	NS	NS	N/A	Not used
Total rainfall in previous December to February before crop sown	NS	NS	N/A	Not used
Total rainfall in previous December before crop sown	NS	NS	N/A	Not used
Total rainfall in previous January before crop sown	NS	NS	N/A	Not used
Total rainfall in previous February before crop sown	NS	NS	N/A	Not used
Total rainfall in May before crop sown	NS	NS		Not used

Table 6. Continued.

Explanatory Variable	Significant result in permutation test (Yes / Not Significant)		Conditional importance (selected variables only)	Grouping/ Threshold Value
	ORV	TRV		
Total rainfall in July before crop sown	NS	NS	N/A	Not used
Total rainfall in August	NS	NS	N/A	Not used
Total rainfall in September to November	NS	NS	N/A	Not used
Total rainfall in November	NS	NS	N/A	Not used
Total rainfall in December to February	NS	NS	N/A	Not used
Total rainfall in December	NS	NS	N/A	Not used
Total rainfall in February	NS	NS	N/A	Not used

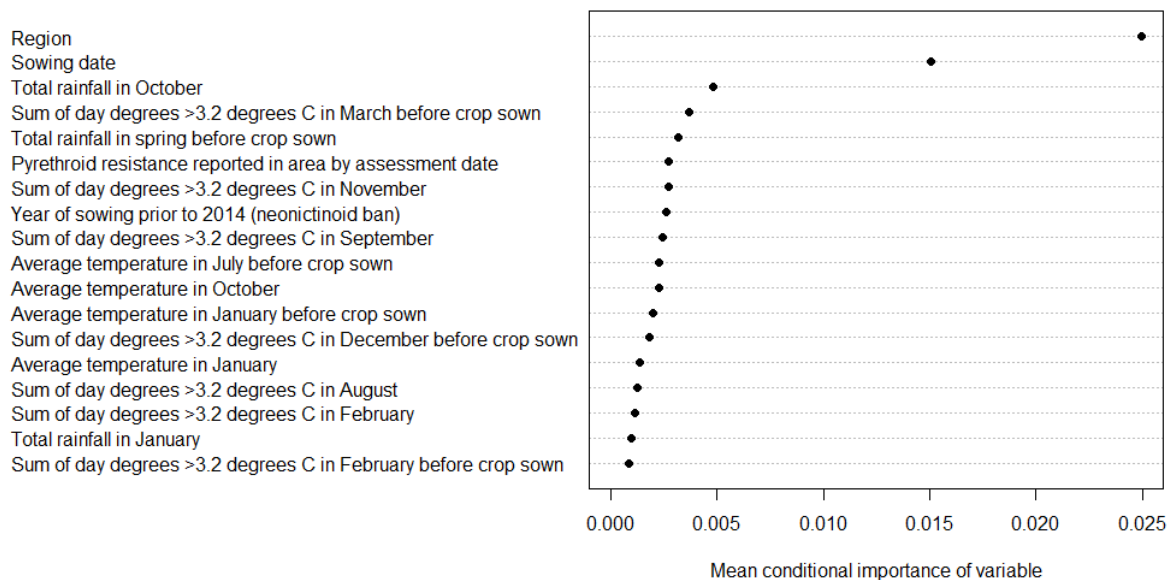


Figure 47. Conditional variable importance of explanatory variables selected through permutation test, log-transformed mean CSFB larval count per plant in spring. The position of the dot relative to the x axis indicates the importance of the variable, with values further from zero being more important.

#### 4.3.2. Stepwise regression and general linear regression

##### **Adult CSFB damage (% leaf area lost)**

The results of the stepwise regression analysis are given in Table 7. This analysis selected eight explanatory variables for inclusion in the general linear regression analysis. The regression model produced by the general linear regression of these variables accounted for 33.3% of the variance (Table 8). Coefficients for non-reference levels of specific factors are added to the constant of 10.01 to calculate the predicted value for a particular field. For example, in a field where short stubble was left, the crop was emerging at the point of CSFB migration, there was lots of rain after sowing, the average temperature in July was 16°C (so  $\leq 17.2^\circ\text{C}$ ), the total rainfall in December (in the year prior to sowing) was 50 mm (so  $\leq 71$  mm), the sum of day degrees  $>3.2^\circ\text{C}$  in December (in the year prior to sowing) was 120 (so  $\leq 187$ ), the total rainfall in August was 50 mm (so  $>38$  mm) and the sum of day degrees  $>3.2^\circ\text{C}$  in May was 250, the predicted leaf area lost, back-transformed from the logit, is calculated as  $\frac{100 \times \exp(10.01 + 2.034 - 2.021 - 0.04020 \times 250)}{1 + \exp(10.01 + 2.034 - 2.021 - 0.04020 \times 250)}$ , which is equal to a prediction of 49.3% leaf area lost. By changing the above to a crop that was beyond the cotyledon stage at the point of CSFB migration but keeping all other variables the same, the predicted value drops to 11.3% leaf area lost.

Table 7. *t*-test and stepwise regression results of variables selected through permutation test for logit-transformed % leaf area lost to CSFB adults. ‘-’ indicates that no significant association either way was found.

Explanatory Variable	Grouping/ Threshold Value	n of group in bold (total n=299)	P value, individual factor t-test	P value, individual linear regression (continuous variable only)	Selected by stepwise regression Yes/No	Group in bold associated with Higher or Lower adult damage?
Crop stage at migration	<b>At or before emergence</b> / Other (Missing, Cotyledon, After cotyledon)	20	0.009	N/A	Yes	H
Region	<b>East</b> / Other	210	<0.001	N/A	No	H
Soil stoniness	<b>Few stones</b> / Other (Missing, No stones, Stony).	118	NS, >0.1	N/A	No	-
Average temperature in August (year of sowing)	<b>&gt;16.9°C</b>	144	<0.001	<0.001	No	H
Total rainfall in July before crop sown	<b>&gt;29mm</b>	249	<0.001	NS, >0.1	No	L
Average temperature in July before crop sown	<b>&gt;17.2°C</b>	170	0.001	NS, >0.1	Yes	H
Average temperature in February before crop sown	<b>&gt;4.6°C</b>	210	<0.001	<0.001	No	L
Rain post sowing	<b>Dry, Some rain</b> / Other (Lots of rain, Missing)	171	0.008	N/A	Yes	H
Proportion of land occupied by OSR in region in previous year	<b>&gt;0.06</b>	155	<0.001	<0.001	No	H
Field area	<b>20-30 ha</b> , Other (Missing, <5ha, 5-20ha, >30ha).	53	<0.001	N/A	No	L
Sum of day degrees >3.2°C in May before crop sown	<b>&gt;275</b>	174	NS, >0.1	0.003	Yes	L
Total rainfall in August (year of sowing)	<b>&gt;38mm</b>	241	<0.001	<0.001	Yes	L
Total rainfall in December (year prior to sowing)	<b>&gt;71mm</b>	45	<0.001	0.019	Yes	L
Average temperature in January before crop sown	<b>&gt;5.2°C</b>	74	NS, 0.07	NS, >0.1	No	-
Sum of day degrees >3.2°C in December before crop sown	<b>&gt;187</b>	78	0.003	NS, >0.1	Yes	Not consistent: Higher in t-test, lower in stepwise regression.

Table 7. Continued.

Explanatory Variable	Grouping/ Threshold Value	n of group in bold (total n=299)	P value, individual factor t-test	P value, individual linear regression (numerical variables only)	Selected by stepwise regression Yes/No	Group in bold associated with Higher or Lower adult damage?
Stubble length	<b>Not left</b> / <i>Short (&lt;15 cm)</i> / Tall (>15 cm), Missing	38 / 135	GLM Not left: <0.001 Short: 0.004	N/A	Yes (2-level factor, 'Not left' vs 'Other')	L ('Not left' is lower than 'Short', which is lower than Tall/Missing)
Nutrient before drilling	<b>P or Unknown</b> / Other (P and N, N, No Fertiliser)	38	<0.001	N/A	No	H
Drilling depth	<b>Less than 2cm</b> / Other (Greater than 2cm, Missing)	110	<0.001	N/A	No	L

*Table 8. General linear regression of variables selected through permutation test and stepwise regression, logit-transformed % leaf area lost to CSFB adults. Values in bold indicate non-reference levels for specific factors. These reference levels indicate increased damage where the coefficient estimate is positive and reduced damage where the coefficient estimate is negative.*

<b>Parameter</b>	<b>Grouping/ Threshold Value</b>	<b>Coefficient Estimate, s.e.</b>	<b>% total variance explained by factor</b>
Constant	N/A	10.01, 1.82	-
Crop stage at migration	<b>At or before emergence</b> / Other (Missing, Cotyledon, After cotyledon)	2.034, 0.476	10.0
Sum of day degrees >3.2°C in May before crop sown	<b>Continuous variable</b>	-0.0402, 0.006	7.0
Average temperature in July before crop sown	<b>&gt;17.2°C</b>	1.712, 0.293	5.5
Total rainfall in December (year prior to sowing)	<b>&gt;71mm</b>	-1.586, 0.375	4.2
Sum of day degrees >3.2°C in December (year prior to sowing)	<b>&gt;187</b>	-1.815, 0.483	3.0
Rain post sowing	<b>Dry, Some rain</b> / Other (Lots of rain, Missing)	1.023, 0.255	2.5
Total rainfall in August (year of sowing)	<b>&gt;38mm</b>	-2.021, 0.498	1.8
Stubble length	<b>Not left</b> / Other (Short (<15 cm), Tall (>15 cm), Missing)	-0.777, 0.362	1.0

### ***Larval CSFB populations in the autumn***

The results of the stepwise regression analysis are given in Table 9. This analysis selected nine explanatory variables for inclusion in the general linear regression analysis. The regression model produced by the general linear regression of these variables accounted for 33.4% of the variance (Table 10). To calculate the predicted value for a particular field, coefficients for non-reference levels of specific factors are added to the constant of (0.968-0.01), where 0.01 is the offset used in calculating the log-transformed variable. As an example, the predicted mean CSFB larval numbers per plant in autumn in a non-HEAR variety crop sown in August after 2013, in an area where



pyrethroid resistance has been reported, with 51 mm of rain in June in the year of sowing (so  $\geq 30$ mm), where the average temperature in the previous December to February prior to sowing was  $4^{\circ}\text{C}$  (so  $< 4.5^{\circ}\text{C}$ ), where the sum of day degrees  $>3.2^{\circ}\text{C}$  in August was 300, the sum of day degrees  $>3.2^{\circ}\text{C}$  in October was 285 and the total rainfall in October was 30 mm, is calculated as  $\exp(0.968 - 0.01 + 1.193 - 0.388 - 0.00552 \times 300 + 0.00594 \times 285 - 0.00558 \times 30)$ , which is equal to 5.1 larvae per plant. By changing the above to a September sown crop but keeping all other variables the same, the predicted value drops to 3 larvae per plant.

Table 9. *t*-test and stepwise regression results of variables selected through permutation test for log-transformed mean CSFB larval count per plant in autumn.

Explanatory Variable	Grouping/ Threshold Value	n of group in bold (total n=1154)	P value, individual factor t- test	P value, individual linear regression (continuous variable only)	Selected by stepwise regression Yes/No	Group in bold associated with Higher or Lower larval count?
Sow date	August / <b>September</b>	731	<0.001	N/A	Yes	L
Region	<b>East</b> / Other	476	< 0.001	N/A	No	H
Average temperature in previous December to February before crop sown	<b>&gt; 4.5°C</b>	631	< 0.001	NS, >0.1	Yes	L
Sum of day degrees >3.2°C in October	<b>&gt; 260</b>	496	< 0.001	< 0.001	Yes	H
Total rainfall in August	<b>&gt; 44 mm</b>	853	< 0.001	NS, >0.1	No	H
Total rainfall in April before crop sown	<b>&gt; 13 mm</b>	930	< 0.001	< 0.001	No	L
Total rainfall in July before crop sown	<b>&gt; 45 mm</b>	814	< 0.001	< 0.001	No	L
HEAR variety	<b>Yes</b> / No & Unknown	37	0.003	N/A	Yes	L
Pyrethroid resistance reported in area by assessment date	<b>Yes</b> / No	160	< 0.001	N/A	Yes	H
Total rainfall in October	<b>&gt; 73 mm</b>	455	0.016	< 0.001	Yes	L
Total rainfall in previous December (year prior to sowing)	<b>&gt; 50 mm</b>	693	< 0.001	< 0.001	No	L
Proportion of land occupied by OSR in region in previous year	<b>&gt; 0.06</b>	400	0.003	< 0.001	No	H
Total rainfall in March before crop sown	<b>&gt; 30 mm</b>	705	< 0.001	< 0.001	No	L
Sum of day degrees >3.2°C in November	<b>&gt; 125</b>	625	< 0.001	< 0.001	No	H
Average temperature in September	<b>&gt; 15°C</b>	365	0.003	NS, >0.1	No	H
Year of sowing prior to 2014	<b>Yes</b> (i.e. 2013 or earlier) / No	941	< 0.001	N/A	Yes	L
Sum of day degrees >3.2°C in August	<b>&gt; 410</b>	609	< 0.001	< 0.001	Yes	L
Total rainfall in May before crop sown	<b>&gt; 70 mm</b>	323	< 0.001	< 0.001	No	H
Total rainfall in June before crop sown	<b>&gt; 30 mm</b>	860	< 0.001	< 0.001	Yes	L
Average temperature in July before crop sown	<b>&gt; 16°C</b>	853	0.001	< 0.001	No	H

*Table 10. General linear regression of variables selected through permutation test and stepwise regression, log-transformed mean CSFB larval count per plant in autumn. Values in bold indicate non-reference levels for specific factors. These reference levels indicate increased larval counts where the coefficient estimate is positive and decreased larval counts where the coefficient estimate is negative.*

<b>Parameter</b>	<b>Grouping/ Threshold Value</b>	<b>Coefficient Estimate, s.e.</b>	<b>% total variance explained by factor</b>
Constant	N/A	0.968, 0.906	-
Whether the crop was sown prior to 2014	Yes (i.e. 2013 or earlier) / <b>No</b>	-1.917, 0.230	23.3
Average temperature in previous December to February before crop sown	<b>&gt; 4.5°C</b>	-0.674, 0.130	4.2
Pyrethroid resistance reported in area by assessment date	<b>Yes / No</b>	1.193, 0.253	2.0
Sum of day degrees >3.2°C in October	<b>Continuous variable</b>	0.00594, 0.001	1.5
Sow date	August / <b>September</b>	-0.528, 0.126	1.1
HEAR variety	<b>Yes / No &amp; Unknown</b>	-1.135, 0.335	0.6
Total rainfall in October	<b>Continuous variable</b>	-0.00558, 0.002	0.4
Sum of day degrees >3.2°C in August	<b>Continuous variable</b>	-0.00552, 0.002	0.4
Total rainfall in June before crop sown	<b>&gt; 30 mm</b>	-0.388, 0.161	0.3

### ***Larval CSFB populations in the spring***

The results of the stepwise regression analysis are given in Table 11. This analysis selected nine explanatory variables for inclusion in the general linear regression analysis. The regression model produced by the general linear regression of these variables accounted for 36.6% of the variance (Table 12). To calculate the predicted value for a particular crop, coefficients for non-reference levels of specific factors are added to the constant of (-1.994-0.01), where 0.01 is the offset used in calculating the log-transformed variable. As an example, the predicted mean CSFB larval number per plant in spring in a crop sown in the Yorkshire and Humber region in August after 2013, in an area where pyrethroid resistance had been reported, where the percentage of land occupied by OSR in the region in the previous year was 5% (so  $\leq 7\%$ , or 0.07), where the total rainfall in the summer before the crop sown was 155 mm (so  $\leq 160\text{mm}$ ), the sum of day degrees  $>3.2^\circ\text{C}$  in December was 130 (so  $>129$ ), the average temperature in January was  $4^\circ\text{C}$  (so  $>3.4^\circ\text{C}$ ) and the total rainfall in January was 66 mm, is calculated as  $\exp(-1.994 - 0.01 + 0.824 + 1.507 + 0.914 +$

$1.066 + 0.00533 \times 66$ ), which is equal to 14.3 larvae per plant. By changing the above to a September sown crop but keeping all other variables the same, the predicted value drops to 9.5 larvae per plant.

Table 11. *t*-test and stepwise regression results of variables selected through permutation test for log-transformed mean CSFB larval count per plant in spring. ‘-’ indicates that no significant association either way was found.

Explanatory Variable	Grouping/ Threshold Value	n of group in bold (total n=585)	p value, individual factor t-test	p value, individual linear regression (continuous variable only)	Selected by stepwise regression Yes/No	Group in bold associated with Higher or Lower (log-transformed) larval count?
Region	<b>Yorkshire and The Humber / <i>North East, North West and West Midlands</i> / East, East Midlands, South East and South West</b>	95 / 89	GLM Yorkshire <0.001 N East, N West and W Midlands: 0.001	N/A	Yes (2-factor variable only, Yorkshire/ Other)	<b>Yorkshire: Higher</b> East, E Midlands and South: Intermediate <i>N East, N West and W Midlands: Lower</i>
Sow date	August / <b>September</b>	213	<0.001	N/A	Yes	L
Total rainfall in October	<b>&gt;74mm</b>	234	NS, >0.1	NS, >0.1	No	-
Sum of day degrees >3.2°C in March before crop sown	<b>&gt;100</b>	388	NS, >0.1	NS, >0.1	No	-
Total rainfall in March to May before crop sown	<b>&gt;148mm</b>	222	<0.001	0.001	No	L
Pyrethroid resistance reported in area by assessment date	<b>Yes / No</b>	83	<0.001	N/A	Yes	H
Sum of day degrees >3.2°C in November	<b>&gt;152</b>	191	<0.001	<0.001	No	H
Year of sowing prior to 2014	<b>Yes</b> (i.e. 2013 or earlier) / No	471	<0.001	N/A	Yes	L
Sum of day degrees >3.2°C in September	<b>&gt;309</b>	459	0.003	0.043	No	L
Average temperature in July before crop sown	<b>&gt;17.7°C</b>	174	<0.001	0.003	No	H
Average temperature in October	<b>&gt;10.9°C</b>	346	<0.001	<0.001	No	H
Average temperature in January before crop sown	<b>&gt;4.8°C</b>	265	<0.001	NS, >0.1	No	L
Proportion of land occupied by OSR in region in previous year	<b>&gt;0.07</b>	152	<0.001	<0.001	Yes	H

Table 11. Continued.

Explanatory Variable	Grouping/ Threshold Value	n of group in bold (total n=585)	p value, individual factor t-test	p value, individual linear regression (numerical variables only)	Selected by stepwise regression Yes/No	Group in bold associated with Higher or Lower (log-transformed) larval count?
Total rainfall in September	<b>&gt;70mm</b>	79	<0.001	0.004	No	L
Sum of day degrees >3.2°C in December	<b>&gt;129</b>	60	<0.001	<0.001	Yes	H
Average temperature in January	<b>&gt;3.4°C</b>	453	<0.001	<0.001	Yes	H
Sum of day degrees >3.2°C in August	<b>&gt;400</b>	395	NS, >0.1	NS, >0.1	No	
Sum of day degrees >3.2°C in February	<b>&gt;72</b>	207	NS, >0.1	NS, >0.1	No	-
Total rainfall in January	<b>&gt;73mm</b>	208	0.010	0.002	Yes	H
Sum of day degrees >3.2°C in previous February before crop sown	<b>&gt;43</b>	358	<0.001	0.010	No	L
Average temperature in previous December before crop sown	<b>&gt;4.7°C</b>	325	0.002	NS, >0.1	No	H
Total rainfall in June to August before crop sown	<b>&gt;160mm</b>	355	<0.001	<0.001	Yes	L

Table 12. General linear regression of variables selected through permutation test and stepwise regression, log-transformed mean CSFB larval count per plant in spring. These reference levels indicate increased larval counts where the coefficient estimate is positive and decreased larval counts where the coefficient estimate is negative.

Parameter	Grouping/ Threshold Value	Coefficient Estimate, s.e.	% total variance explained by factor
Constant	N/A	-1.994, 0.474	-
Pyrethroid resistance reported in area by assessment date	Yes / No	0.824, 0.380	17.8
Year of sowing prior to 2014	Yes (i.e. 2013 or earlier) / No	-1.552, 0.357	5.3
Region	Yorkshire and The Humber / Other	1.507, 0.250	4.1
Sowing date	August / September	-0.407, 0.185	3.7
Average temperature in January	>3.4°C	0.914, 0.223	3.2
Sum of day degrees >3.2°C in December	>129	1.066, 0.376	1.3
Total rainfall in June to August before crop sown	>160mm	-0.553, 0.185	1.0
Total rainfall in January	Continuous variable	0.00533, 0.002	0.6
Proportion of land occupied by OSR in region in previous year	>0.07	0.675, 0.231	0.5

#### 4.3.3. Validation

##### **Adult CSFB damage (% leaf area lost)**

There was a poor relationship between the observed and predicted values (Figure 48). T-test/regression analyses of individual explanatory variables found that only 'Crop stage at migration' was significantly associated with damage ( $t = -2.79$ ,  $df = 13.8$ ,  $P = 0.014$ ), with higher damage associated with crops in which CSFB migration started at or before crop emergence, which is in line with the general linear regression analysis. A number of observed values came from field trial plots, resulting in variable responses within the same field. These differences could not be accounted for by the general linear regression analysis and may be due trial treatments (e.g. seed rate and variety) or within field variation.

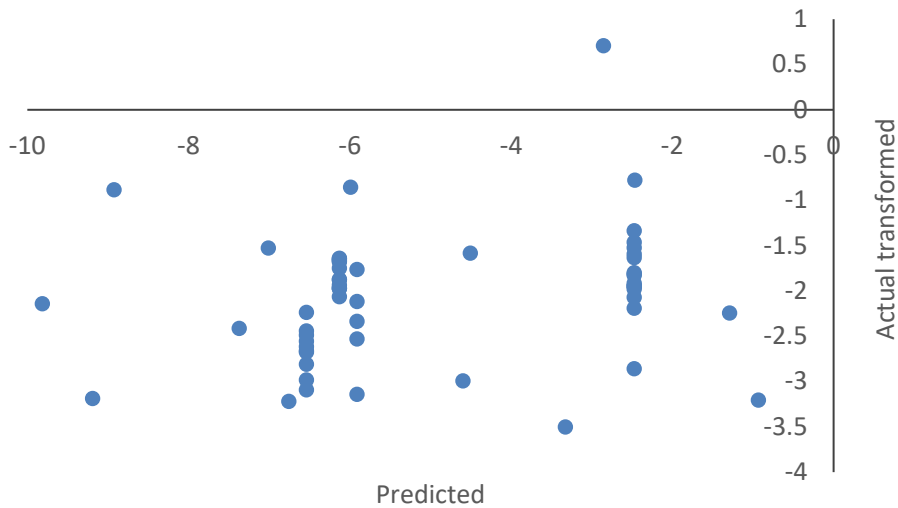


Figure 48. Plot of predicted and observed values for adult CSFB feeding damage. Observed values have been logit transformed.

**Larval CSFB populations in the autumn**

There was a poor relationship between the observed and predicted values (Figure 49). T-test/regression analyses of individual explanatory variables found that only ‘Total rainfall in June before crop sown’ ( $t = 3.16$ ,  $df = 73$ ,  $P = 0.002$ ) and ‘Total rainfall in October’ ( $F = 8.3$ ,  $df = 73$ ,  $P = 0.005$ ) were significantly associated with larval number. A total of >30 mm in June was associated with lower larval numbers, which is in line with the general linear regression analysis. Higher rainfall in October was associated with higher larval numbers, which is counter to the model finding. A number of observed values came from field trial plots, resulting in variable responses within the same field. These differences could not be accounted for by the general linear regression analysis and may be due trial treatments (e.g. seed rate and variety) or within field variation.

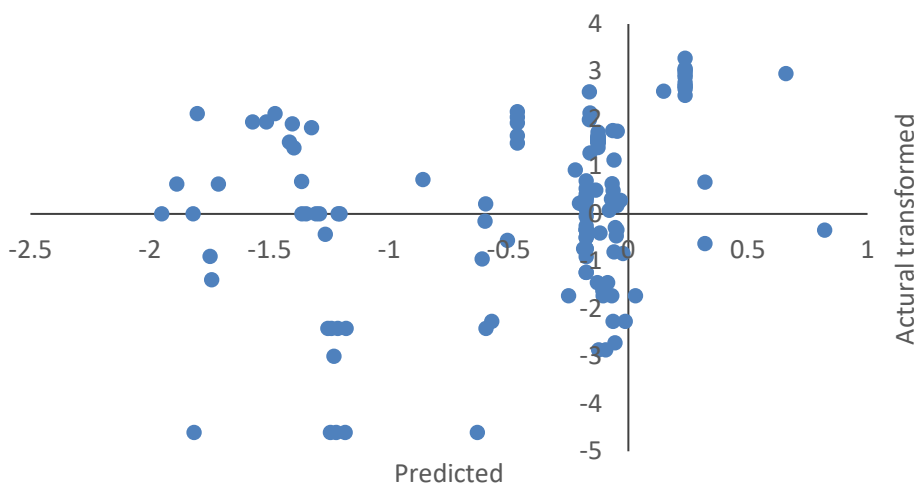
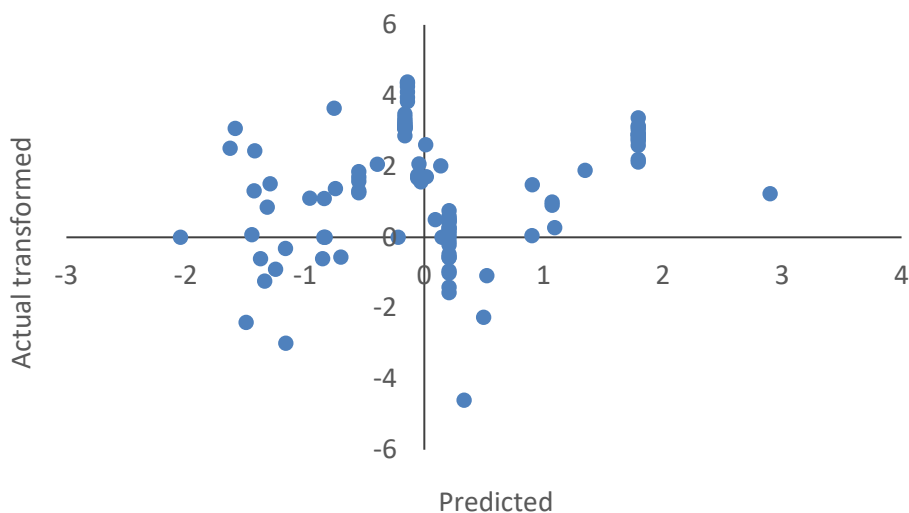


Figure 49. Plot of predicted and observed values for autumn larvae per plant. Observed values have been log transformed.



### **Larval CSFB populations in the spring**

There was a poor relationship between the observed and predicted values (Figure 50). T-test/regression analyses of individual explanatory variables found that only 'Proportion of land occupied by OSR in region in previous year' was significantly associated with larval number, with a higher proportion of OSR land associated with higher larval number, which is in line with the general linear regression analysis. 'Pyrethroid resistance reported in area by assessment date' and 'Sowing date' were significant at the  $P < 0.01$  level, with trends in line with the general linear regression analysis. A number of observed values came from field trial plots, resulting in variable responses within the same field. These differences could not be accounted for by the model and may be due trial treatments (e.g. seed rate and variety) or within field variation.



*Figure 50. Plot of predicted and observed values for spring larvae per plant. Observed values have been log transformed.*

## **4.4. Discussion**

A novel approach was used to identify agronomic and weather factors with potential power to explain and predict CSFB pressure in crops. Use of a random forest ensemble learning approach enabled screening of a large number of explanatory variables. The factors identified by this approach are good candidates for further practical work to confirm their effects. The percentage variance accounted for by the regression analyses and the validation suggest that the final models produced (the general linear regressions) have relatively poor predictive ability on average. However, the general linear regressions do identify variables that appear to be associated with the respective measures of CSFB pressure (e.g. crop stage at CSFB migration for adult feeding damage). The majority of explanatory variables identified were weather-related, which is to be expected given insects are ectothermic. The importance of temperature and rainfall in affecting insect populations and pest pressures have been identified for a range of pests, including for CSFB

(Alford, 1979; Mathiasen *et al.*, 2015a), bird cherry-oat aphid (Morgan, 2000) and slugs (Shirley *et al.*, 2001).

Relatively few explanatory variables identified in the analyses were factors that could be directly influenced by growers. This is helpful as many approaches have been reported anecdotally to reduce CSFB pressure in recent years, and this work identifies those most likely to be important so that further research can be most cost-effectively targeted. Further practical work should be done to confirm the effects of these factors on CSFB adult feeding damage and larval populations in the autumn and spring.

There are mechanistic rationales for why the explanatory variables identified in the analyses should be important in determining CSFB pressure. These are discussed below for each response variable. Explanatory variables are discussed in order of those that can be controlled by growers, regional effects and weather variables. While the general linear regression analyses produced predictive models using the explanatory variables selected by the stepwise regression analyses, it would be remiss to disregard the importance of other explanatory variables for which associations with CSFB pressure were identified at earlier stages of the statistical process (e.g. Random Forests and stepwise regression analyses). That they did not appear in the general linear regression analyses may simply be an artefact of the statistical approach and so it is likely that they also influence CSFB pressure to some degree. Some of these are also discussed below.

#### **4.4.1. Adult feeding damage**

*Crop stage at migration:* The analysis identified that highest damage was associated with crops that are at or before emergence when CSFB begin migrating into the crop. WOSR is recognised as being more vulnerable to damage in the early phase of establishment. For example, the treatment thresholds for adult CSFB is lower at the cotyledon to two-true leaf stage (25% leaf area lost) than at the three to four leaf stage (50% leaf area lost) (AHDB, 2016). This analysis suggests that the critical stage for adult feeding damage is when the crop is emerging, and this is supported by anecdotal reports from growers and advisors. Emergence date can be adjusted by changing sow date, although the timing of emergence is affected by other factors such as moisture, soil conditions and temperature (Blake *et al.*, 2004). The effect of sow date has been noticed for some time, with Williams & Garden (1961) commenting that crop sown before mid-July or after August tended to suffer less damage while sow dates most suitable for optimal overwintering (August) were most susceptible. Kaufman (1941 in Williams & Garden, 1961) also noted that early sown crops were less susceptible, late sown crops suffered least damage and intermediate sow dates suffered the most damage. Bonnemaïson (1965) also suggests that sowing early where CSFB migration is late will be beneficial, presumably because the crop is well established by the time the pest arrives.

*Stubble length:* The analysis found that lower damage was associated with crops grown in the absence of stubble from the previous crop (i.e. the stubble was cultivated or the previous crop was a crop that left no stubble following harvest), and higher where stubble was left. This is counter to anecdotal reports suggesting that leaving high stubble reduces CSFB damage. Damage from flea beetle has also been found to be lower in mustard grown in high stubble compared to no stubble (Thomas, 2018). This analysis indicates that stubble actually increases feeding damage, perhaps by slowing crop establishment or by increasing slug damage, which can be difficult to distinguish from CSFB damage and can be higher in the presence of chopped straw as it provides shelter. Alternatively, the model may reflect underlying bias within the dataset. For instance, a relatively high numbers of the sites at which no stubble was left were drilled before 11 August or after 14 September and there was a trend for lower CSFB damage during these periods (Figure 16).

*Rain post-sowing:* The analysis found that crops that experienced dry conditions or some rain after sowing tended to have higher damage than where more rain was recorded. It is recognised that dry conditions around sowing can result in uneven emergence and slow crop establishment (Blake *et al.*, 2004). Uneven emergence is likely to result in adult CSFB feeding being concentrated on the proportion of plants emerging at any one time, before moving onto later emerged plants in the field. Plants that are growing slowly are less able to compensate for adult feeding damage by growing more rapidly than the CSFB feed.

*Sum of day degrees >3.2°C in December in year prior to sowing:* The analysis found that lower levels of leaf damage were associated with situations in which the sum of day degrees above 3.2°C in the previous December was greater than 187. When compared with 1981-2010 averages (Met Office, 2020), such conditions are very warm for December. However, December conditions in recent years have been exceptionally warm (e.g. 2016, 2018 and 2019). While mild winter conditions are likely to encourage late egg-laying and -hatch (Alford, 1979; Mathiasen *et al.*, 2015a), it is possible that exceptionally warm conditions may be harmful for CSFB. For example, the activity of natural enemies may increase and so reduce the CSFB population migrating into crops the following autumn. It is also possible that very warm conditions may accelerate larval development so that adult emergence and migration does not coincide with WOSR sowing in the autumn.

*Total rainfall in December in year prior to sowing:* The analysis found that lower damage was associated with >71 mm of total rainfall in the December prior to sowing in comparison with where less was recorded. This is above average rainfall for much of the main WOSR-producing areas of the UK. High rainfall may reduce adult activity, limiting egg-laying, and wash away eggs and newly hatched larvae, increasing mortality.

*Sum of day degrees >3.2°C in May before crop sown:* The analysis identified that adult damage was highest following cool conditions in May. During this month most CSFB are in the soil as pupae, with adult emergence usually occurring in June. The relationship identified by the model may be due several reasons. Warm May conditions are likely to result in early emergence of adult CSFB. Following emergence, adult CSFB feed for a period before entering aestivation. As the duration of aestivation is thought to be genetically fixed, and not influenced by environmental factors (e.g. temperature) (Såringer, 1984), the early onset of aestivation may have a knock-on effect on the subsequent timing of key stages in the CSFB lifecycle. Both the end of aestivation and the start of adult CSFB migration would likely occur earlier, potentially meaning that migration is less synchronised with sowing of WOSR, resulting in less damage. Warm May conditions may also increase the activity of natural enemies of CSFB pupae and emerging adults, thereby increasing CSFB mortality and reducing pest pressure on WOSR in the autumn. Knowledge of the natural enemies of pupal and adult CSFB is limited but is likely to include entomopathogenic fungi, ground beetles and parasitoids. *Microctonus brassicae*, a parasitic wasp that targets adults, has recently been identified as a parasitoid of CSFB in the UK (see Section 9.1).

*Average temperature in July before crop sown:* The analysis found that feeding damage in WOSR was higher at an average July temperature  $\geq 17.2^{\circ}\text{C}$  than at temperatures below this threshold. This temperature is warmer than average for most of the country. In July CSFB adults tend to be found feeding in the crop from which they emerged. The relationship identified by the model may be due to a combination of factors. Warm conditions have been shown to increase the intensity of adult CSFB feeding during the period prior to aestivation and shorten the duration of this feeding (Ankersmit, 1964), meaning aestivation begins earlier. Warm conditions in July also accelerates senescence in WOSR, and the resulting lack of food may also encourage early onset of aestivation. As the period of aestivation is fixed (Såringer, 1984), the early onset of diapause is also likely to mean that it will finish earlier. This may mean that adult migration into crops coincides with more vulnerable stages of the plants. It is also possible that warm July conditions interact with parasitoid-prey relationships in complex ways, for example by reducing activity of parasitoids.

*Total rainfall in August prior to sowing:* The analysis found that damage was lower when there was more than 38 mm of total rainfall in August than when less was recorded. This is likely to be below average rainfall for much of the UK. Increased soil moisture due to rain would improve crop establishment, helping crops to grow away from CSFB feeding damage. Rainfall may also limit adult CSFB flight and feeding activity.

Other factors identified in the survey (Section 3.4.4) and at earlier stages in the statistical process as being associated with adult damage included August temperature, the proportion of land used

for OSR cultivation in the previous year and drill depth. Warmer than average temperatures in August (>16.9°C) were associated with higher damage and it is likely that such conditions would increase adult CSFB activity in general. The occurrence and magnitude of migration has also been linked with temperature (Ebbe-Nyman, 1952; Ballanger, 1984; Sivčev *et al.*, 2016) and so warmer conditions may increase the frequency and size of migration events. The impact of the proportion of land used for OSR cultivation in the previous year is discussed in Section 4.4.3 below. Seed drilled less than 2 cm deep was associated with less adult feeding damage (Figure 21, Table 7), suggesting that deep drilling does not minimise damage by reducing access to the germinating seed by the pest. In fact, deep drilling has been shown to reduce emergence (Blake *et al.*, 2004).

The absence of a relationship between damage of some explanatory variables is also notable. For example, there has been much discussion regarding the benefits of early sowing in reducing damage, with a resulting trend for crops to be sown increasingly early. This analysis suggests that it is not so much sow date as the coincidence of CSFB migration with crop emergence that is key. This highlights that crop emergence and CSFB activity timings are not fixed, instead being influenced by environmental conditions such as temperature and rainfall (Blake *et al.*, 2004). An improved understanding of these relationships would assist growers in selecting appropriate sow dates to minimise adult CSFB damage. That WOSR rotation frequency, proximity to previous WOSR or CSFB pressure in previous, nearby WOSR did not appear to be associated with adult damage likely reflects the distance CSFB are able to fly when migrating into crops. It has been reported that CSFB can fly two miles (Bonnemaison, 1965) and this work supports this in that crops 1 km away from the previous WOSR appear to suffer a similar risk to those adjacent to the previous WOSR. Distances greater than 1 km were not analysed in this work because only a relatively small proportion of the sites fell into this category and it was felt that siting WOSR more than 1 km from the previous WOSR would be impractical for many growers. Ultimately, this means that reducing WOSR rotation frequency on-farm is unlikely to be beneficial if nearby farms continue to grow the crop regularly in the rotation.

#### **4.4.2. Larval CSFB populations in the autumn**

*Sow date*: The analysis found that September sown crops had lower numbers of larvae per plant than August sown crops. This effect is supported by trial work in Germany in which the date of CSFB arrival in the crop was experimentally manipulated by caging plots for different periods. Plots in which adult CSFB were introduced at the start of September had significantly greater numbers of larvae in the autumn and spring than those in which adults were introduced at the end of September (Conrad *et al.*, 2018). This effect is likely due to several factors. Firstly, late September sown crops may emerge after the peak of adult CSFB migration has occurred. This is supported by Bonnemaison (1965), who suggested timing emergence to occur after migration flights are complete. Secondly, the pest is thought to gradually lose the ability to fly once it has arrived in a

crop (Bonnemaison, 1965). Therefore, if it migrates into WOSR in August it is likely to remain there, laying eggs. Thirdly, temperature has an important effect on reproduction in CSFB, with the pre-oviposition period (the time between sexual maturity and first egg-laying) decreasing with increasing temperature, and oviposition and egg development rates increasing with increasing temperature (Alford, 1979; Mathiasen *et al.*, 2015a). If CSFB migrate during August temperatures are likely to be warmer than in September so egg-laying and -hatch is likely to start earlier which is in turn likely to result in higher autumn larval numbers. The relationship between sow date and autumn larvae was clear from the raw data (see Figure 35 in Section 3.4.4), with highest populations in early August sown crops and lowest in late September sown crops. The relationship between sow date and CSFB larvae is worth confirming experimentally, especially the effect different September sowing dates have on CSFB larval numbers. Also the impact of sow date on crop tolerance to larval feeding should be considered as there is some suggestion that taller plants in the late autumn/early winter suffer less of yield impact than shorter plants (Williams & Garden, 1961).

*HEAR variety:* The analysis found that lower autumn larval pressures were associated with HEAR WOSR varieties than other varieties. HEAR varieties have higher levels of erucic acid and their oil is used in a range of industrial food applications. HEAR varieties are a niche market so widespread adoption would be limited. Rather this finding presents a potential trait to investigate in breeding programmes, either because high erucic acid levels affect CSFB or HEAR varieties contain differing glucosinolate levels to traditional varieties, although this is unlikely as they are thought to involve different metabolic pathways in the plant (see Sections 5.1, 5.4.1 and 7.1 for discussion of the impact of glucosinolates). Alternatively, the germplasm for HEAR varieties is fairly different to that for other varieties (V. Gegas, pers. comm.) and so this effect could be due to an unknown mechanism only present in this germplasm. It is possible that high erucic acid levels are detrimental to CSFB larvae, although it is worth noting that the presence of high levels of erucic acid in the seed does not necessarily mean that erucic levels in plant tissue would be high during the autumn. Unfortunately, as no HEAR varieties were investigated in the variety trials described in Section 5 the effect on CSFB larvae could not be confirmed experimentally. It is also possible that HEAR varieties are less attractive to adult CSFB but as none appeared in the CSFB survey (see Section 3) it was not possible to include this variable in the modelling analysis of adult CSFB damage. Alternatively, the finding may reflect underlying bias within the dataset. HEAR varieties made up only 3.2% of the dataset (37 sites), a reflection of how rarely they are cultivated. While they were proportionally well represented in high CSFB areas (e.g. the east and Yorkshire) in the dataset, they do not figure at all in data from 2016/17 and 2017/18. These were years with high larval pressures so the absence of HEAR varieties may have affected the analyses. On the other hand, HEAR varieties were proportionally well represented in 2014/15 and 2015/16, which were also years with high larval pressures. Experimental work is needed to confirm this finding.

*Year of sowing prior to 2014:* The analysis showed a clear trend for increasing autumn larval populations from 2014 onwards which was independent of other variables such as weather. This effect is likely due to two factors. Firstly, pyrethroid resistance in CSFB was detected for the first time in the UK in 2014 (Højland *et al.*, 2015) and has since spread to most areas of England (S. Foster, pers. comm.). This means that pyrethroids are largely ineffective in controlling CSFB. Secondly, neonicotinoid seed treatments provided protection from CSFB for approximately six weeks from sowing but restrictions preventing their use were introduced on 1 December 2013. This has likely contributed to a general increase in CSFB populations.

*Pyrethroid resistance status:* The analysis found that autumn larval populations tended to be highest in areas where pyrethroid resistance had been reported. Pyrethroid resistance confers high levels of tolerance to pyrethroids in CSFB (Højland *et al.*, 2015). As described in Section 3.4.2, this resistance has drastically reduced the efficacy of pyrethroid sprays against larvae. In areas where resistance is widespread, growers have no effective chemical control options and so high levels of damage would be expected.

*Average temperature in the previous December to February before crop sown:* The analysis found that lower autumn larval populations were associated with average temperatures in the previous winter of  $>4.5^{\circ}\text{C}$  than at temperatures below this threshold. Such conditions are warmer than an average for most of the UK (Met Office, 2020). It is possible that warm conditions in winter may increase the activity of natural enemies, such as ground beetles that feed on CSFB eggs (Warner *et al.*, 2003). This may reduce the number of CSFB emerging from crops in the summer and, in turn, CSFB pressure in crops the following autumn. Warm winter conditions may also accelerate larval development so that adult emergence and migration does not coincide with WOSR sowing in the autumn resulting in low larval populations in the autumn.

*Total rainfall in June before crop sown:* The analysis found that  $>30$  mm of total rainfall in June was associated with lower autumn larval populations than rainfall levels below this threshold. Such rainfall is typical for most of the UK and so is likely to occur in most years. In June, most CSFB adults are emerging from soil-borne pupae and it is possible that rainfall hinders this process. Increased moisture may also increase their susceptibility to pathogens such as entomopathogenic fungi. This factor explained the least amount of the variance in the final model and had a relatively low conditional variable importance score, so it is difficult to determine its true importance.

*Sum of day degrees  $>3.2^{\circ}\text{C}$  in August:* The analysis found that autumn larval populations decreased with increasing sum of day degrees  $>3.2^{\circ}\text{C}$  in August. Other work has shown that the total number of eggs laid per female CSFB (life-time fecundity) is greatest at  $16^{\circ}\text{C}$  and decreases

at lower and higher temperatures (Mathiasen *et al.*, 2015a). Survival time of adult CSFB is also lower at 20°C than 16°C (50% survival at 78 and 186 days respectively, Mathiasen *et al.*, 2015a). It is more likely that average UK August temperatures will be above than below 16°C and so this relationship will regularly describe the effect warm temperatures have on reducing life-time fecundity and adult survival of CSFB. Low numbers of eggs laid per adult and a shortened lifespan will likely result in lower larval infestation in the autumn. It is also possible that warm August conditions increase the activity of natural enemies which could reduce larval populations. This factor explained a small amount of the variance in the final model and had a relatively low conditional variable importance score, so it is difficult to determine its true importance.

*Sum of day degrees >3.2°C in October:* The analysis found that autumn larval populations increased with increasing sum of day degrees >3.2°C in October. In October average temperatures will be likely be below 16°C so the effect of this explanatory variable is less having an effect on life-time fecundity (as in August above) and more on oviposition rate, egg development time and egg mortality. The rate of CSFB oviposition and egg development increases with temperature (Alford, 1978; Mathiasen *et al.*, 2015a) as does egg survival rate (Mathiasen *et al.*, 2015a) and so it is likely that warm October conditions will result in high numbers of larvae hatching in the autumn and invading plants.

*Total rainfall in October:* The analysis identified that autumn larval populations decreased with increasing rainfall in October whereas the validation suggested the opposite. The validation results may be due to the very high rainfall experienced in October 2019 skewing the results as CSFB pressure in autumn 2019 was unusually high. The egg stage is considered to have relatively high levels of mortality compared to other developmental stages of the pest (Thioulouse, 1987) and can be eaten by beetles and infected by bacteria (Bonnemaison, 1965). It is possible that increased rainfall increases bacterial infections and high rainfall may wash away eggs, which are laid in the soil (Thioulouse, 1987).

Several other explanatory variables, primarily weather related, were identified at earlier stages in the statistical process as being associated with high autumn larval populations. Where these relate to temperature or rainfall effects at particular times of year, the explanations given above are likely also to apply. For instance, warm temperatures throughout autumn are likely to increase larval populations by increasing egg-laying and development.

#### **4.4.3. Larval CSFB populations in the spring**

*Sow date:* The analysis found that a lower number of larvae per plant in the spring was associated with September sown rather than August sown crops. The mechanistic rationale for this effect is described in Section 4.4.2 above. It is interesting to note that the impact of sow date persists until



the spring. As in the effect of autumn larval populations, this was supported by the work of Conrad *et al.* (2018).

*Region:* The analysis identified that higher spring larval populations were more likely in the Yorkshire and The Humber region than elsewhere. This area has traditionally been an area of high CSFB risk (likely due to the long history of WOSR cultivation) and it is possible that climatic conditions here are particularly conducive to late larval invasion. Conversely, this could be an artefact of the adjustment made to the raw data to account for insecticide use as Yorkshire and The Humber had a relatively high proportion of sites that had applied sprays, resulting in large adjustments in the raw data compared to other regions.

*Proportion of land occupied by OSR in region in previous year:* The analysis found that the highest larval populations were associated with crops grown in areas in which the proportion of land on which OSR was grown the previous year was greater than 7%. It is likely that this reflects the large populations of CSFB in areas with high OSR cultivation. It is notable that CSFB pressures have continued to increase in recent years despite a general trend for decreasing areas of OSR (Defra, 2019), suggesting that reductions in WOSR cultivation need to continue for several years for CSFB pressure to be reduced.

*Year of sowing prior to 2014:* The analysis found that spring larval populations were higher after rather than before 2013. See Section 4.4.2 above for the mechanistic rationale.

*Pyrethroid resistance status:* The analysis found that spring larval populations tended to be higher in areas where pyrethroid resistance had been reported compared to areas where there was no resistance. See Section 4.4.2 above for the mechanistic rationale.

*Total rainfall in June to August before crop sown:* The analysis identified that lower spring larval populations were associated with total rainfall the previous summer >160 mm in comparison with levels of rainfall below this threshold. This amount of rain is fairly average or below average for much of the UK. In the summer, CSFB are adults and will be aestivating for several weeks, before migrating into freshly sown WOSR. Rain may hinder flight, resulting in smaller crop infestations. It is also possible that high rainfall in the summer may increase infection of aestivating adults by entomopathogenic fungi, resulting in increased mortality. Both of these effects are likely to reduce larval infestations.

*Sum of day degrees >3.2°C in December:* The analysis found that higher autumn larval populations were associated with a sum of day degrees above 3.2°C in December of 129 or above. Such conditions are warm compared to the long-term UK average but are likely to have occurred

relatively frequently in recent winters. The analyses for adult CSFB damage and autumn larval populations suggest that warm conditions in the previous December are detrimental to CSFB populations later in the year, so reducing pressures in following crops. However, the model for spring larval populations suggests that such conditions increase larval pressure in the current crop. CSFB activity is traditionally thought to subside during the winter months, with egg-laying and larval invasion resuming in the spring (Bonnemaison, 1965). Egg-laying has been shown to be minimal below 4°C (Mathiasen *et al.*, 2015a) and egg development ceases at or below either 3.2°C (Alford, 1979) or 5.1°C (Mathiasen *et al.*, 2015a). The rate of egg-laying and development increases with temperature (Mathiasen *et al.*, 2015a). Egg mortality has also been shown to decrease with increasing temperature between 4°C and 12°C (Mathiasen *et al.*, 2015a). All these factors are likely to result in increased larval invasion in late winter and in the spring following warm winters especially, it seems, warm Decembers. Other work has recognised the same effect of recent warm winters on increasing spring larval populations (Collins, 2017). Overall, the analyses suggest that mild winter temperatures may increase pest pressures in the current crop but decrease pressures in the following season.

*Average temperature in January:* The analysis found that higher spring larval populations were associated with average temperatures in January >3.4°C. Such conditions are likely to be slightly above average for much of the UK. The rationale for this effect is described above for the influence of warm Decembers on spring larval populations. The value selected by the model is very close to the egg development threshold of 3.2°C identified by Alford (1979), suggesting that this threshold is likely most relevant for UK CSFB populations.

*Total rainfall in January:* The analysis found that spring larval populations were negatively correlated with increasing rainfall in January. High rainfall may reduce adult activity, limiting egg-laying, and wash away eggs and newly hatched larvae, increasing mortality.

Several other explanatory variables, primarily weather related, were identified at earlier stages in the statistical process as being associated with spring larval populations. For example, a sum of day degrees above 3.2°C in September of greater than 309 (which is average or above average for most of the UK) tended to result in lower spring larval populations than where temperatures were at or below this threshold. Each CSFB adult can lay a finite number of eggs (Mathiasen *et al.*, 2015a). If warm temperatures stimulate oviposition in the autumn there will be fewer eggs laid in the winter (Mathiasen *et al.*, 2015a). Where other variables relate to temperature or rainfall effects at particular times of year, the explanations given above are likely also to apply. For instance, warm temperatures throughout winter are likely to increase larval populations in general because they increase egg-laying and development.

#### 4.4.4. Model summary and improvements

This work identifies several factors associated with CSFB pressure. For adult feeding damage the most important factors (those that explained the greatest total variance) were the stage of the crop at the point CSFB migration, the sum of day degrees above 3.2°C in May and the average temperature in July (see Table 8 for % total variance explained of all selected factors). For autumn larval populations the most important factors were whether the crop was sown prior to 2014, average temperature in the previous winter and whether pyrethroid resistance is present in the area (see Table 10 for % total variance explained of all selected factors). For spring larval populations the most important factors were whether pyrethroid resistance is present in the area, whether the crop was sown prior to 2014 and region (see Table 12 for % total variance explained of all selected factors).

The only factor that had a consistent effect on reducing CSFB pressure in regards all three response variables was wet summers. Wetter conditions in August appeared to reduce adult CSFB damage, possibly due to better crop establishment and less CSFB flight activity, and wetter conditions in June and the summer as a whole reduced autumn and spring larval populations respectively, possibly due increased mortality of adults from disease. Warmer conditions in the autumn and winter generally increased larval pressures, with strong associations found with specific months. That other factors were not consistent between the three response variables likely reflects the complex interactions between weather, the pest, the crop and natural enemies.

The general linear regression analyses are unlikely to be able to accurately predict pest pressure without further refinement. The approach used illustrates some of the issues with survey data and highlights the caution with which such data should be treated. Examination of the survey data suggested a number of potential associations between CSFB pressure and other factors (see Section 3.4.4 and Section 3.5). Examples for adult CSFB damage include the apparent high levels of leaf area lost seen in DK Exalte compared with other varieties, and lower levels of damage seen with crop cultivated using less intensive methods, low and very high seed rates, good soil moisture and low slug pressure. Higher larval loads were associated with large rather than small fields. It would be easy to take these trends at face value but to do so would likely be incorrect as the statistical approaches used here (Section 4) found no association with CSFB pressure. For example, it is likely that DK Exalte appeared in the dataset more regularly in years with high CSFB damage than other varieties and large fields were more common in areas of the country with high CSFB pressure. Good soil moisture is likely to have a similar effect to (and be correlated with) rainfall post sowing, and slug damage can appear similar to CSFB feeding, especially where CSFB pressure is high as shot-holes coalesce, and so the two can be mistaken. The novel modelling approach allowed a dataset containing missing values, many candidate explanatory factors of different types, any of which could be correlated or confounded with other factors, to be analysed.

Nevertheless, the factors for which trends were identified in Section 3.4.4 and Section 3.5 but were not identified in this analysis may still have some importance and so could be worth further investigation, though should of lower priority than those identified in this analysis. For instance, as field edges are often the source of natural enemies (Holland & Oakley, 2007), larger fields may be associated with higher larval loads because natural enemy activity (e.g. predation from ground beetles) may be reduced as the distance to field edges increases. The effect of seed rate is investigated further in Section 5.

This is one of the first instances that we are aware of Random Forest analysis being used to model insect pests and, while it was successful in identifying key risk factors, undoubtedly improvements could be made. The analysis of adult CSFB feeding damage contained a good range of explanatory factors but the number of data points in the dataset were small relative to the larval population analyses. Improved confidence in the findings of the analysis would be gained by using a larger dataset. Conversely, the larval populations analyses used suitably large datasets but would likely be improved by the addition of further explanatory factors, which were not available. The number of explanatory variables available for the larval analyses was limited by the data sources, which were largely collected prior to this project and for other uses. Whereas the adult damage dataset was largely collected within this project (primarily through the adult damage survey – see Section 3.3), meaning that a greater list of questions could be asked and a more comprehensive set of explanatory variables included in the analyses.

Explanatory variables may not have been at the correct scale. For example, given that the most severe CSFB adult damage occurs over a relatively short period of time it is likely that weekly weather data would be more appropriate than the monthly averages used in this study. Being able to relate weather data to a more time-specific response variable would also be helpful, e.g. percent leaf area lost was recorded at a specific range of crop stages but these could occur at any time over several weeks. Additional explanatory variables that are likely to be relevant include altitude (anecdotally CSFB pressure appears to be lower at higher altitudes) and sum of day degree thresholds more relevant to adult activity during summer months, e.g. flight temperature thresholds. As few novel control methods (e.g. companion cropping) were being used on farms when this project started, they did not feature in the analysis. However, many farmers/agronomists are now using such methods and it would be interesting to include them in future modelling work.

The analysis assumed that all data points were independent, although some were actually from the same field. Treating data points as non-independent would have significantly increased the complexity of the analysis and required the use of mixed effects random forest analyses, and though these have been reported (Hajjem *et al.*, 2014), they are not well known or widely implemented. It is also not thought that they have been implemented with the permutation test,

which was used in this work (see Section 4.2.2). The permutation test was considered important in this project to give a cut-off point for variable selection. Assumptions were also made in adjusting the raw values to account for insecticide use and it is possible that these do not reflect what the CSFB pressure would have been in the absence of these sprays. For example, the level of control achieved by a pyrethroid is likely more variable than the control efficacy used to adjust the raw values in this work. Additionally, this work also assumed that CSFB populations were resistant if resistance had already been detected in that county, based on resistance monitoring work. However, recent results from this monitoring work suggest that resistance levels can vary on a farm by farm basis (Farmers Guardian, 2020). Nevertheless, accounting for insecticide use is an unavoidable issue with survey data and the approach used in this work was reasonable based on the available data.

The analysis used all the available data in order to investigate as wide a range of potential explanatory variables as possible. However, there are potential sources of bias in the dataset. For example, some data came from the same geographical location, in the same or different years. There may also be other sources of bias that are less obvious, such as agronomic decisions taken by farmers who perceive their crop to be at greater risk of CSFB damage (e.g. varietal choice or sowing date), which could confound potential risk factors such as region. These risks were balanced against the need to assemble a large dataset to enable the analysis to proceed. Further practical work to confirm the effects of the factors identified in this analysis on CSFB adult feeding damage and larval populations should use experimental design to control for potential sources of bias.

The regression models performed poorly when validated, however this may reflect issues with the validation dataset as much as the models themselves. The validation data include substantial data from 2018/19, when CSFB pressure was generally higher than in any year in the dataset used to parameterise the models. As such it is perhaps unsurprising the models were unable to predict CSFB pressure where these pressures continue to increase year on year. Additionally, values in the validation dataset for important factors in the adult CSFB damage model (specifically crop stage at migration, rainfall post-sowing and stubble length) had to be estimated as this data was not available. Errors in these estimates would have affected the accuracy of the model predictions.

Some of the variables selected through the conditional random forest permutation test were not significant as two- or three-level factors, analysed using individual t-tests, linear regression (for numerical variables) and stepwise regression. As Random Forest analysis has a great deal of additional flexibility in how explanatory variables are used compared to a stepwise regression, this indicates that the source of the explanatory power of these variables is more complex than that captured by a two-level factor or simple linear regression. For example, an agronomic explanatory

factor may only be important in a subset of cases, such as years with a cool summer. Further consideration of these variables may be worthwhile, however Random Forest algorithms alone are not well suited to examining the effects of specific variables, as the fitted effect may vary between the multiple decision trees forming the ensemble. This is why the ensemble learning approach was coupled with regression analysis in this work.

Several risk factors were identified in the final model for each CSFB response variable, and are therefore potentially useful tools in managing CSFB. These factors were selected using rigorous statistical approaches to produce models that maximise explanatory power while minimising the number of parameters (explanatory variables). However, several other risk factors were identified at earlier stages of the modelling process and, despite their absence from the final model, they also present potentially useful means of reducing CSFB damage. There is a wealth of additional data in the CSFB dataset and gathered elsewhere in this project for which there was not the resource to analyse, including plant populations, plant establishment rate, proportional change in larval numbers overwinter within a site, differences in larval size and adult CSFB numbers. There would be value in analysing this data to identify the factors that govern their variability as all are potentially important in determining crop damage to CSFB. CSFB phenology models are used elsewhere in Europe to predict timings of CSFB activity (e.g. Johnen *et al.*, 2010) and are worth adapting for the UK.

CSFB-crop interactions are complex and this work illustrates this as, despite assembling and analysing a considerable dataset on the pest, the final models were capable of explaining only approximately a third of the variability. Nevertheless, the work achieved the primary objective, which was to identify the most important risk factors from a wide range of candidates. Several were identified for each response variable. The importance of these now need to be confirmed experimentally as they present potential tools in managing the pest.

#### **4.5. Conclusions**

- Novel statistical methods were used to model large datasets on CSFB damage and incidence.
- Several risk factors were identified by the models for adult CSFB damage and larval populations in the autumn and spring.
- The majority of risk factors were weather related, presenting the potential to gauge seasonal risk in pest pressure.
- A small number of risk factors are to some extent in the control of growers. These were:
  - The crop stage at CSFB migration and stubble management for adult CSFB damage.
  - Sow date and HEAR variety status for autumn larval populations.

- Sow date for spring larval populations.
- The importance of these risk factors should be confirmed in experimental work and their usefulness in managing CSFB determined.
- Other explanatory variables identified at early stages of the modelling process may still be important in managing CSFB risk.

## 5. Understanding the impact of OSR variety and seed rate on CSFB pressure

### 5.1. Introduction

Varietal resistance/tolerance is an important component of any IPM strategy and yet there has been little work comparing varieties for any indication of resistance or tolerance to CSFB. Newman (1984) investigated the effects of insect larval damage, including CSFB, upon the incidence of canker in OSR. Although the varieties studied are old and no longer on the recommended list (Jet Neuf, Rafal, Elvira, 76/315/1) there were large differences between cultivars in the amount of insect damage to the upper stems and crown. Unpublished work at ADAS Boxworth (Table 13) compared the level of CSFB larval infestation of 10 varieties sown in unreplicated plots in September 2015. There were clear differences between varieties with larval numbers ranging between 8.4 and 29.2 per plant. There was also a tendency for most larvae to be present in plants with the highest GAI suggesting that plant size is an important factor.

*Table 13. Mean number of CSFB larvae on 9 March 2016 in the stems and petioles of 10 coded OSR varieties sown at 80 seeds per m<sup>2</sup> on 27 August 2015 at ADAS Boxworth. (Shading red to green gives an indication of the variability in larval numbers and GAI, red = lowest value and green the highest value).*

Variety	Mean larvae/stem	Mean larvae/petiole	Mean of total larvae	Average of GAI
ADAS1	0.4	8	8.4	0.27
ADAS2	0.2	8.8	9	0.75
ADAS3	0.6	12.8	13.4	0.66
ADAS4	1	14.2	15.2	0.55
ADAS5	0.6	15.4	16	0.7
ADAS6	1.4	15.6	17	0.64
ADAS7	1	17	18	0.92
ADAS8	1.8	16.4	18.2	0.52
ADAS9	2.2	21	23.2	0.86
ADAS10	1.6	27.6	29.2	0.93

A number of studies have also suggested that varietal differences in glucosinolate or isothiocyanate content can influence attractiveness to CSFB adults and crop damage. In general, the higher the levels of these products the greater the attractiveness of the plants to CSFB and the higher the level of leaf damage. (Bartlet and Williams, 1991; Bartlet *et al.* 1992; Giamoustaris and Mithen, 1995).

The recommended list (RL) trials provide an excellent opportunity to be able to compare varieties for their level of infestation by CSFB and it was planned to monitor three RL trials in each of years 1 and 2 of the project. As RL trials are increasingly being situated in locations with low CSFB



pressure it was also decided to establish two ADAS managed variety trials in areas of moderate CSFB pressure in years 2 and 3 of the project. Previous ADAS work has investigated adjusting seed rate and in turn, plant populations as a means of compensating for pest attack from different arable pests such as slugs (Kendall *et al.*, 2013), wheat bulb fly (Storer *et al.*, 2018) and pollen beetle (Ellis & Berry, 2012). It was therefore decided to include drilling a specific variety in the ADAS trials at a range of seed rates to investigate the impact on CSFB pressure

The ultimate aim of this objective was to investigate any variability in the resistance/tolerance of WOSR varieties to CSFB in both RL and ADAS trials and also to determine if varying seed rate had any impact on pest pressure (Objective 2).

## **5.2. Materials and methods**

### **5.2.1. Recommend list trial monitoring**

Three RL trial sites were selected in year 1 (Cowlinge, Suffolk; Colne, Essex and Benniworth, Lincolnshire) and two in year 2 (Cowlinge, Suffolk and Great Tey, Essex). It was agreed with AHDB to not monitor a third RL trial in year 2 as the remaining sites were in areas of low CSFB pressure. Up to 17 varieties were selected for assessments at each site, depending on permission from breeders and the varieties available. Each variety was drilled in three replicate plots at each site. Varieties chosen for assessments were selected to provide a range of varietal types (e.g. conventional open pollinated, hybrid, semi-dwarf), specialised oil types (i.e. high oleic, low linolenic types), disease resistance (e.g. canker resistance, turnip yellows virus resistance) and a range of traits that may affect CSFB attractiveness and/or tolerance (e.g. autumn vigour, spring vigour, timing of flowering, stem stiffness, and glucosinolate content).

#### **Assessments**

An adult CSFB feeding damage assessment was done at approximately the five-true leaf stage. The assessment involved estimating the percentage leaf area lost in 50 randomly selected plants per plot. At the same time a plant population assessment was done by placing a 0.5 m rod between two rows and recording all emerged crop plants on each side. This was repeated five times in each plot.

Larval infestation was assessed in December by counting the number and position of scars created by larvae on 10 randomly selected plants per plot for 10 selected varieties. Leaf scarring was used as plants could not be destructively sampled. The 10 varieties were selected to represent a range of levels of adult feeding damage (as measured during establishment) and varietal traits that may affect tolerance to larval infestation. With agreement from AHDB it was decided to not do this assessment at Benniworth in 2016/17 or at any site in 2017/18 due to the low levels of CSFB damage recorded at establishment. Instead additional assessments were

included in the variety-seed rate trials in 2018/19, where for instance plants could be destructively sampled allowing larval numbers to be assessed.

### **Statistical analysis**

All data were subjected to the analysis of variance. Bar charts are presented as summaries using the standard error of the difference (SED) between means as an indication of data variability.

#### **5.2.2. Variety-seed rate trials**

Two variety and seed rate experiments were set up in both 2017/18 and 2018/19 in an area with a moderate CSFB pressure. These experiments used varieties common to the RL trial sites. There were 10 varieties, with one of these being sown at five seed rates to determine if this had any impact on CSFB infestation. Varieties were chosen to represent a range of levels of adult feeding damage (as observed in the RL trials in 2016/17) and provide a range of varietal types (e.g. conventional open pollinated, hybrid, semi-dwarf), specialised oil types (i.e. high oleic, low linolenic types), disease resistance (e.g. canker resistance, turnip yellows virus resistance) and a range of traits that may affect CSFB attractiveness and/or tolerance (e.g. autumn vigour, spring vigour, timing of flowering, stem stiffness, and glucosinolate content). Where possible the same varieties were investigated in both years to determine if any trends were consistent. To ensure a susceptible variety was used for the seed rate work, a variety for which high adult and larval CSFB damage had been observed in the 2016/17 RL trials was chosen.

Additionally, three treatments designed to create varying levels of CSFB larval infestation (insecticide sprays to kill adult CSFB and hatching eggs) were superimposed to five varieties and two seed rates (of one variety) in one of the variety-seed rate trials in each of years 2 and 3 in order to provide more data for Objective 3 to 'Understand crop tolerance to adult feeding damage and larval infestation and use this to revise thresholds for adults and larvae' (Section 6). Having three treatments to manipulate CSFB larval populations provided a range of larval infestations upon which to test crop tolerance. Varieties were chosen from the 10 selected for the variety-seed rate experiment; these varied in their vigour and developmental rate and whether they were open pollinated or hybrids. The treatments to manipulate CSFB larval populations were applied to one variety-seed rate trial in each of 2017/18 (Boxworth) and 2018/19 (High Mowthorpe). These data on larval manipulation were combined with that generated in Section 6.2.2.

Ten varieties were sown at Boxworth and High Mowthorpe in 2017/18 and 2018/19. Varieties were sown at 120 seeds per m<sup>2</sup> with the exception of Alizze which was sown at 10, 20, 40, 80 and 120 seeds per m<sup>2</sup> (Table 14). There were three replicates of each variety. In 2018/19, Troy was replaced by Django, Angus by Aquila and Cracker by Windozz. There were six additional plots (two per replicate) of Alizze, Amalie, Aquila, Nikita and Troy (replaced by Django in 2018/19) sown at

120 seeds per m<sup>2</sup> and six further plots (two per replicate) of Alizze sown at 40 seeds per m<sup>2</sup> at one site in each of the two years. These additional plots were at Boxworth in 2017/18 and at High Mowthorpe in 2018/19. These additional plots were used to manipulate CSFB larval populations as part of Objective 3 (Table 15).

*Table 14. Treatment list for variety-seed rate experiments and additional plots to manipulate CSFB larval populations in 2017/18 and 2018/19. See Table 15 for further details on larval manipulation treatments. \* = extra sown plots to which insecticides were applied to manipulate larval numbers.*

Treatment no.	Cultivar 2017/18	Cultivar 2018/19	Seed rate (seeds/m <sup>2</sup> )	Larval manipulation & spray treatment	Seed rate x variety
1	Alizze	Alizze	10		✓
2	Alizze	Alizze	20		✓
3	Alizze	Alizze	40	✓1*	✓
4	Alizze	Alizze	40	✓2	
5	Alizze	Alizze	40	✓3	
6	Alizze	Alizze	80		✓
7	Alizze	Alizze	120	✓1*	✓
8	Alizze	Alizze	120	✓2	
9	Alizze	Alizze	120	✓3	
10	Amalie	Amalie	120	✓1*	✓
11	Amalie	Amalie	120	✓2	
12	Amalie	Amalie	120	✓3	
13	Angus	Aquila	120	✓1*	✓
14	Angus	Aquila	120	✓2	
15	Angus	Aquila	120	✓3	
16	Cracker	Windozz	120		✓
17	Wembley	Wembley	120		✓
18	Elgar	Elgar	120		✓
19	Nikita	Nikita	120	✓1*	✓
20	Nikita	Nikita	120	✓2	
21	Nikita	Nikita	120	✓3	
22	Mentor	Mentor	120		✓
23	Troy	Django	120	✓1*	✓
24	Troy	Django	120	✓2	
25	Troy	Django	120	✓3	
26	V316OL	V316OL	120		✓

No insecticide treatments were applied to the variety-seed rate experiments. The insecticide treatments designed to manipulate CSFB larval populations were only applied to the extra plots of the five varieties and two seed rates of Alizze sown specifically for this purpose. The insecticide treatments were as per Table 15.

The rate of application of Hallmark Zeon (lambda-cyhalothrin, Syngenta) in treatments 2 and 3 was three times the approved rate so crop destruction was required for all plots of this treatment. The triple rate of Hallmark Zeon was used as an experimental tool to create different levels of larval infestation by attempting to control pyrethroid resistant CSFB. Using this approach in a non-research situation is illegal and may drive further resistance. A single overspray in November or

December to control peach-potato aphid was applied to the trials. In 2017/18, Biscaya (thiacloprid, Bayer) @ 0.3 l/ha was applied and in 2018/19 Plenum (pymetrozine, Syngenta) @ 0.3 kg/ha was applied once migration of peach-potato aphid was complete. This was to minimise any impact of Turnip yellows virus (TuYV) which potentially could confound the effect of CSFB on crop growth and yield. This has minimal if any impact on CSFB larvae. Where necessary, an insecticide was also applied in the spring for pollen beetle. This would have also had limited impact on CSFB larvae due to the widespread incidence of insecticide resistance.

*Table 15. Insecticide treatments used to manipulate CSFB larval populations in additional plots at Boxworth in 2017/18 and High Mowthorpe in 2018/19.*

Insecticide treatment	Product & rate	Timing
1. Untreated	N/A	N/A
2. Lamda-cyhalothrin	Hallmark Zeon or alternative @ 225ml/ha	100% emergence
3. Lamda-cyhalothrin	Hallmark Zeon or alternative @ 225ml/ha	100% emergence, two weeks later and in mid/end November

### **Assessments**

Crop establishment was assessed in 2018/19 but not in 2017/18 to improve understanding of this trait, which is potentially important in determining susceptibility to adult CSFB but are not assessed in RL trials. The establishment index was assessed in the variety plots (but not the seed rate or additional plots for manipulating larval numbers) once they had all emerged, at about the three true leaf stage. All plots were assessed on a scale of 1-5, where 1 = poorly established plots with low and uneven plant emergence, and 5 = well established plots with high and even plant emergence.

A plant population assessment was done during establishment in both years. This was done in the 10 varieties (with three replicates of each), all five seed rates of Alizze and all the additional plots of five varieties and two seed rates where CSFB larval numbers were manipulated using insecticides. The assessment was done by placing a 0.5 m rod between two rows and recording all emerged crop plants on each side. This was repeated five times in each plot.

Green area index (GAI) was also assessed in the variety and seed rate plots (but not the additional plots for manipulating larval numbers) at the same time as the establishment assessment. An overhead photograph was taken in each plot and the GAI determined using the BASF online tool: [https://www.agricentre.basf.co.uk/agroportal/uk/en/services\\_1/website\\_tools/gai\\_cereals\\_cat\\_online/cat\\_online.html](https://www.agricentre.basf.co.uk/agroportal/uk/en/services_1/website_tools/gai_cereals_cat_online/cat_online.html).

An adult CSFB feeding damage assessment was done at the same time as the plant population assessment. This was undertaken in the 10 varieties (with three replicates of each), all five seed rates of Alizze and all the additional plots of five varieties and two seed rates where CSFB larval numbers were manipulated using insecticides. The assessment involved estimating the percentage leaf area lost in 50 randomly selected plants per plot.

In 2017/18 and 2018/19 a larval population assessment was done in December/January in all plots. This included the 10 varieties (with three replicates of each), all five seed rates of Alizze and all the additional plots of five varieties and two seed rates where CSFB larval numbers were manipulated using insecticides. A total of 10 plants were sampled randomly from each plot and returned to the laboratory where the number of feeding scars due to CSFB larvae along the petioles and stem was counted. Separate counts of feeding scars were made for the petioles and stem. The stems and petioles were then dissected with a sharp scalpel and the number of larvae counted. Separate counts of larvae were made for the petioles and stem. In 2018/19, the larval count assessment was repeated in early March. The larval count assessment in March was added to provide additional information on late larval invasion. It was not considered necessary to include scarring assessments in March. In 2018/19, larval assessments occurred in one of the replicates of Alizze at 10 seeds per m<sup>2</sup> at Boxworth instead of all replicates. This was done to ensure that not all plants were removed from the low seed rate plots.

In 2018/19, stem diameter and crop height were also assessed in the variety and seed rate plots (but not the additional plots for manipulating larval numbers). These factors potentially could influence crop tolerance to CSFB larval damage. A plant with a wide stem could potentially tolerate higher CSFB larval populations than a plant with a thin stem. Taller plants, if infested with CSFB larvae, may be more prone to lodging than shorter plants infested with a similar level of the pest. At the end of flowering the stem diameter of the main stem on 20 plants per plot was measured using digital callipers. At the same time the height to the top of the terminal raceme was also measured on six plants per plot.

The plots were harvested with a small plot combine and yield assessed at 91% dry matter, together with oil content and thousand seed weight.

### ***Statistical analysis***

All data were subjected to the analysis of variance, except for data on larval number in varieties under different insecticide regimes, which was analysed using regression analysis for varietal tolerance to larval damage. In this case, larval number was compared with yield at harvest, however to account for intrinsic differences in yield potential between varieties, and so better identify differences between varieties due to larval load, the yield at harvest data was adjusted by

the seed yield given as a percentage of the control as reported in AHDB RL trials for the specific season in question. For example, in 2017/18 RL lists Angus had a seed yield of 106% of the control (AHDB, 2018) so the yield data for Angus in the 2017/18 trial in this work was adjusted down 6%. Note that yield was not adjusted in this way for other comparisons, e.g. comparing seed rate or varietal yield per se. Significant differences between treatments were identified using Duncan's multiple range test indices. Bar charts are presented as summaries using the standard error of the difference (SED) between means as an indication of data variability. Data for which no statistical analysis was done (e.g. High Mowthorpe in 2018/19) are presented as summaries using standard error of the means as an indication of data variability.

Linear plus exponential seed rate response curves were fitted to the seed yield for each seed rate treatment of the form

$$Y = A + BR^S + CS \quad \text{Equation 1}$$

where  $Y$  is the seed yield (t/ha),  $A$ ,  $B$ ,  $C$  and  $R$  are constants and  $S$  is the seed rate (seeds per m<sup>2</sup>). A linear plus exponential function was chosen because this curve was found to describe the yield response best which was frequently typified by a steep initial yield increase, followed by a plateau, followed by a yield reduction at high seed rates particularly where lodging occurred.

The equation for the best-fit curve was then used to calculate the economically optimum seed rate for each trial by assuming OSR seed costs of £12/kg and an OSR seed price of £350/t. The cost of each seed rate treatment (seeds per m<sup>2</sup>) was calculated by using the seed costs described above and the thousand seed weight of the seed of 5 g.

### **5.3. Results**

#### **5.3.1. Recommend list trial monitoring**

##### **2016/17**

There were variable levels of CSFB infestation between the three RL trial sites in 2016/17. Most adult feeding damage was recorded at Colne where between 35 and 50% of leaf area was lost (Figure 51), followed by Cowlinge where 17-28% of leaf area was lost (Figure 52). The least adult feeding damage was found at the Benniworth where there was never more than 2% of leaf area lost (Figure 53). There were no statistically significant differences in percentage leaf area lost between varieties at Cowlinge and Colne. At Benniworth, percentage leaf area lost was statistically different between the varieties ( $P = 0.002$ , Figure 53), although this should be interpreted with caution due to the low levels of damage. At Cowlinge, the least leaf damage was recorded in coded variety I and most in coded variety B with the latter losing 40% more leaf area than the

former. At Colne, the least leaf damage was found in coded variety M and most in coded variety N. Coded variety N had 29% more leaf damage at this site than coded variety M.

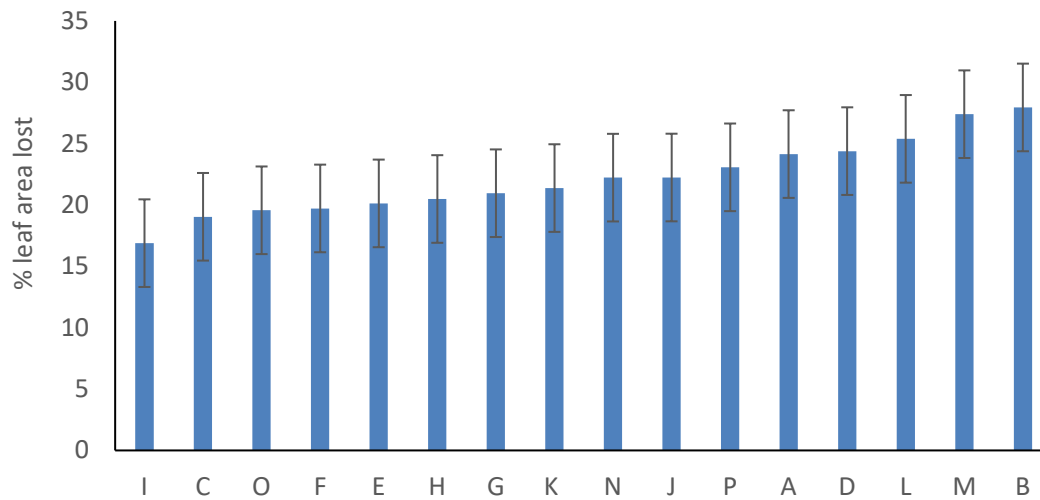


Figure 51. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 15 in a range of coded varieties at the Cowlinge RL trial in 2016/17. Bars indicate the SED.

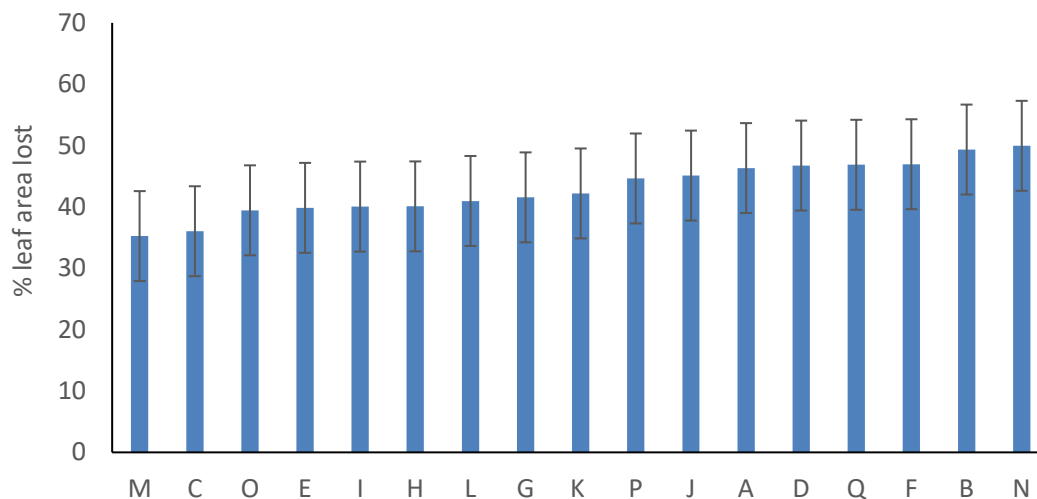


Figure 52. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 16 in a range of coded varieties at the Colne RL trial in 2016/17. Bars indicate the SED.

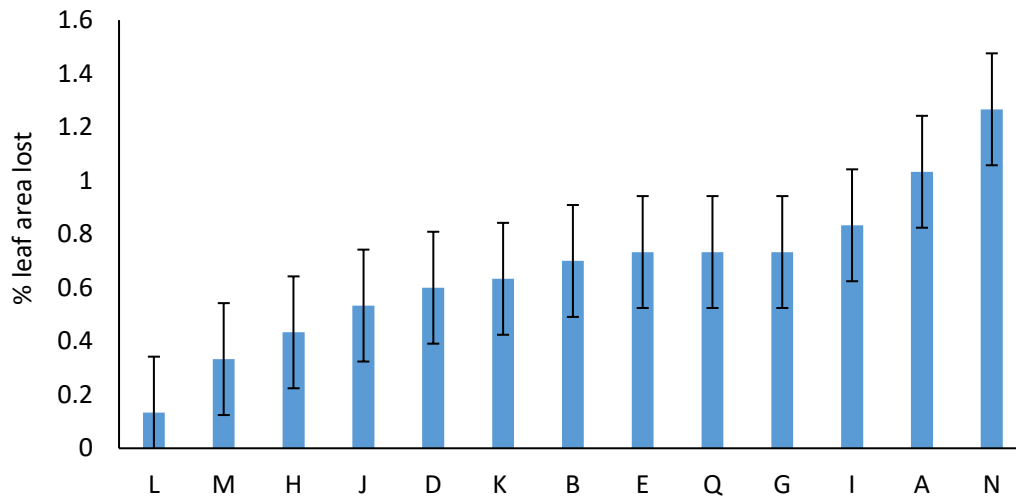


Figure 53. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 15 in a range of coded varieties at the Benniworth RL site in 2016/17. Bars indicate the SED.

There were no significant differences in plant populations between the varieties at any of the RL sites in 2016/17 (Figure 54-56). Coded varieties E, A and K had the highest plant populations at Cowlinge, Colne and Benniworth respectively. Coded varieties J, M and B had the lowest plant populations at Cowlinge, Colne and Benniworth respectively.

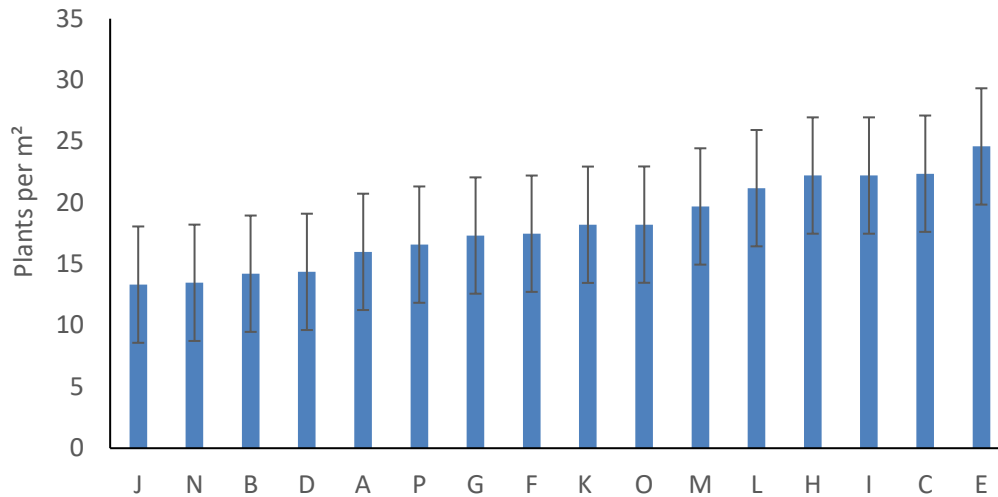


Figure 54. Mean plant per m² at BBCH 15 in a range of coded varieties at the Cowlinge RL trial in 2016/17. Bars indicate the SED.



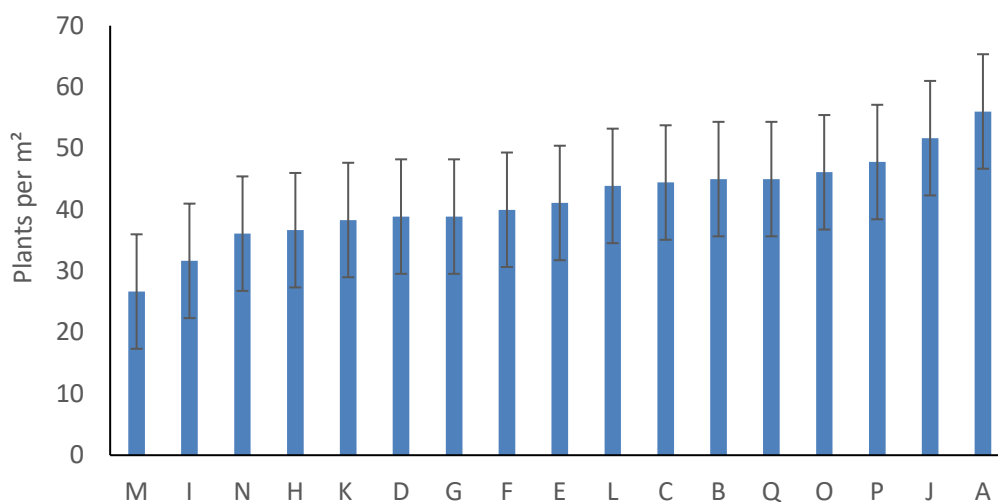


Figure 55. Mean plant per m<sup>2</sup> at BBCH 16 in a range of coded varieties at the Colne RL trial in 2016/17. Bars indicate the SED.

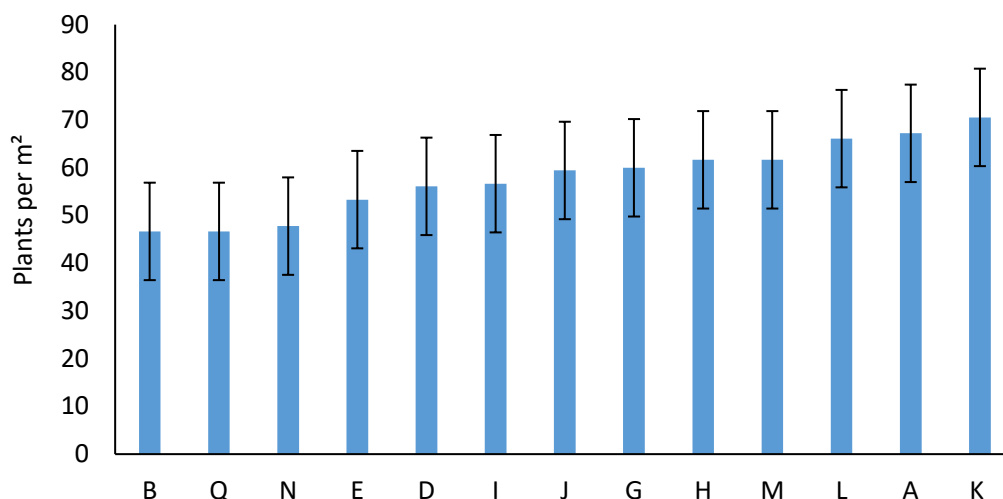


Figure 56. Mean plant per m<sup>2</sup> at BBCH 15 in a range of coded varieties at the Benniworth RL site in 2016/17. Bars indicate the SED.

Levels of larval scarring were similar between the Cowlinge (Figure 57) and Colne (Figure 58) sites (no assessment occurred at Benniworth due to low damage levels at establishment). Very little stem scarring was recorded so total scarring per plant was analysed. There were no statistically significant differences in scarring at either site. Scar data showed that at Cowlinge coded variety N had the least damage and coded variety B most, with coded variety B having 39% more leaf scarring than coded variety N. At Colne, coded variety A had 43% more leaf scarring than the least scarred variety, coded variety I.

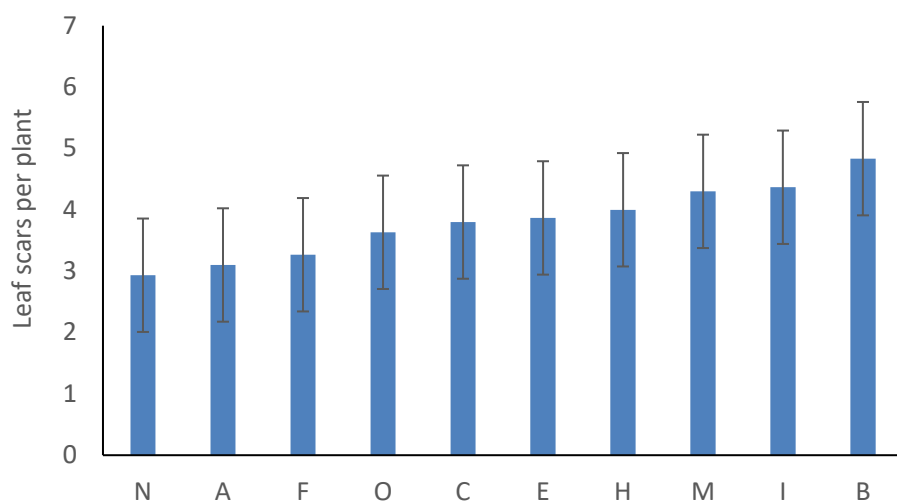


Figure 57. Mean number of scars per plant caused by CSFB larval feeding in a range of coded varieties at the Cowlinge RL trial in December 2016. Bars indicate the SED.

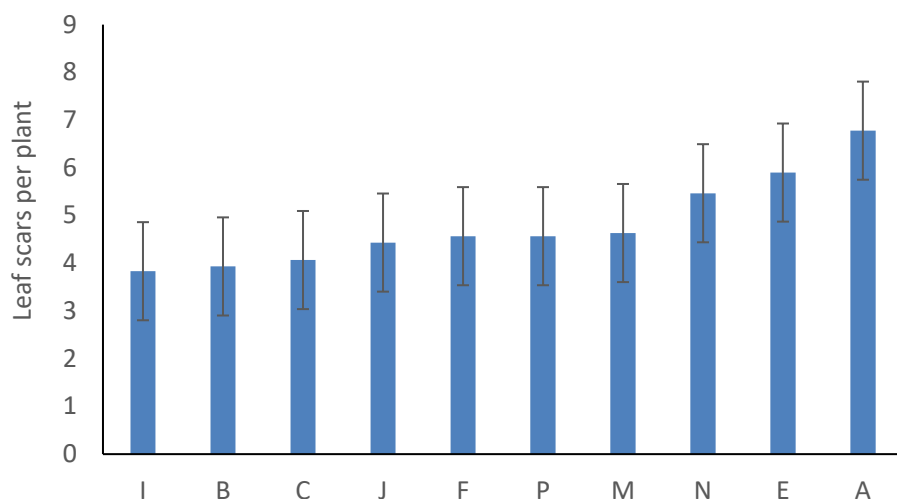


Figure 58. Mean number of scars per plant caused by CSFB larval feeding in a range of coded varieties at the Colne RL trial in December 2016. Bars indicate the SED.

There was good agreement in the ranking of varieties for adult CSFB damage at Cowlinge and Colne. In 11 of 17 varieties (A, B, C, D, E, G, H, J, K, O and P) ranking of adult damage was the same or only different by one ranking position at Cowlinge as at Colne. In contrast there was little agreement in ranking for the number of larval scars or plant populations between sites, except for coded variety N which consistently had low plant populations.

### 2017/18

Low levels of adult CSFB feeding damage were seen at both RL trial sites in 2017/18 (Figure 59-60). There were no statistically significant differences in percentage leaf area lost at either site. There was little agreement in the ranking of varieties between sites.

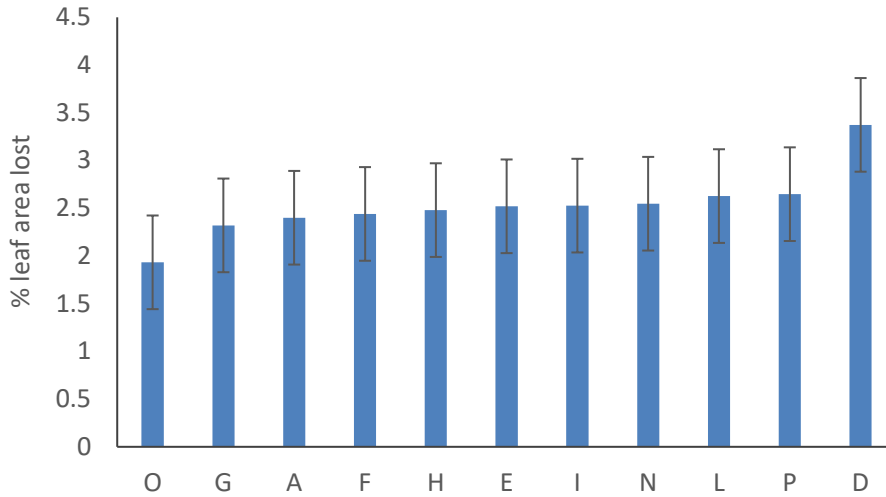


Figure 59. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 18 in a range of coded varieties at the Cowlinge RL trial in 2017/18. Bars indicate the SED.

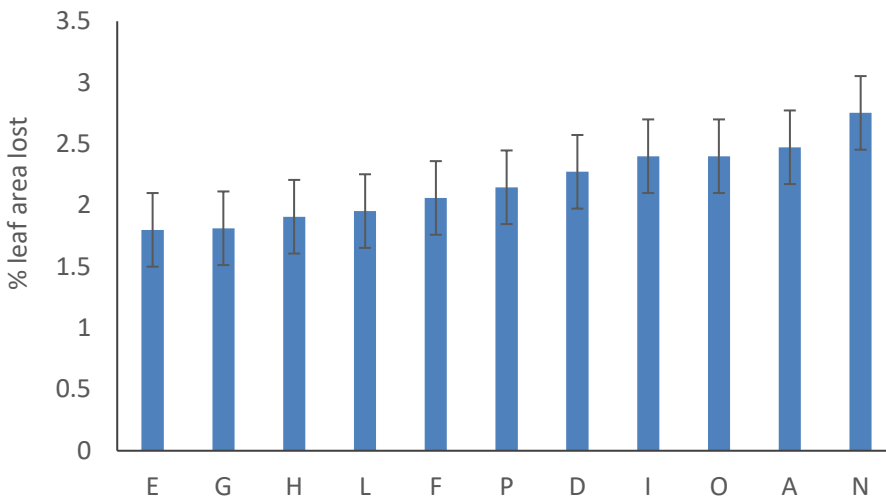


Figure 60. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 15 in a range of coded varieties at the Great Tey RL trial in 2017/18. Bars indicate the SED.

Plant populations were similar at both RL trial sites in 2017/18 (Figure 61-62). There were no statistically significant differences in plant populations at either site. There was little agreement in the ranking of varieties between sites, except for coded variety D which had low populations at both sites.

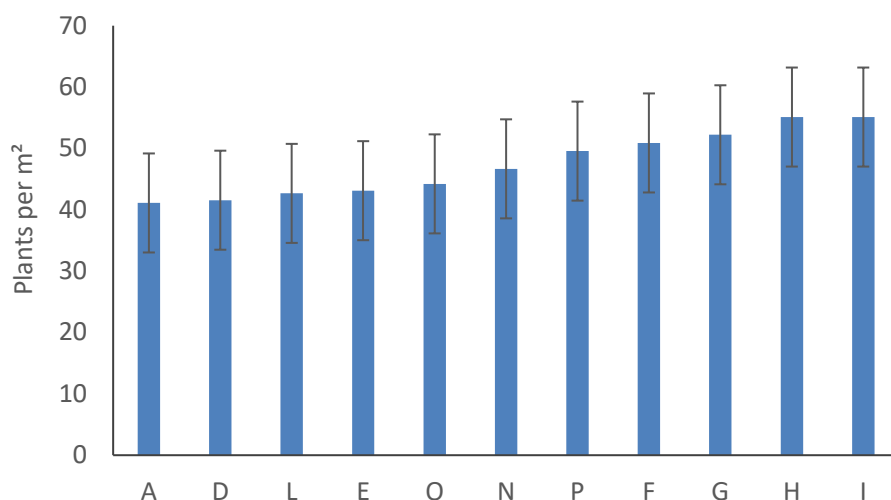


Figure 61. Mean plants per m<sup>2</sup> at BBCH 18 in a range of coded varieties at the Cowlinge RL trial in 2017/18. Bars indicate the SED.

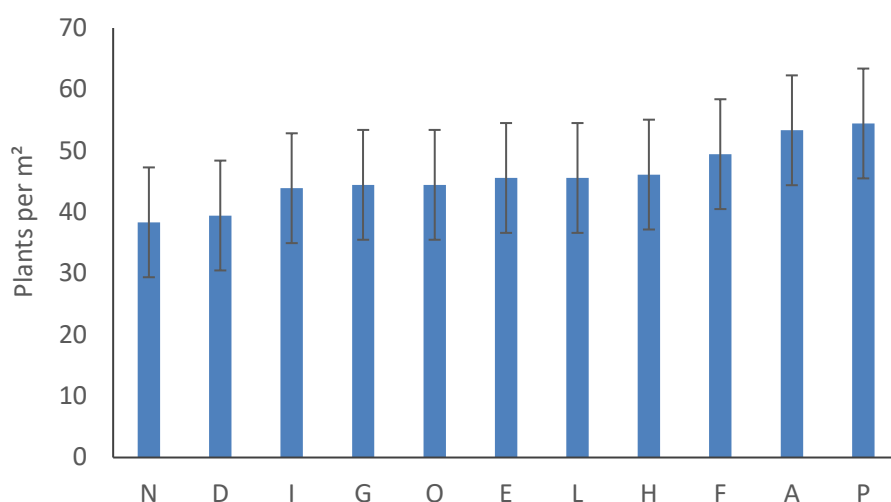


Figure 62. Mean plants per m<sup>2</sup> at BBCH 15 in a range of coded varieties at the Great Tey RL trial in 2017/18. Bars indicate the SED.

### 5.3.2. Seed rate-variety trials

Data are presented for trials at Boxworth in both 2017/18 and 2018/19 and 2017/18 at High Mowthorpe. Only limited data is available for presentation from the High Mowthorpe trial in 2018/19 as the trial succumbed to extreme CSFB adult attack and plots were not taken to yield.

#### Varietal differences

##### Establishment and GAI

Establishment index differed significantly between varieties at Boxworth in 2018/19 ( $P < 0.001$ , Figure 63), with Mentor establishing best and Django the worst. Mentor established significantly better than Wembley, Aquila, Amalie, Nikita and Django ( $P < 0.05$ ). Windozz established significantly better than Aquila, Amalie, Nikita and Django ( $P < 0.05$ ), Alizze, V316OL and Elgar

established significantly better than Nikita and Mentor ( $P < 0.05$ ) and Wembley established significantly better than Django ( $P < 0.05$ ). There was no significant difference in GAI between varieties (Figure 64).

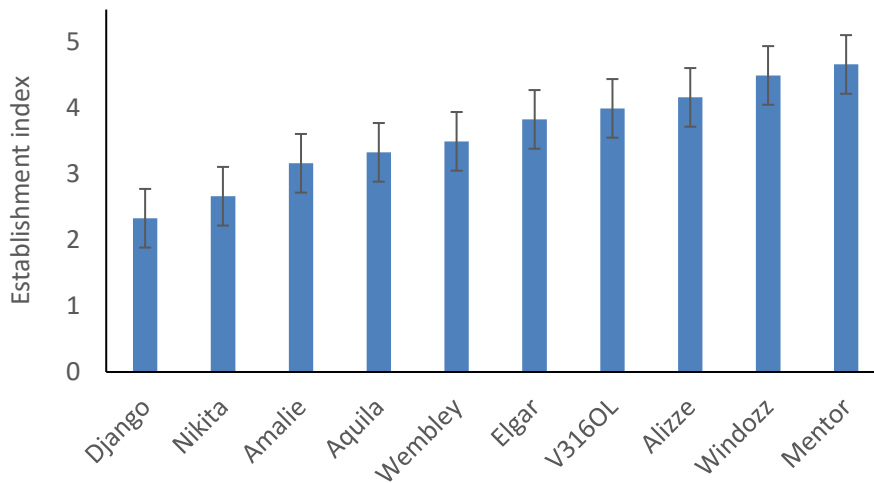


Figure 63. Mean establishment indices of 10 WOSR varieties at BBCH 13 at Boxworth in 2018/19. Bars indicate the SED.

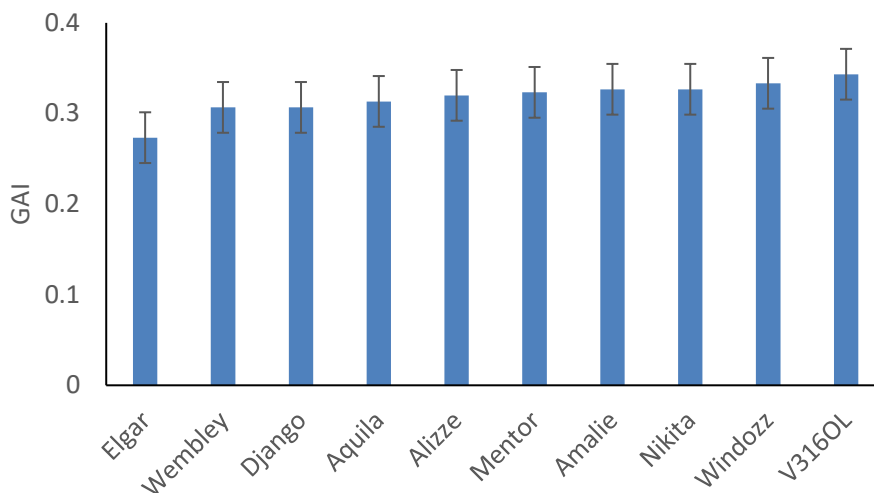


Figure 64. Mean GAI of 10 WOSR varieties at BBCH 13 at Boxworth in 2019/19. Bars indicate the SED.

There was no significant difference in establishment index between varieties at High Mowthorpe in 2018/19 (Figure 65). Establishment was generally poor due to significant CSFB adult attack. GAI was not assessed due to the poor establishment of the crop. The ranking of most varieties in terms of establishment was fairly consistent across the trials, with V316OL and Mentor tending to establish well, and Django and Nikita tending to establish poorly.

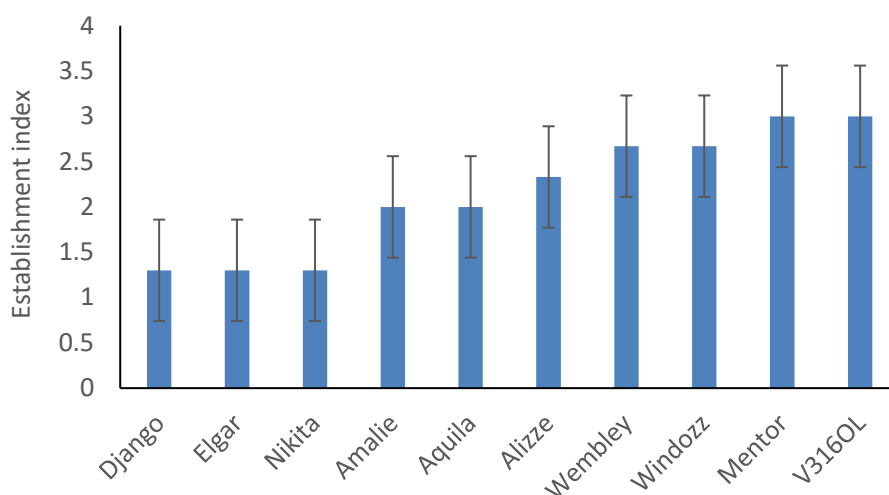


Figure 65. Mean establishment indices of 10 WOSR varieties at BBCH 14 at High Mowthorpe in 2018/19. Bars indicate the SED.

### Plant population

Plant populations differed significantly between varieties at Boxworth in 2017/18 ( $P < 0.001$ , Figure 66). Plant numbers for Angus and Troy were significantly higher than for all other varieties except Wembley and Cracker. At High Mowthorpe in 2017/18 and Boxworth in 2018/19 there was no significant difference in plant populations between varieties (Figure 67 & Figure 68 respectively). Plant populations at High Mowthorpe in 2018/19 are shown in Figure 69 but differences were not statistically analysed as some plots were lost. Comparison of the plant populations across the trials reveals consistency in the rankings of some of the varieties, with Mentor, Troy and Windozz all tending to have higher plant populations, although the latter two were only investigated in one year. Populations of Mentor were low in one trial in which conditions were dry at establishment. Nikita, Amalie and Django all tended to have lower plant populations, although the latter was only investigated in one year.

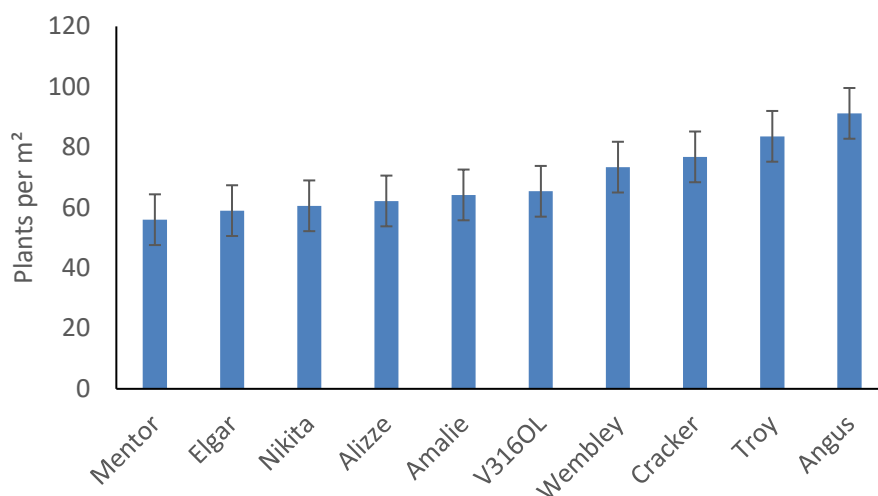


Figure 66. Mean plant populations (plants per m<sup>2</sup>) of 10 WOSR varieties at Boxworth in 2017/18 at BBCH 11. Bars indicate the SED.

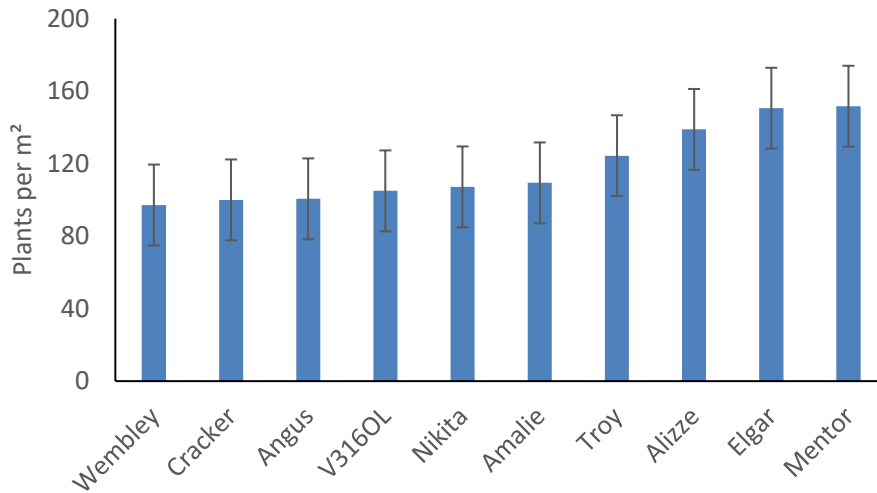


Figure 67. Mean plant populations (plants per m<sup>2</sup>) of 10 WOSR varieties at High Mowthorpe in 2017/18 at BBCH 13. Bars indicate the SED.

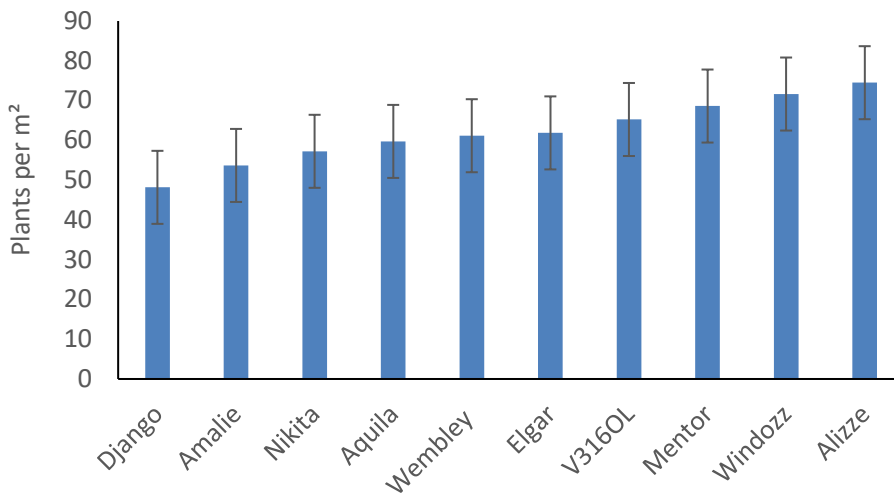


Figure 68. Mean plant populations (plants per m<sup>2</sup>) of 10 WOSR varieties at Boxworth in 2018/19 at BBCH 13. Bars indicate the SED.

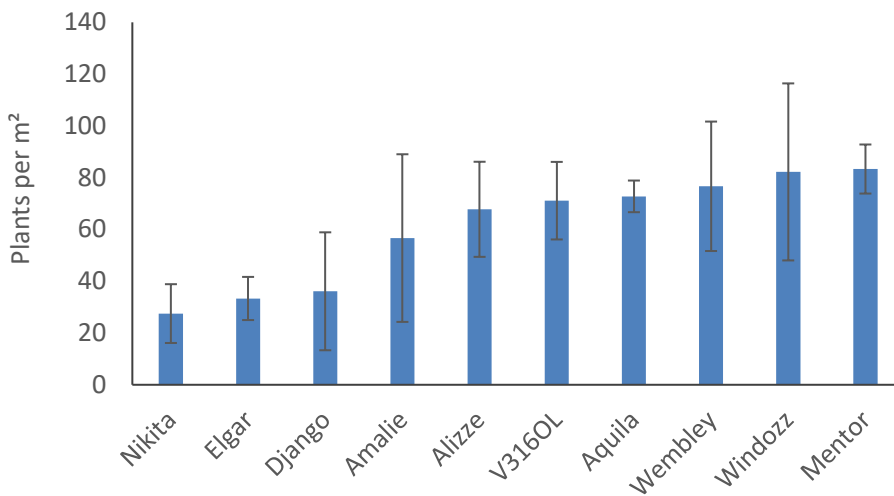


Figure 69. Mean plant populations (plants per m<sup>2</sup>) of 10 WOSR varieties at High Mowthorpe in 2018/19 at BBCH 14. Bars indicate the standard of the mean.

### Adult feeding damage

The level of leaf area lost due to CSFB adult feeding was relatively low at both Boxworth and High Mowthorpe in 2017/18 but higher in 2018/19 at both sites. Assessments were made at approximately BBCH13 and the current threshold to justify insecticide treatment is 50% leaf area lost from BBCH13 to BBCH14. At Boxworth in both 2017/18 and 2018/19 and at High Mowthorpe in 2017/18 this threshold was not exceeded. This was also the case at High Mowthorpe in 2018/19 although the trial was lost suggesting that some other factor was primarily responsible for poor establishment. There was no significant difference between varieties in the % leaf area lost as a result of adult CSFB feeding damage in any of the trials (Figures 70-73). Comparing the adult feeding damage across the trials shows consistency in the rankings for some varieties, with Amalie and Nikita tending to suffer higher levels of damage and Mentor tending to suffer lower level.

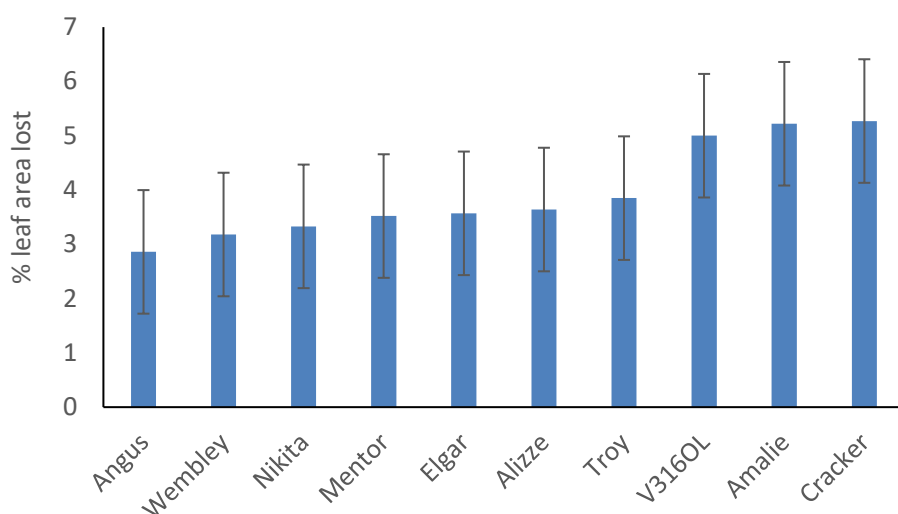


Figure 70. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 13 in 10 WOSR varieties at Boxworth in 2017/18. Bars indicate the SED.

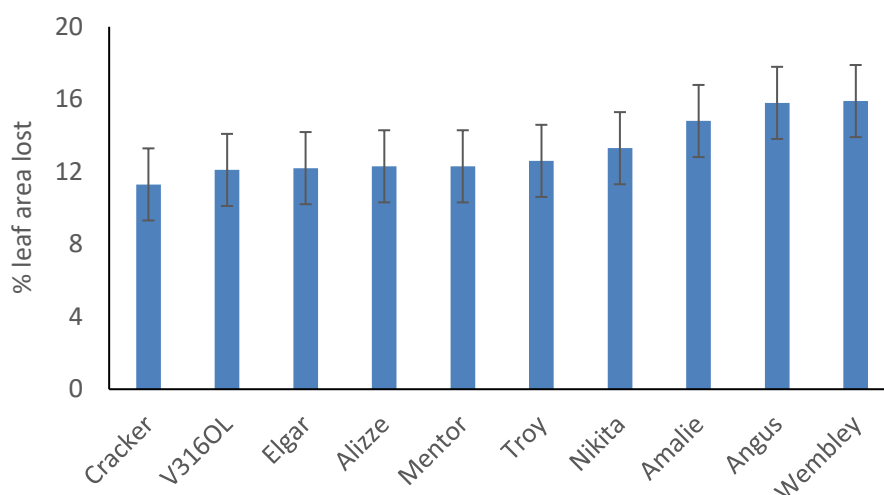


Figure 71. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 13 in 10 WOSR varieties at High Mowthorpe in 2017/18. Bars indicate the SED.



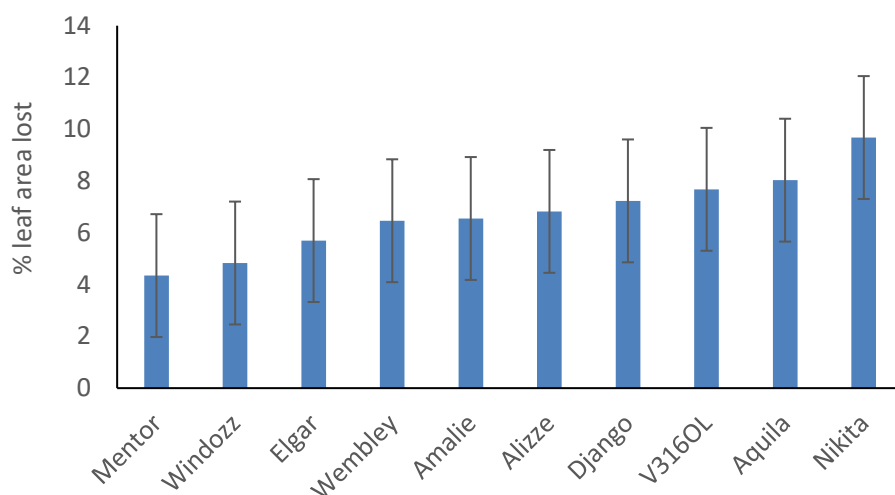


Figure 72. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 13 in 10 WOSR varieties at Boxworth in 2018/19. Bars indicate the SED.

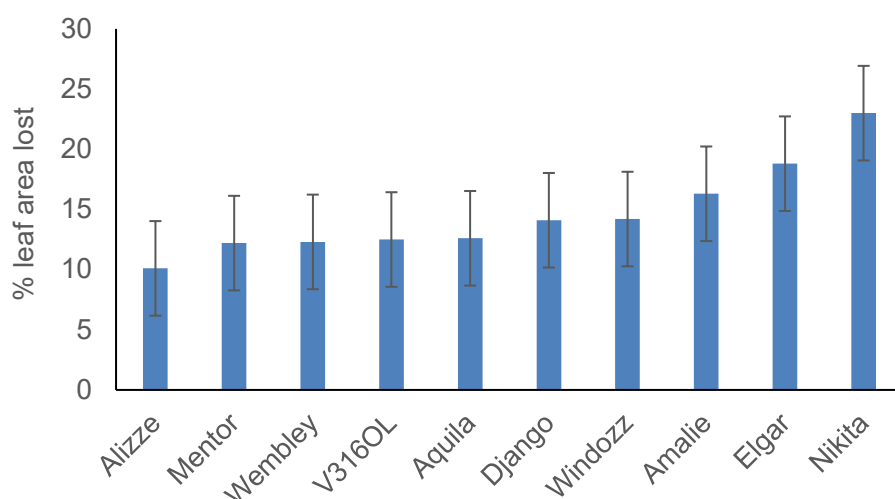


Figure 73. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 14 in 10 WOSR varieties at High Mowthorpe in 2018/19. Bars indicate the SED.

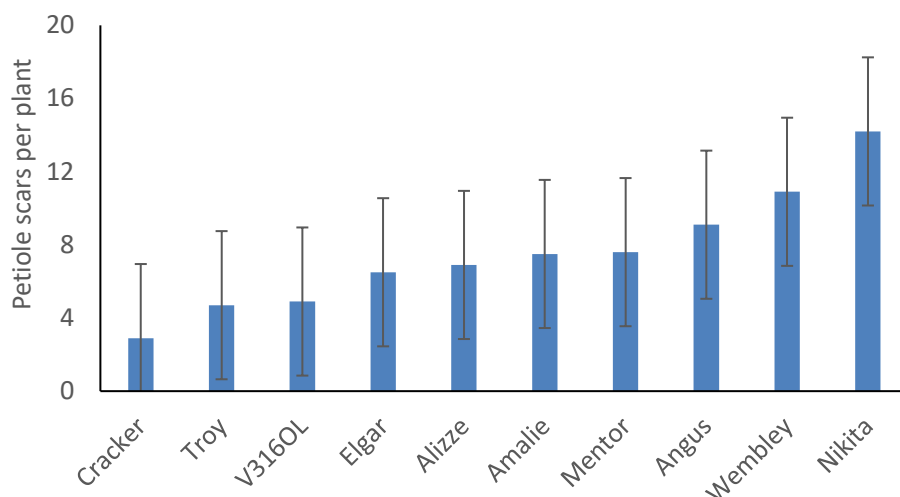
### CSFB larval infestation

To estimate CSFB infestation both scarring on the leaf petioles and stems and numbers of larvae in the petioles and stems were assessed. For the 2017/18 trials the first larval assessment was done in December at Boxworth and January at High Mowthorpe. For the 2018/19 trial at Boxworth the first larval assessment was done in January and the second in March. There was no larval assessment at High Mowthorpe in 2019 as the trial failed due to significant CSFB adult feeding.

Levels of CSFB larval infestation were much higher in 2018/19 at Boxworth than at Boxworth or High Mowthorpe in 2017/18. In 2017/18 numbers of larvae did not exceed eight per plant which is only just above the current autumn threshold of five per plant. In 2018/19 at Boxworth, numbers in January ranged between 17 and 33 larvae per plant which is significantly above the autumn

threshold and would be expected to have an impact on crop yield. This level of infestation provided a very robust test of the tolerance/resistance of varieties to CSFB attack.

At the first larval assessment there was no significant difference in the number of petiole scars caused by CSFB larvae (Figure 74-76) or in the numbers of CSFB larvae within the petioles between varieties in 2017/18 or 2018/19 (Figure 77-79). Very few larvae or scars were found in or on the stems in the December/January assessments, with no stem larvae in either year at High Mowthorpe and a total of 1 and 13 at Boxworth in 2017/18 and 2018/19 respectively (data not shown but varietal differences were not significant). Comparing the December/January larval pressure in the varieties across the trials shows little consistency in the rankings, with only Troy and Amalie showing a slight tendency to have lower larval numbers (though the former was investigated in only two trials) and only Alizze showing a slight tendency to have higher larval numbers.



*Figure 74. Mean number of petiole scars per plant caused by CSFB larval feeding in 10 WOSR varieties at Boxworth in December 2017. Bars indicate the SED.*

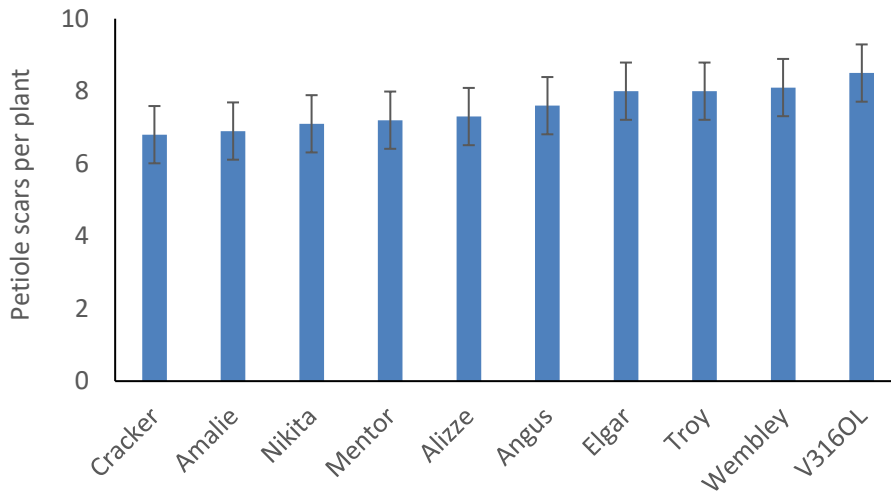


Figure 75. Mean number of petiole scars per plant caused by CSFB larval feeding in 10 WOSR varieties at High Mowthorpe in January 2018. Bars indicate the SED.

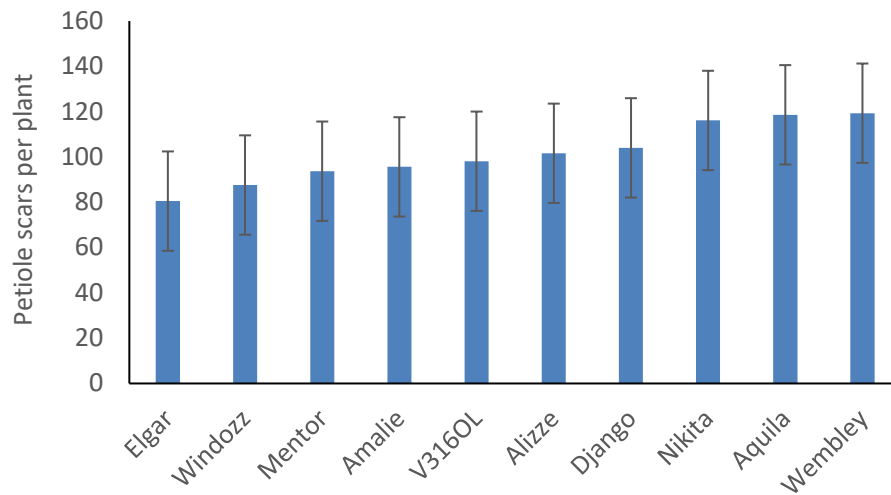


Figure 76. Mean number of petiole scars per plant caused by CSFB larval feeding in 10 WOSR varieties at Boxworth in January 2019. Bars indicate the SED.

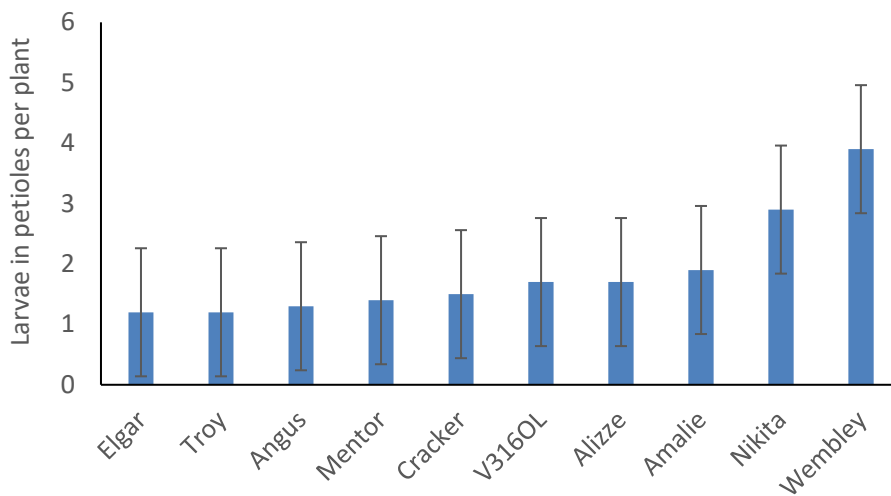


Figure 77. Mean number of CSFB larvae in petioles per plant in 10 WOSR varieties at Boxworth in December 2017. Bars indicate the SED.

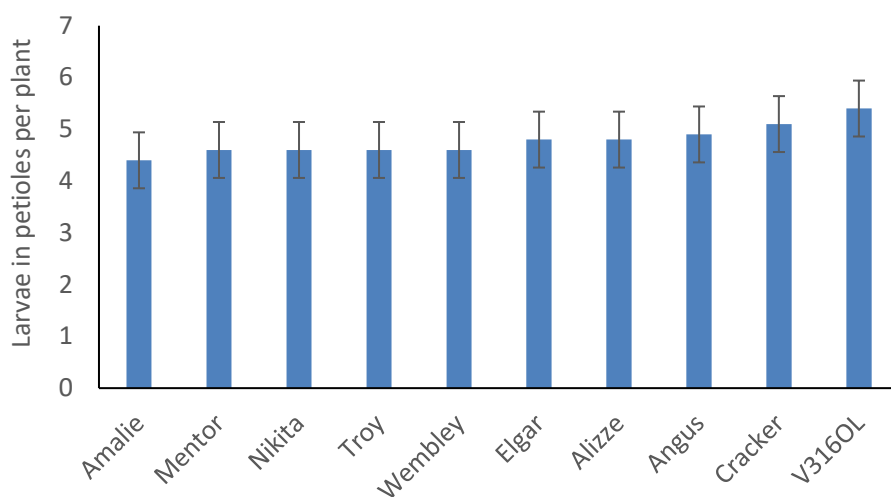


Figure 78. Mean number of CSFB larvae in petioles per plant in 10 WOSR varieties at High Mowthorpe in January 2018. Bars indicate the SED.

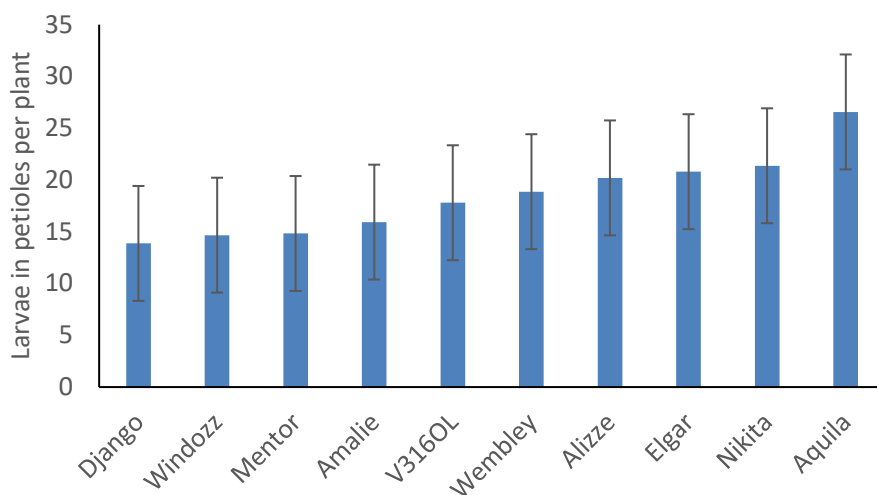


Figure 79. Mean number of CSFB larvae in petioles per plant in 10 WOSR varieties at Boxworth in January 2019. Bars indicate the SED.

By the second assessment at Boxworth in 2018/19 (March), larval numbers had increased in all varieties, except for Elgar which saw a 16% reduction. Increases ranged from 10% in Aquila to 83% in Mentor. There was no significant difference in CSFB larval numbers in the petioles, stems or the combined total of petioles and stems (Figure 80-82 respectively) between the varieties.

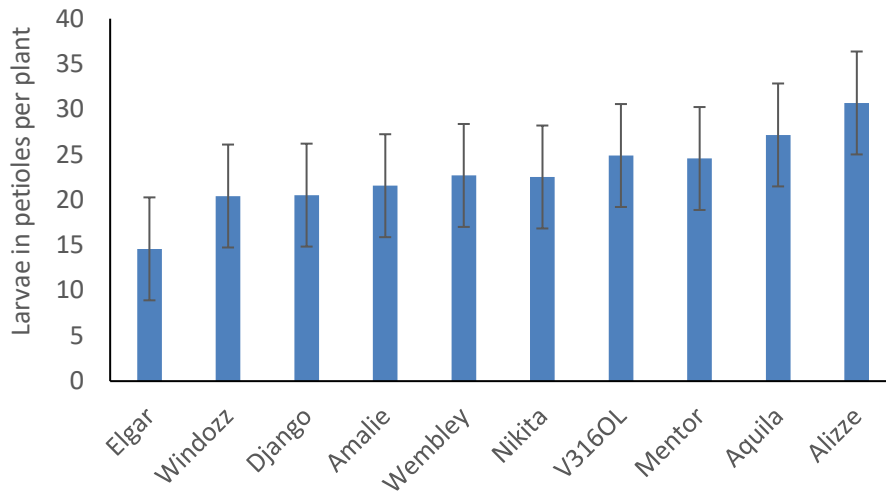


Figure 80. Mean number of CSFB larvae in petioles per plant in 10 WOSR varieties at Boxworth in March 2019. Bars indicate the SED.

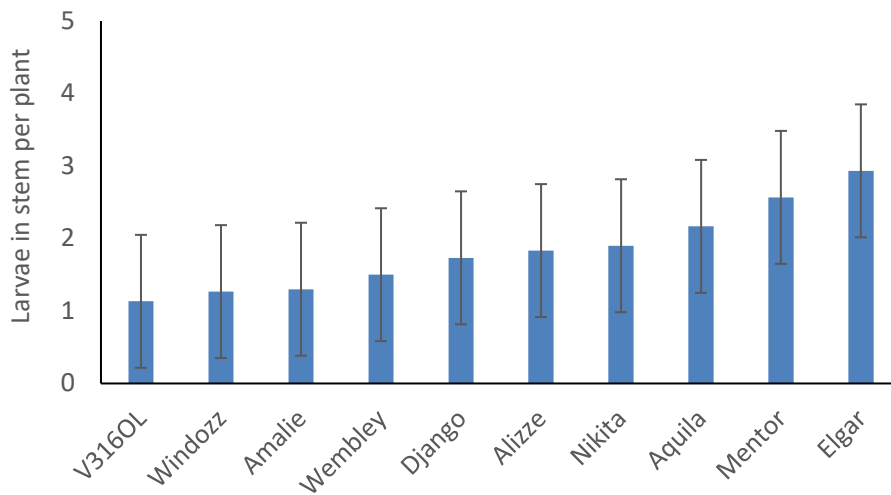


Figure 81. Mean number of CSFB larvae in stems per plant in 10 WOSR varieties at Boxworth in March 2019. Bars indicate the SED.

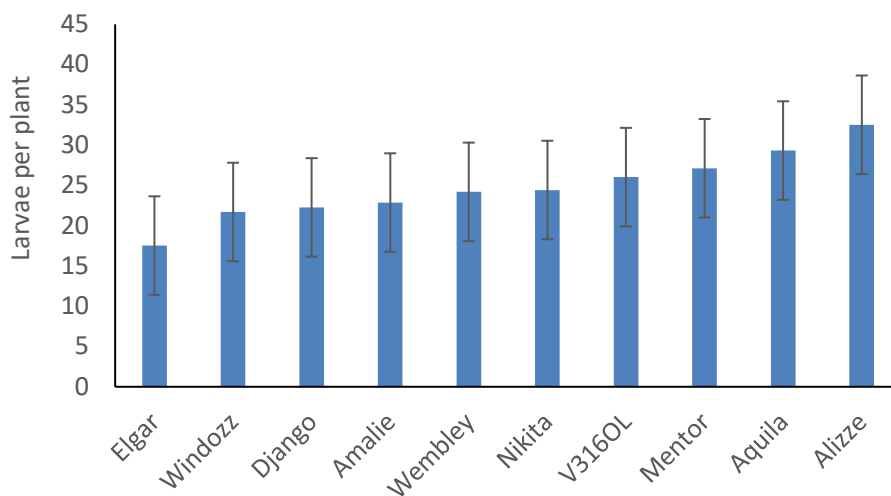


Figure 82. Mean number of combined total of CSFB larvae in the petioles and stems per plant in 10 WOSR varieties at Boxworth in March 2019. Bars indicate the SED.

The insecticide treatments used to manipulate CSFB larval populations produced some differences in larvae per plant, with higher larval numbers in the untreated plots and lower in the treated plots in most varieties (Figure 83). As these treatments were designed to create differing larval populations, the differences in larval populations were not statistically analysed. Instead the relationship between larval load and yield (adjusted for yield potential) was analysed using regression analysis. This analysis found no significant differences between varieties, with the slope of the yield response to larval number similar for all five varieties (data for varieties not shown here. Yield response to larval load is further discussed in Section 6).

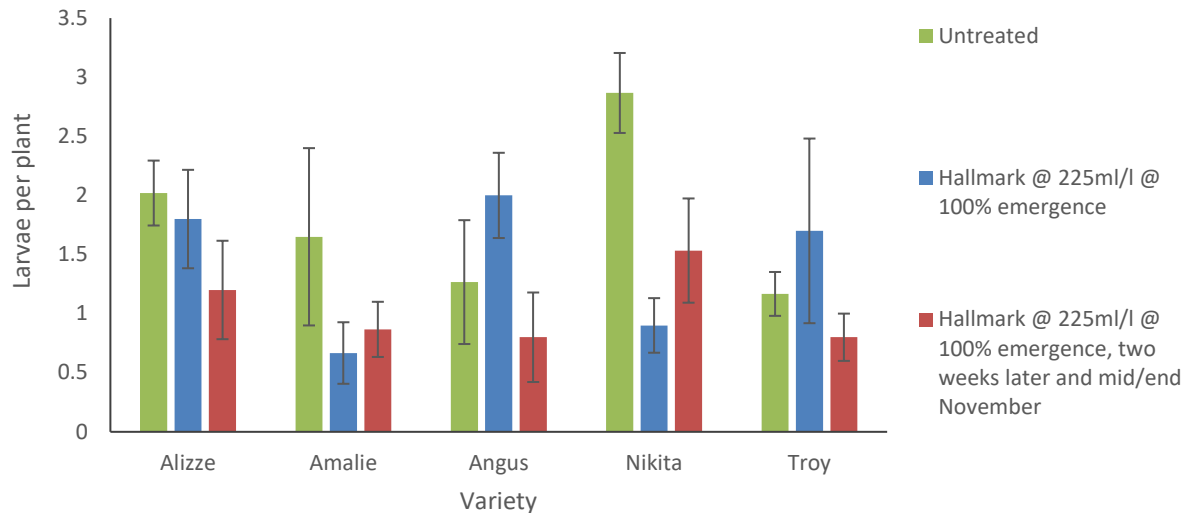


Figure 83. Larvae per plant in five varieties treated with three different insecticide regimes as an experimental tool to create different levels of larval infestation.

### Stem width and plant height

Stem width did not differ significantly between varieties at Boxworth in 2018/19 (Figure 84) but there was a difference in crop height ( $P < 0.05$ , Figure 85). Plants of V316OL were significantly taller than all other varieties ( $P < 0.05$ ).

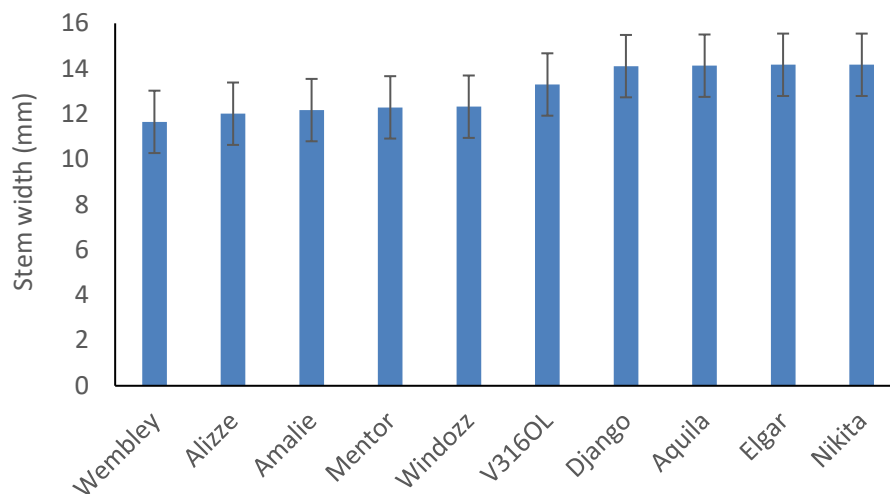


Figure 84. Stem width (mm) at the end of flowering in 10 WOSR varieties at Boxworth in 2018/19. Bars indicate the SED.

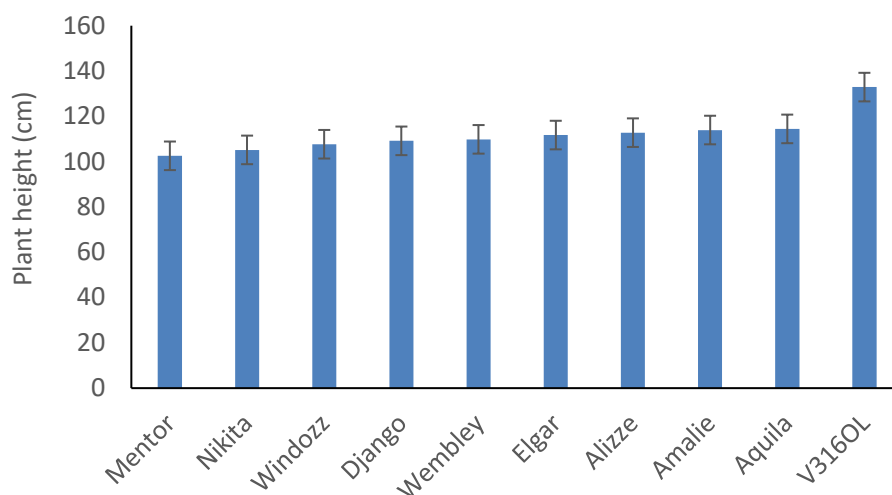


Figure 85. Plant height (cm) at the end of flowering in 10 WOSR varieties at Boxworth in 2018/19. Bars indicate the SED.

### Yield

Crop yield differed significantly between varieties in 2017/18 and 2018/19 at Boxworth and in 2017/18 at High Mowthorpe ( $P < 0.05$  in each case, Figure 86-88). In 2017/18 at Boxworth, Amalie was the highest yielding variety and Cracker the lowest yielding. Amalie yielded significantly higher than Elgar, V316OL, Troy, Nikita and Cracker ( $P < 0.05$ ). Alizze and Wembley yielded significantly higher than Troy, Nikita and Cracker ( $P < 0.05$ ) and Angus yielded significantly higher than Cracker ( $P < 0.05$ ). At High Mowthorpe in 2017/18, Wembley produced the highest yield and Cracker the lowest. Wembley yielded significantly higher than all other varieties except Nikita ( $P < 0.05$ ) and Nikita yielded significantly higher than Elgar and Cracker ( $P < 0.05$ ). In 2018/19 at Boxworth, V316OL had the highest yield and Aquila the lowest. V316OL yielded significantly higher than Nikita, Elgar and Aquila ( $P < 0.05$ ) and Amalie and Django yielded significantly higher than Elgar and Aquila ( $P < 0.05$ ). Comparing the yield across the trials shows consistency in the rankings of some varieties, with Amalie and Wembley consistently performing well and Elgar consistently performing less well.

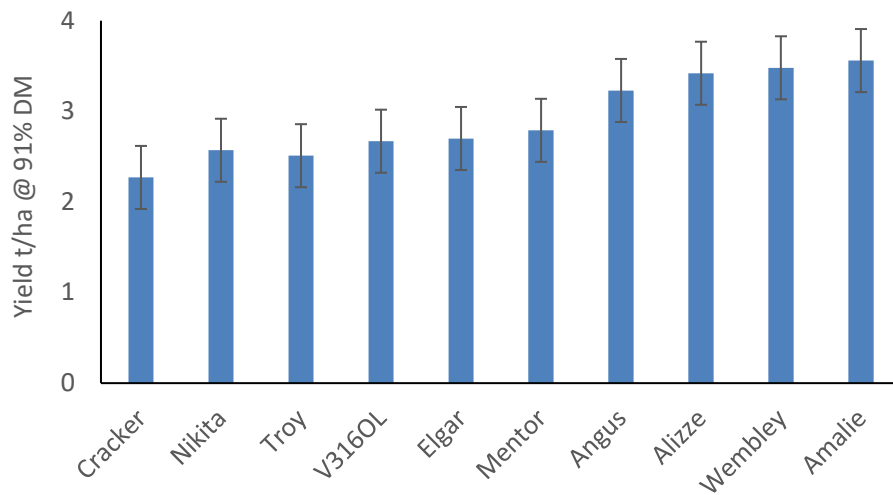


Figure 86. Yield of WOSR (t/ha @ 91%DM) in 10 WOSR varieties at Boxworth in 2017/18. Bars indicate the SED.

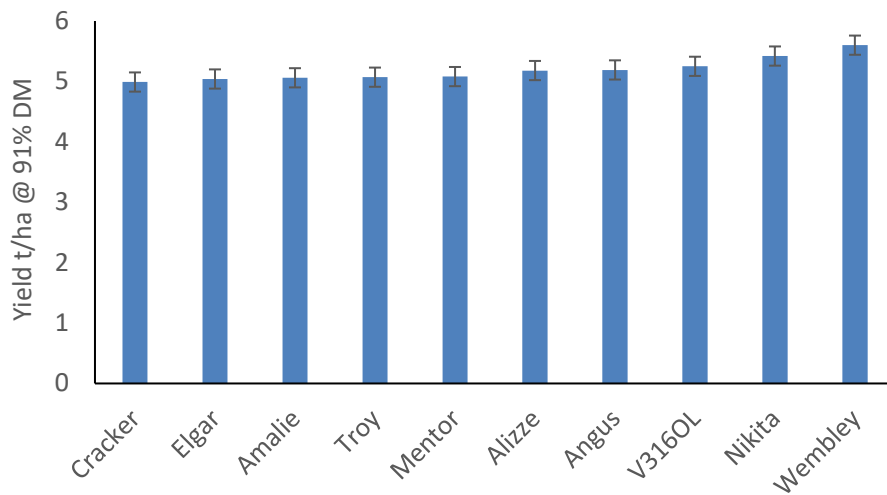


Figure 87. Yield of WOSR (t/ha @ 91%DM) in 10 WOSR varieties at High Mowthorpe in 2017/18. Bars indicate the SED.

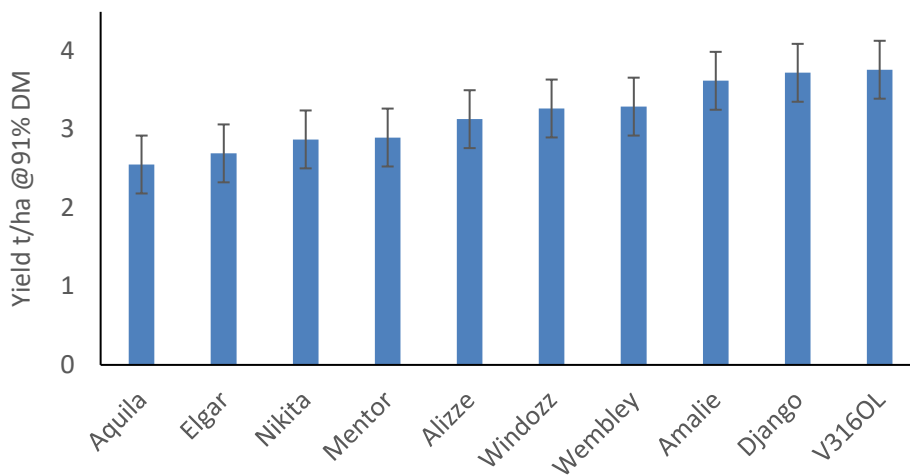


Figure 88. Yield of WOSR (t/ha @ 91%DM) in 10 WOSR varieties at Boxworth in 2018/19. Bars indicate the SED.



### Seed rate differences

For all parameters measured across five seed rates of cv Alizze the horizontal axis gives both the seed rate per m<sup>2</sup> and the number of plants established per m<sup>2</sup>. This was to show the number of plants that ultimately established and also to take account of any emergence of volunteers. This was not possible at High Mowthorpe in 2018/19 as the trial succumbed to very high adult CSFB pressure and the plots were lost.

### Plant populations and GAI

Plant populations increased with seed rate (Figure 89-91), as might be expected. Establishment rate (the plant population as a % of the seeds sown) differed between the trials. At Boxworth in 2017/18, establishment rate was similar between the seed rates, ranging from 53% at 10 seeds per m<sup>2</sup> to 55% at 120 seed per m<sup>2</sup> (Figure 89). At Boxworth in 2018/19, establishment rate was higher at lower seed rates, with 82% establishing at 10 seeds per m<sup>2</sup> and 62% establishing at 120 seed per m<sup>2</sup> (Figure 90). At High Mowthorpe in 2017/18, establishment rate was much higher at the lowest seed rate (439% establishing at 10 seeds per m<sup>2</sup>) but similar between 20 and 120 seed per m<sup>2</sup> (181% and 116% respectively) (Figure 91). The higher plant population at High Mowthorpe in 2017/18 indicates that there was a high number of volunteer OSR plants at this site.

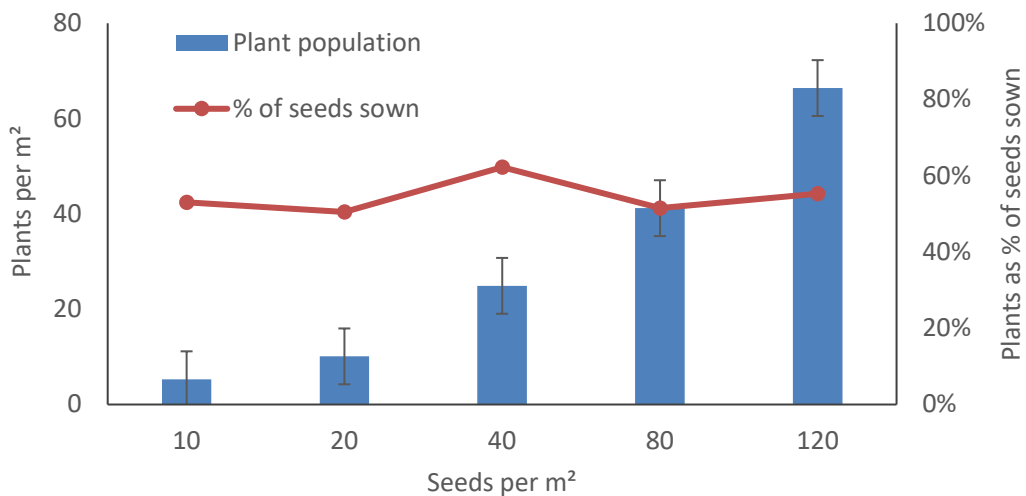


Figure 89. Mean plant populations (plants per m<sup>2</sup>) and establishment rate (plant numbers as a % of the seeds sown) at BBCH 11 in cv Alizze sown at five seed rates at Boxworth in 2017/18. Bars indicate the SED.

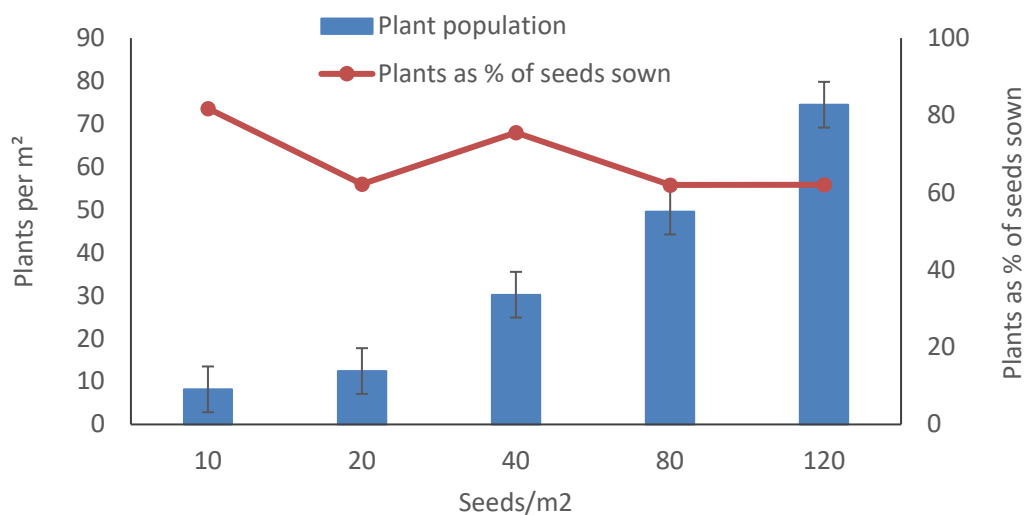


Figure 90. Mean plant populations (plants per m<sup>2</sup>) and establishment rate (plant numbers as a % of the seeds sown) at BBCH 13 in cv Alizze sown at five seed rates at Boxworth in 2018/19. Bars indicate the SED.

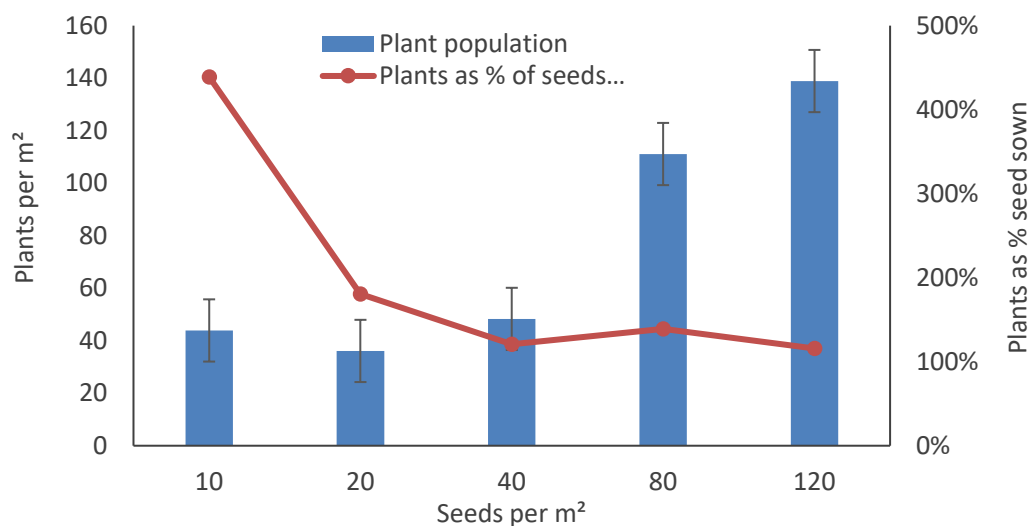


Figure 91. Mean plant populations (plants per m<sup>2</sup>) and establishment rate (plant numbers as a % of the seeds sown) at BBCH 13 in cv Alizze sown at five seed rates at High Mowthorpe in 2017/18. Bars indicate the SED.

GAI differed significantly between seed rates ( $P=0.05$ , Figure 92), with GAI significantly higher at the highest seed rate than the lowest, as might be expected.

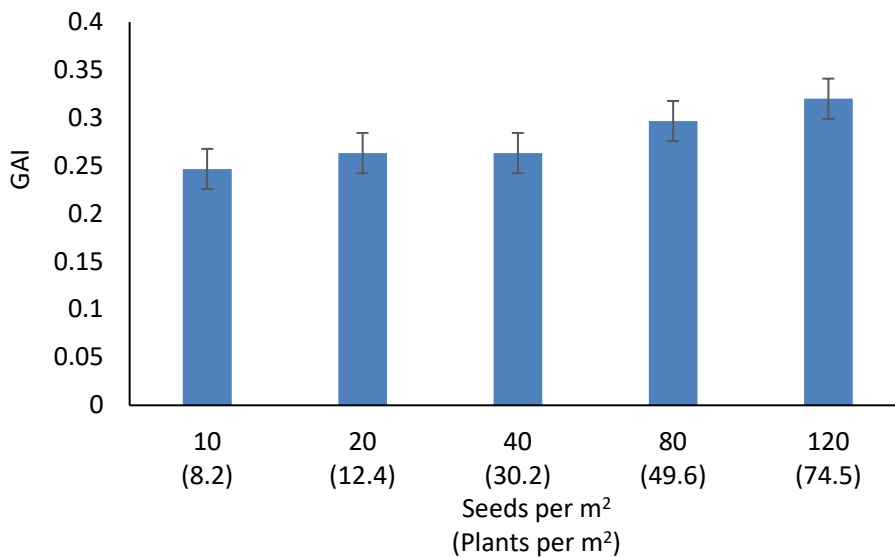


Figure 92. Mean GAI at BBCH 13 of cv Alizze sown at five seed rates at Boxworth in 2019/19. Bars indicate the SED.

#### Adult feeding damage

The % leaf area lost as a result of adult CSFB feeding did not differ significantly between seed rates at Boxworth in both 2017/18 and 2018/19 (Figure 93 & Figure 94). At this site, there was no clear evidence to suggest that increasing the seed rate helped to dilute adult CSFB feeding damage. Even in the presence of high plant populations data suggested that a similar % of leaf area would be lost as at much lower plant populations.

At High Mowthorpe the % leaf area lost differed significantly between seed rates in 2017/18 ( $P < 0.05$ , Figure 95) and in 2018/19 ( $P < 0.01$ , Figure 96). There was a trend for less damage to be recorded at the two highest seed rates in comparison with the lower seed rates. In 2017/18, significantly less damage was recorded at 80 and 120 seeds per m<sup>2</sup> than the other seed rates. In 2018/19, there was significantly less damage at 120 seeds per m<sup>2</sup> than at 10-40 seeds per m<sup>2</sup>, and significantly less damage at 10 and 40 seeds per m<sup>2</sup> than at 20 seeds per m<sup>2</sup>. This was in contrast to the data for Boxworth. Current thresholds suggest that between the cotyledon and the two-leaf stage and between the three- and four-leaf stage control is justified if 25% and 50% of leaf area is lost respectively. These thresholds were not reached in either 2017/18 or 2018/19 at either site.

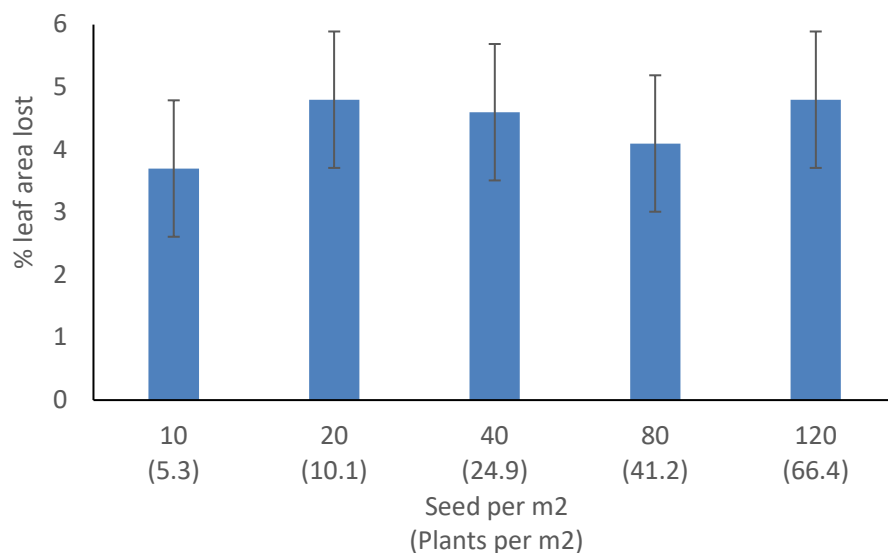


Figure 93. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 11 in cv Alizze sown at five seed rates at Boxworth in 2017/18. Bars indicate the SED.

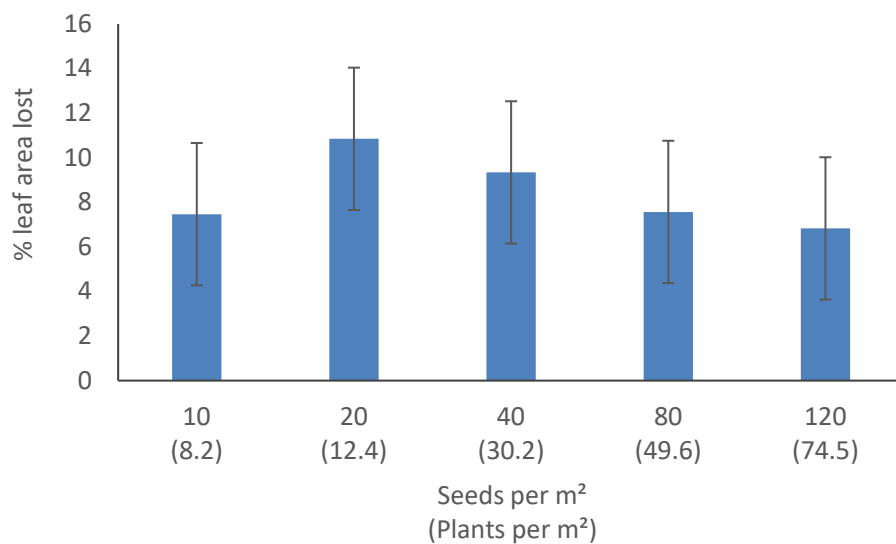


Figure 94. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 13 in cv Alizze sown at five seed rates at Boxworth in 2018/19. Bars indicate the SED.

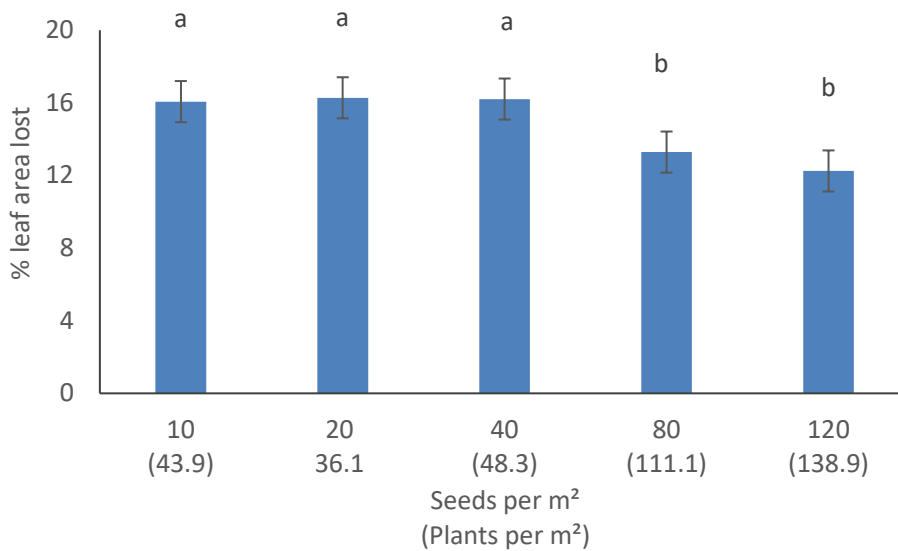


Figure 95. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 13 in cv Alizze sown at five seed rates at High Mowthorpe in 2017/18. Bars indicate the SED. Letters indicate where significant differences between treatments were observed.

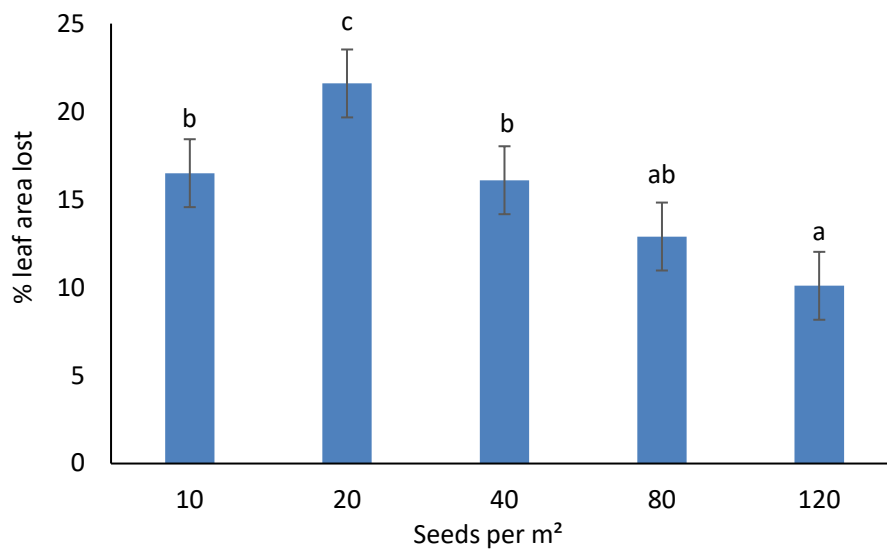


Figure 96. Mean percent leaf area lost to CSFB adult feeding damage at BBCH 14 in cv Alizze sown at five seed rates at High Mowthorpe in 2018/19. Bars indicate the SED. Letters indicate where significant differences between treatments were observed.

### CSFB larval infestation

There was no significant difference between seed rates in the number of leaf scars or larvae in the petioles (Figure 97-103), stems (Figure 104) or whole plant (Figure 105) in the autumn/winter or spring at Boxworth or High Mowthorpe in both 2017/18 and 2018/19. Very few larvae or scars were found in or on the stems in the December/January assessments, with no stem larvae in either year at High Mowthorpe and a total of 6 and 1 at Boxworth in 2017/18 and 2018/19 respectively (data not shown but seed rate differences were not significant). Between the January (Figure 101) and March assessments (Figure 103-105) at Boxworth in 2018/19, larval numbers increased at all seed

rates except 10 seeds per m<sup>2</sup>, which saw a 22% reduction. Increases in the other seed rates ranged from 19% at 20 seeds per m<sup>2</sup> to 96% at 80 seeds per m<sup>2</sup>.

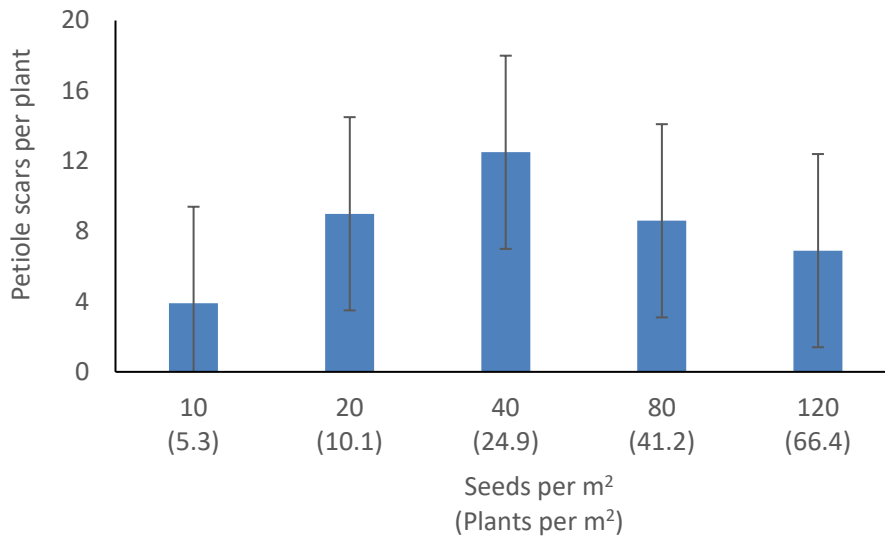


Figure 97. Mean number of petiole scars per plant caused by CSFB larval feeding in cv Alizze sown at five seed rates at Boxworth in December 2017. Bars indicate the SED.

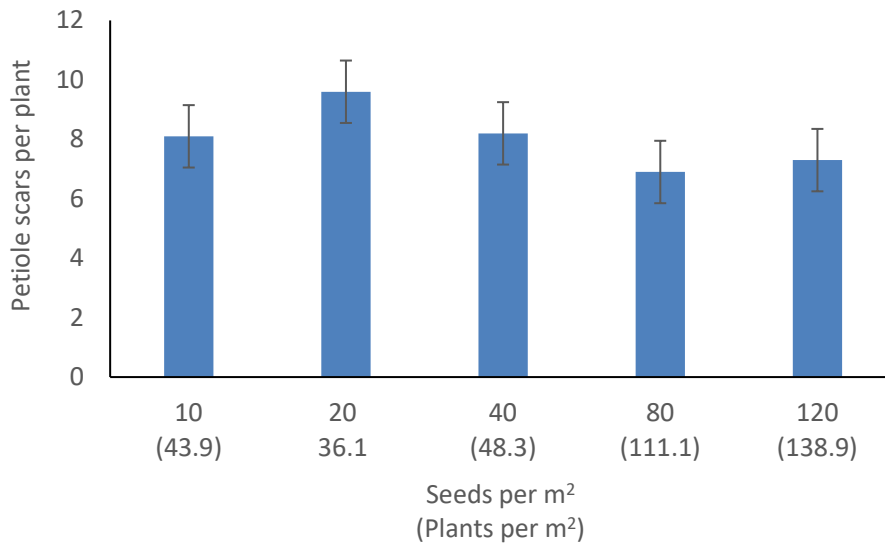


Figure 98. Mean number of petiole scars per plant caused by CSFB larval feeding in cv Alizze sown at five seed rates at High Mowthorpe in January 2018. Bars indicate the SED.

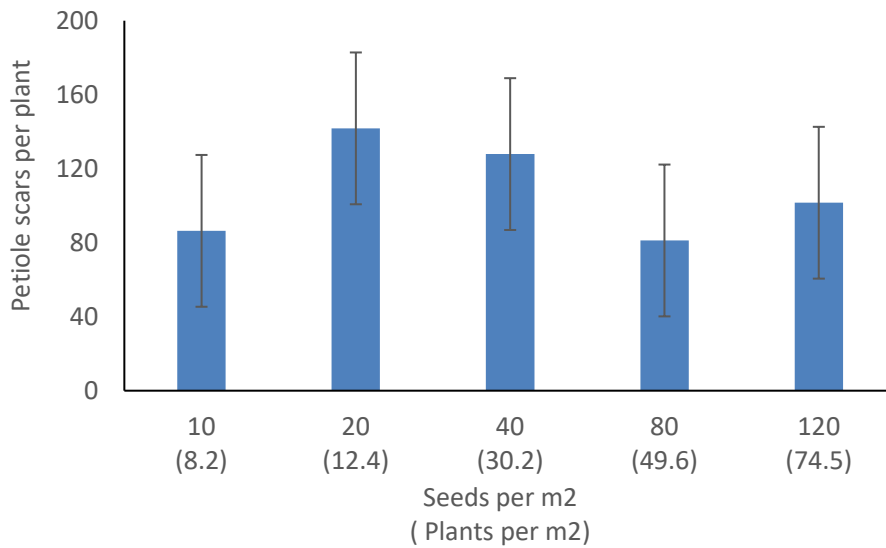


Figure 99. Mean number of petiole scars per plant caused by CSFB larval feeding in cv Alizze sown at five seed rates at Boxworth in January 2019. Bars indicate the SED.

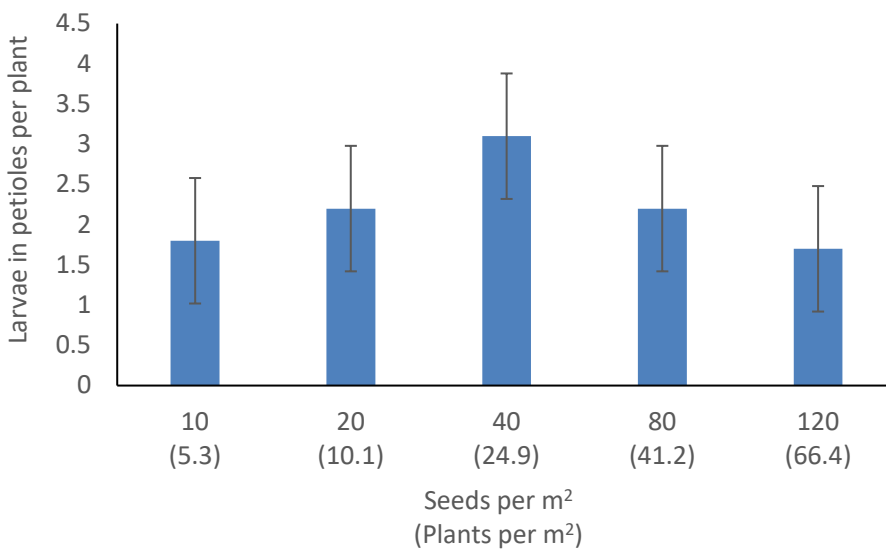


Figure 100. Mean number of CSFB larvae in petioles per plant in cv Alizze sown at five seed rates at Boxworth in December 2017. Bars indicate the SED.

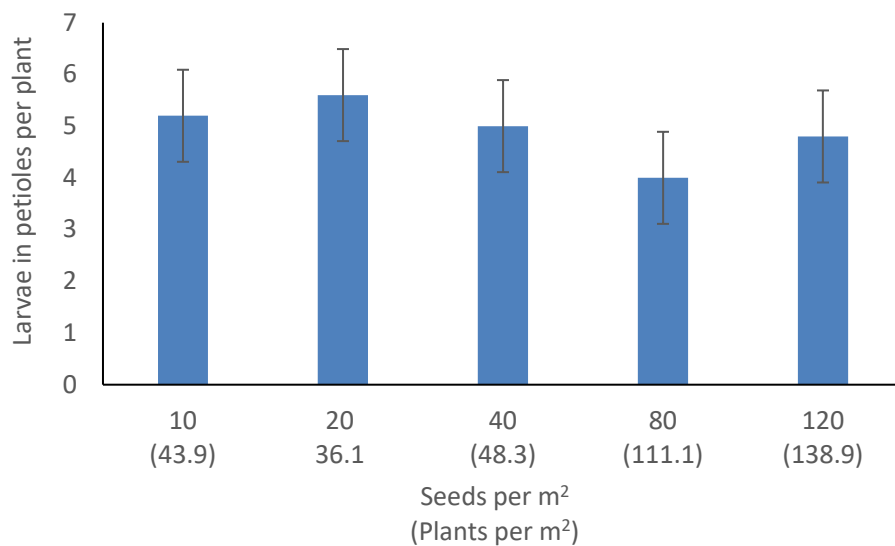


Figure 101. Mean number of CSFB larvae in petioles per plant in cv Alizze sown at five seed rates at High Mowthorpe in January 2018. Bars indicate the SED.

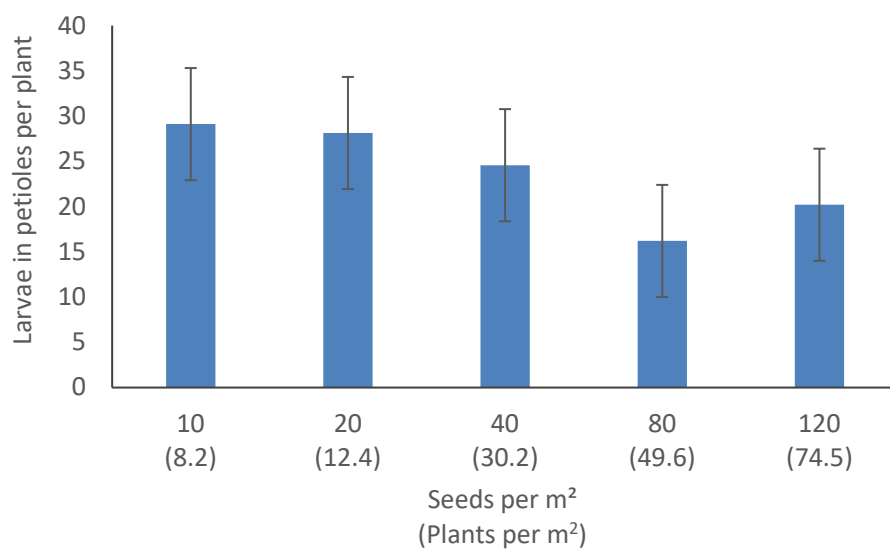


Figure 102. Mean number of CSFB larvae in petioles per plant in cv Alizze sown at five seed rates at Boxworth in January 2019. Bars indicate the SED.



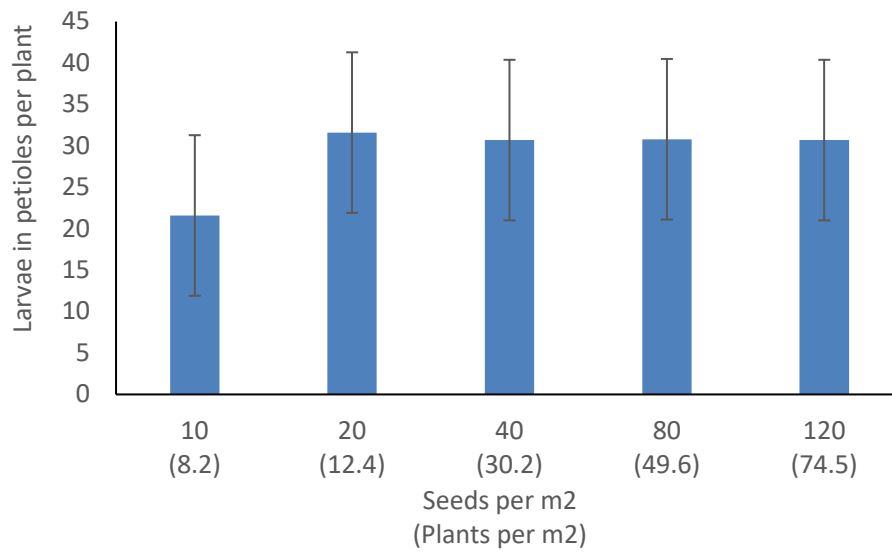


Figure 103. Mean number of CSFB larvae in petioles per plant in cv Alizze sown at five seed rates at Boxworth in March 2019. Bars indicate the SED.

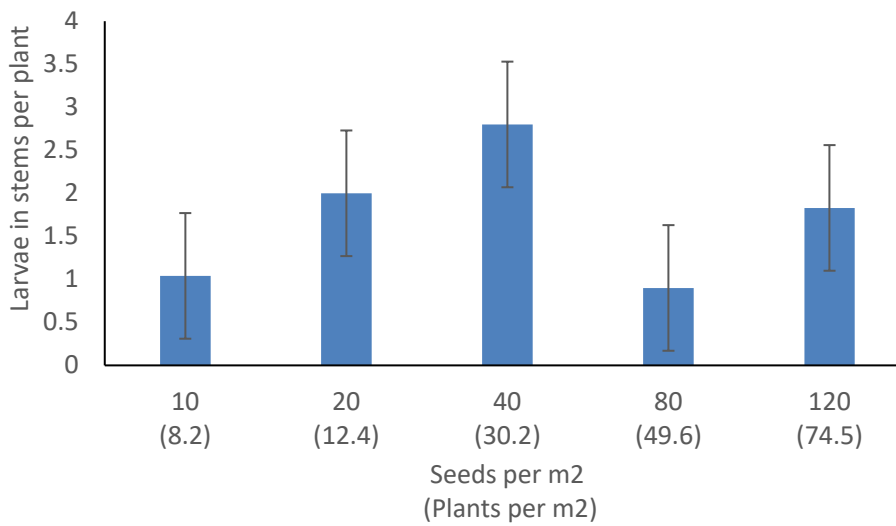
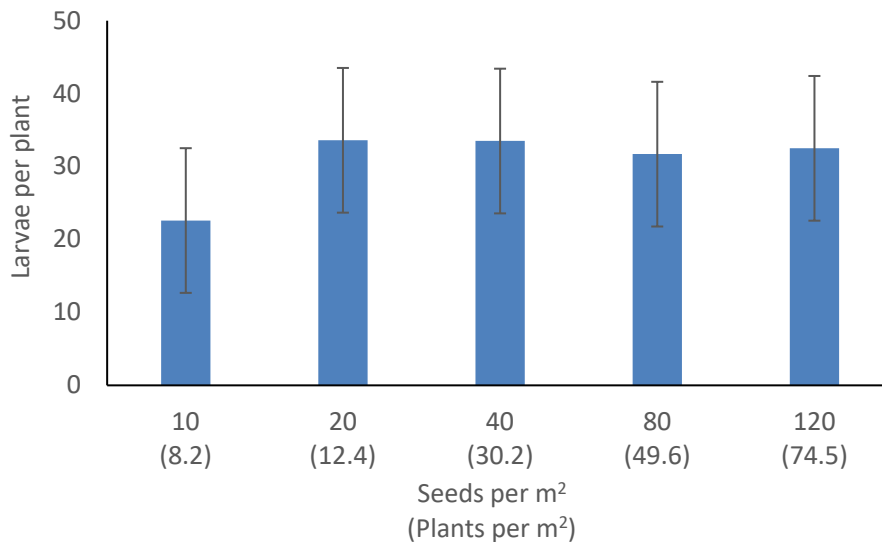


Figure 104. Mean number of CSFB larvae in stems per plant in cv Alizze sown at five seed rates at Boxworth in March 2019. Bars indicate the SED.



*Figure 105. Mean total number of CSFB larvae per plant in cv Alizze sown at five seed rates at Boxworth in March 2019. Bars indicate the SED.*

By multiplying the mean number of larvae per plant by the mean number of plants per m<sup>2</sup> at each seed rate it was possible to estimate the mean number of larvae per m<sup>2</sup> at each site in December 2017/18 and January 2018/19. There was a clear trend for increasing numbers of larvae per m<sup>2</sup> with increasing seed rate, with significant differences ( $P < 0.05$ ) between seed rates in December 2017 in Boxworth (Figure 106) and High Mowthorpe (Figure 107) but not in December 2018 in Boxworth (Figure 108). However, differences between seed rates in March 2019 at Boxworth were significant ( $P > 0.05$ ; Figure 109). This suggests that increasing seed rate has the potential to increase CSFB populations for subsequent seasons. Note that as the plant population assessment, upon which this calculation is based, occurred in September/October, any later loss of plants may mean these calculations are slight overestimations. Nevertheless they provide an indication of differences between seed rates.

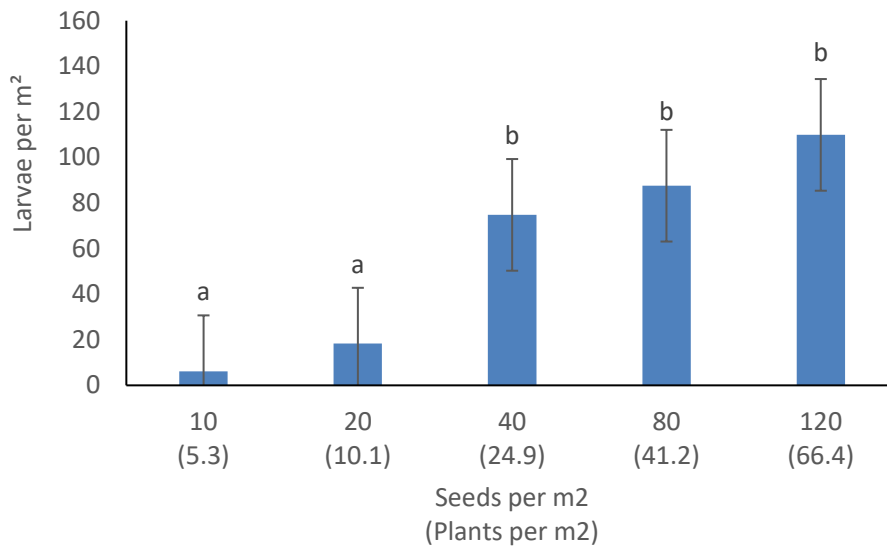


Figure 106. Mean number of larvae per m<sup>2</sup> in Alizze sown in cv Alizze sown at five seed rates at five different seed rates in December 2017 at Boxworth. Bars indicate the SED. Bars followed by the same letter or by no letter are not significantly different ( $P=0.05$ ).

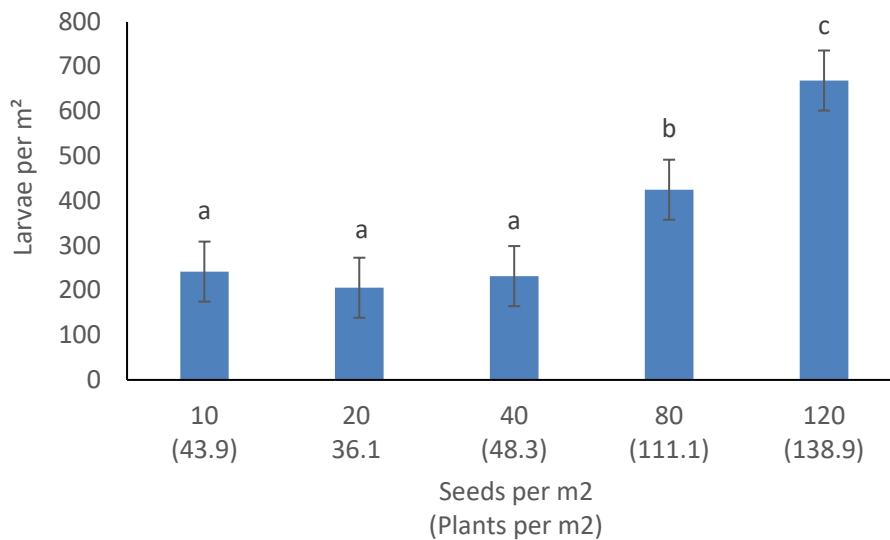


Figure 107. Mean number of larvae per m<sup>2</sup> in Alizze sown in cv Alizze sown at five seed rates at five different seed rates in December 2017 at High Mowthorpe. Bars indicate the SED. Bars followed by the same letter or by no letter are not significantly different ( $P=0.05$ ).

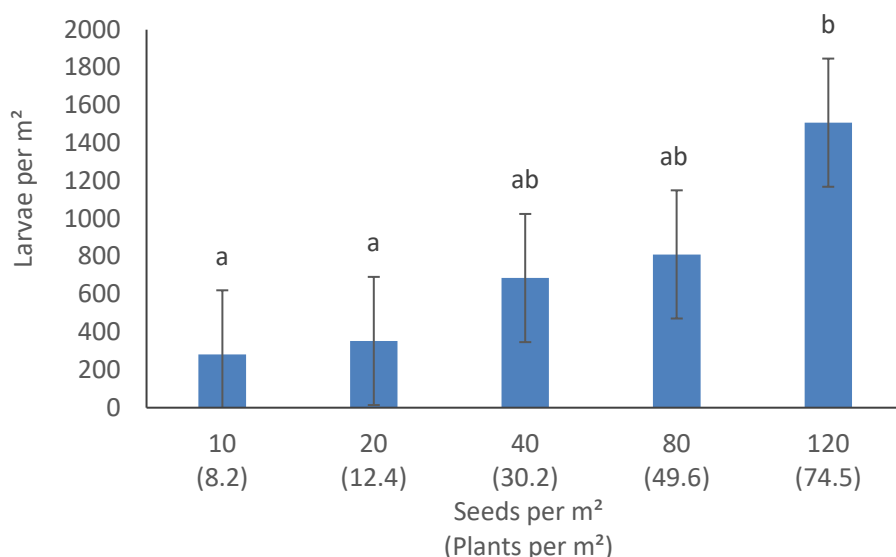


Figure 108. Mean number of larvae per m<sup>2</sup> in Alizze sown in cv Alizze sown at five seed rates at five different seed rates in January 2019 at Boxworth. Bars indicate the SED. Bars followed by the same letter or by no letter are not significantly different ( $P=0.05$ ).

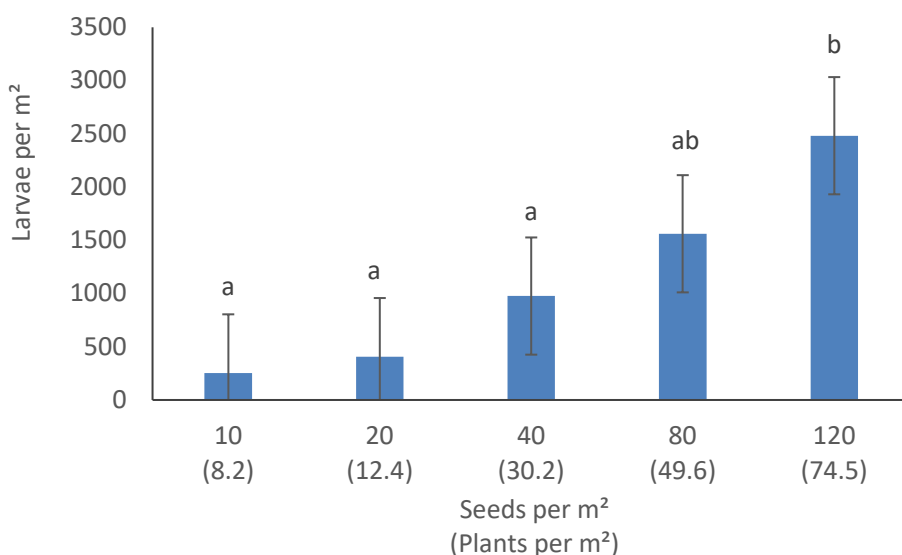


Figure 109. Mean number of larvae per m<sup>2</sup> in Alizze sown in cv Alizze sown at five seed rates at five different seed rates in March 2019 at Boxworth. Bars indicate the SED. Bars followed by the same letter or by no letter are not significantly different ( $P=0.05$ ).

### Stem width and plant height

Neither stem width or plant height differed significantly between seed rates at Boxworth in 2018/19 (Figure 110 & Figure 111). There was however, a trend for decreasing stem width with increasing seed rate (Figure 110), which is to be expected.

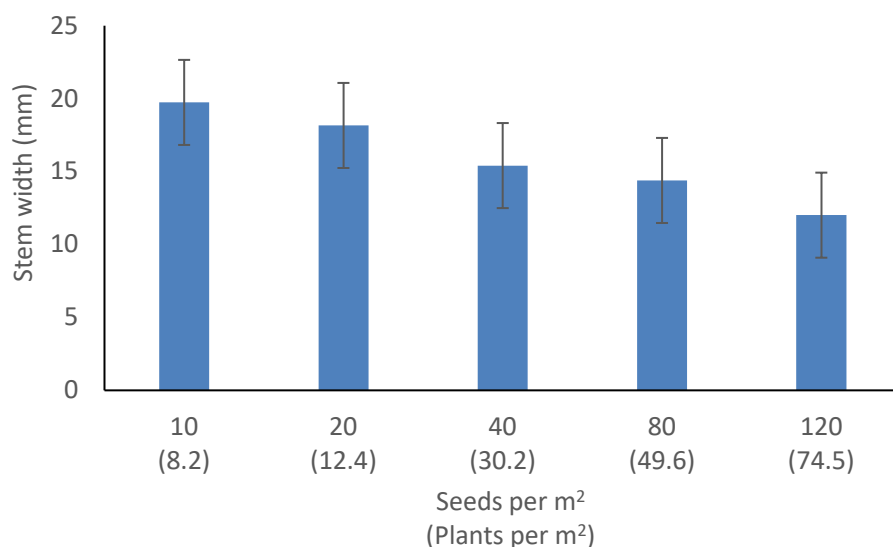


Figure 110. Mean stem width (mm) at the end of flowering in cv Alizze sown at five different seed rates at Boxworth in 2018/19. Bars indicate the SED.

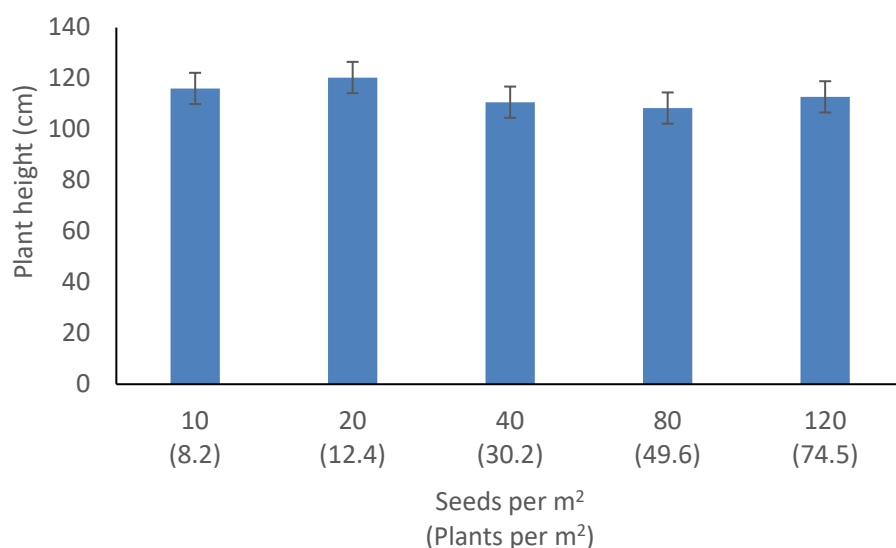


Figure 111. Mean plant height (cm) at the end of flowering in cv Alizze sown at five different seed rates at Boxworth in 2018/19. Bars indicate the SED.

## Yield

Yield differed significantly between seed rates at Boxworth in 2017/18 and 2018/19 ( $P < 0.01$  &  $P < 0.05$  respectively) and at High Mowthorpe in 2017/18 ( $P < 0.01$ ). At Boxworth in 2017/18, yield was significantly higher at 80 and 120 seed per m<sup>2</sup> than the other seed rates, and significantly higher at 40 seeds per m<sup>2</sup> than at 10 seed per m<sup>2</sup> (Figure 112). The economically optimum seed rate was 86 seeds per m<sup>2</sup>. At High Mowthorpe in 2017/18, yield was significantly lower at 10 seeds per m<sup>2</sup> than all other seed rates (Figure 113), and the economically optimum seed rate lower than at Boxworth in 2017/18 at just 34 seeds per m<sup>2</sup>. At Boxworth in 2018/19, yield was significantly

lower at 10 and 20 seeds per m<sup>2</sup> than all other seed rates (Figure 114). Here, the economically optimum seed rate was 96 seeds per m<sup>2</sup>.

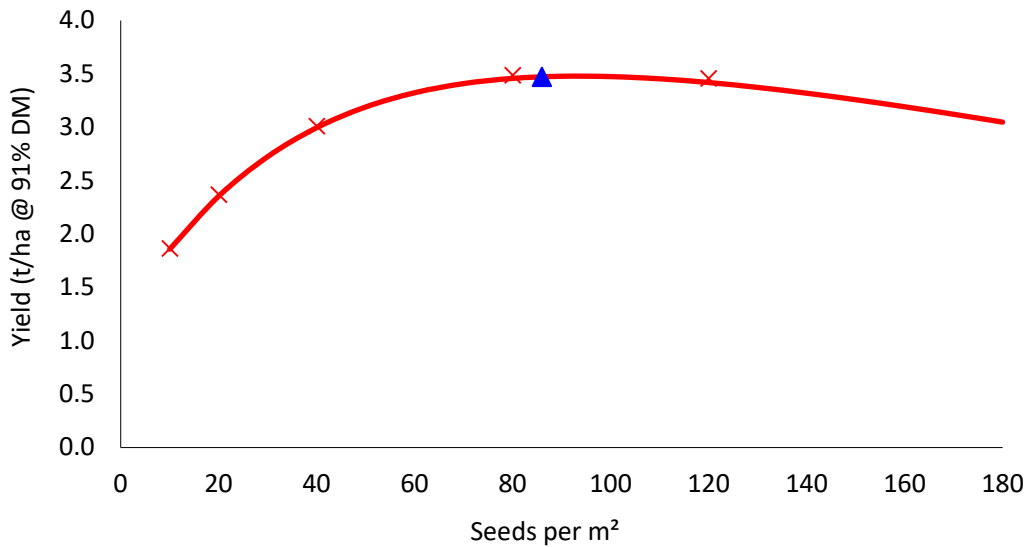


Figure 112. Yield of cv Alizze (t/ha @ 91%DM) sown at five different seed rates at Boxworth in 2017/18 and fitted yield response curve. Blue triangle indicates optimal seed rate.

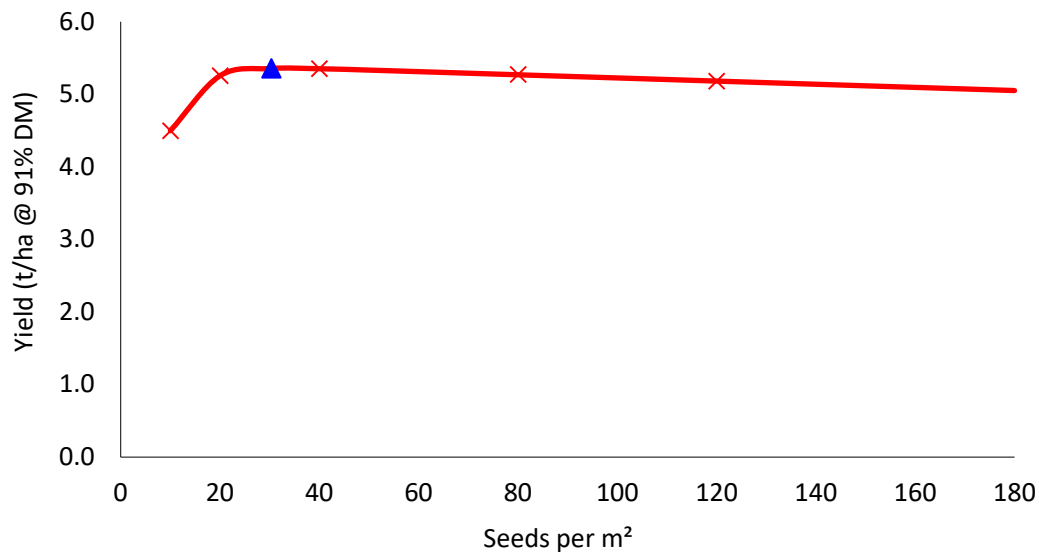


Figure 113. Yield of cv Alizze (t/ha @ 91%DM) sown at five different seed rates at High Mowthorpe in 2017/18 and fitted yield response curve. Blue triangle indicates optimal seed rate.

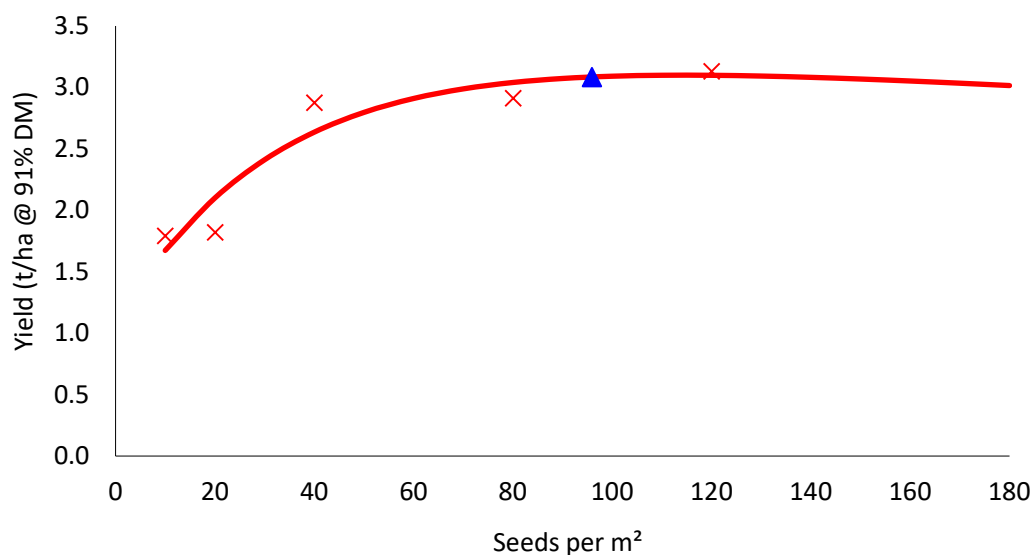


Figure 114. Yield of cv Alizze (t/ha @ 91%DM) sown at five different seed rates at Boxworth in 2018/19 and fitted yield response curve. Blue triangle indicates optimal seed rate.

## 5.4. Discussion

### 5.4.1. Varieties

CSFB pest pressure was relatively low in 2017/18 at both Boxworth and High Mowthorpe. The leaf area lost as a result of adult CSFB feeding at approximately BBCH 13 was below the threshold level that justifies treatment. Also, levels of larval infestation were lower than the current autumn threshold of five larvae per plant at Boxworth and only just above this level at High Mowthorpe. In 2016/17 at the Cowlinge and Colne RL trials and in 2018/19 at Boxworth and High Mowthorpe, CSFB pressure was much greater and sufficiently high at High Mowthorpe that the plots were destroyed. At Boxworth in 2018/19, leaf area loss was below the threshold for treatment at approximately BBCH 13 but larval numbers were about three to five times above threshold. Therefore, the CSFB pest pressure at the Cowlinge and Colne RL trials in 2016/17 and at Boxworth in 2018/19 provided the most robust test of the varieties.

The lack of significant differences between the adult feeding damage or larval pressure (scars or larval counts) between the varieties suggests that there are few inherent differences in the attractiveness and/or palatability of these varieties to CSFB. Comparing across the variety-seed rate and RL trials reveals little consistency in the ranking of varieties in terms of adult feeding damage, although Amalie did tend to experience high levels of feeding damage and Mentor, Elgar and Windozz low levels of feeding damage. It is interesting to note that these varieties have contrasting glucosinolate content in their seed, with Amalie having 13.7  $\mu$ moles per g of seed (AHDB, 2018) and Mentor, Elgar and Windozz having 10.2, 10.5 and 10.6  $\mu$ moles per g of seed respectively (AHDB, 2018; AHDB, 2019). It should be noted that glucosinolate levels in plant tissue during crop establishment are not necessarily correlated with that in the seed. Nevertheless, it is

known that glucosinolates stimulate feeding in CSFB (Bartlet *et al.*, 1994; Giamoustaris and Mithen, 1995) so if the same trends in glucosinolate levels in the seed are seen in establishing foliage then it is possible that this may be involved in the feeding damage trends seen in Angus, Amalie, Mentor, Elgar and Windozz. While it is unlikely that any benefit in terms of reducing adult CSFB damage would be seen by sowing Mentor, Elgar or Windozz on a field scale (based on this data), this does illustrate the potential for breeding varieties that are less attractive/palatable to CSFB.

There were few similarities between the trials in the ranking of varieties in terms of larval numbers or scars. The reduction in larval numbers in Elgar between January and March in the Boxworth 2018/19 trial is intriguing and may suggest that larvae can complete their lifecycle more rapidly in this variety. The large increase in larval numbers over the same period in Mentor may be due to either slower larval development in this variety or that adult CSFB were preferentially choosing to lay late-laid eggs (which result in later larval invasion) by this variety. However, these findings are from a single trial and require further investigation.

Comparison between the variety-seed rate and RL trials of the ranking of varieties in terms of plant populations and establishment indices shows that Mentor tended to establish well (in relation to the number and evenness of plant emergence) and Amalie relatively poorly. V316OL was the tallest variety, which is in line with RL results (AHDB, 2019). Few differences in GAI and stem width were found.

Potential resistance/tolerance to CSFB was assessed by ranking varieties from 1-10 in terms of their yield (1 = lowest yield, 10 = highest yield), the % leaf area lost due to adult CSFB feeding (1 = lowest damage, 10 = highest damage) and the population of CSFB larvae in the autumn/winter (1 = lowest population, 10 = highest population, Table 16). To better compare varieties, the yield rankings were based on adjusted yields, which accounted for the intrinsic differences in yield potential (as described in Section 5.2.2). Varieties that recorded the highest yields whilst having high levels of leaf loss due to CSFB or high levels of CSFB larvae might be said to be showing some degree of tolerance to the pest. Equally varieties which recorded the highest yield whilst having the lowest level of leaf loss or low levels of larval infestation might be exhibiting some level of resistance to the pest. Some caution is necessary when interpreting these data as values were calculated from between one and three replicate trials.

There was no strong evidence that any of the varieties grown were any more or less susceptible to CSFB than any other. Leaf loss on Amalie and Django was high, yet both were able to yield better than other varieties that experienced less adult CSFB feeding damage. It should be noted that Amalie is resistant to TuYV and tends to yield comparably lower compared to other varieties in RL



trials (ADHB, 2017; AHDB, 2018) so it is possible that its good performance in these trials was due to poor TuYV control, despite a cover spray to control the vector, peach-potato aphid. Django was assessed in a single trial that went to harvest. Wembley had some of the highest levels of CSFB larval infestation experienced over the two years of variety-seed rate trials and was still among the highest yielding varieties. Whether these observations provide any indication of inherent tolerance to loss of green leaf area or larval infestation is difficult to determine but provides a potential starting point for future comparison of WOSR varieties.

*Table 16. Ranking of varieties in the variety-seed rate trials (1-10, where 1 is the lowest and 10 the highest ranking) in terms of the yield, % leaf area loss due to adult CSFB damage and CSFB larval numbers in the autumn/winter. Red values indicate low yield, high adult damage or high larval numbers. Green values indicate high yield, low adult damage or low larval numbers. Bold value 1 = 2017/18 at Boxworth, 2 = 2017/18 at High Mowthorpe, 3 = 2018/19 at Boxworth. \* = potential tolerance to adult CSFB feeding. \*\* = potential tolerance to CSFB larvae. ¥ = potential resistance to adult CSFB feeding. † = potential resistance to CSFB larvae.*

Variety	Yield				% leaf area lost to adult CSFB				CSFB larval numbers			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
Alizze	7	2	5	4.7	7	4	6	5.7	8	7	7	7.3
Amalie	10	7	10	9.0	9	8	5	7.3*	7	1	4	4.0†
Angus	8	4	-	6.0	1	9	-	5.0	4	8	-	6.0
Aquila	-	-	1	1.0	-	-	9	9.0	-	-	10	10.0
Cracker	1	9	-	5.0	8	1	-	4.5	6	9	-	7.5
Django	-	-	9	9.0	-	-	7	7.0*	-	-	1	1.0†
Elgar	3	1	2	2.0	5	3	3	3.7	2	6	8	5.3
Mentor	6	6	4	5.3	4	5	1	3.3	5	4	3	4.0
Nikita	2	5	3	3.3	3	7	10	6.7	9	2	9	6.7
Troy	4	10	-	7.0	6	6	-	6.0	2	2	-	2.0
V316OL	5	3	8	5.3	10	2	8	6.7	1	10	5	5.3
Wembley	9	8	7	8.0	2	10	4	5.3	10	4	6	6.7**
Windozz	-	-	6	6.0	-	-	2	2.0¥	-	-	2	2.0†

- Not included in the trial.

Windozz ranked low for adult feeding damage and larval populations but ranked moderately well for yield. Similarly, Amalie and Django ranked well for yield but low for larval numbers. However, Django and Windozz only appeared in a single trial that went to harvest. Nevertheless, this may or may not indicate some level of resistance to the pest and is at least worth further investigation. The AHDB-sponsored recommended list trials provide a perfect opportunity to screen varieties for tolerance/ resistance/ attractiveness/ palatability to CSFB as all varieties are sown together and experience a similar exposure to the pest. It would be relatively straight forward to design an assessment protocol which would allow comparison of varieties and potentially identify traits that might help future breeding programmes to develop varieties that are better able to cope with CSFB attack.

The lack of differences in yield response to different larval loads between the five varieties in which larval loads were manipulated using different spray regimes suggests that these varieties do not differ in their ability to tolerate larval damage. However, the range of larval loads was relatively small, ranging from 1 to 3 larvae per plant. It is possible that differences in tolerance would be evident at higher larval loads.

Based on current RL list recommended varieties, these trials suggest that plant breeding is unlikely to provide a potential control option for CSFB in the near future, although it is unclear what other varieties might be in the pipeline. It may be necessary to screen a wider gene pool of less agronomically acceptable varieties to detect evidence of tolerance/resistance to the pest. Developing varieties that show tolerance and or resistance to CSFB adults and/or larvae must remain as a long-term goal for future control of this pest. While varieties that are resistant or tolerant to CSFB may be some years from market, other traits of currently available varieties may be important in managing CSFB. For example, varieties with good autumn vigour may be better able to grow away from adult CSFB damage. This was illustrated by results at both sites in 2018/19, where varieties with higher establishment indexes tending to have lower levels of adult CSFB damage. Varieties with good spring vigour may also be more tolerant to larval damage, although this was not assessed in this work. In the short and long term, understanding the potential and role of varieties as a component of an IPM strategy for sustainable CSFB control will be necessary.

#### **5.4.2. Seed rate**

It has been suggested that increasing seed rate might mitigate against CSFB pressure by diluting the impact of the pest on individual plants. In the two trials at High Mowthorpe there were significant reductions in adult feeding damage at the highest seed rates, however the plant population consistently exceeded the seed rate in the 2017/18 trial, suggesting that there were large numbers of volunteer plants. These volunteers increased the plant population to 111 and 139 plants per m<sup>2</sup> at the two highest seed rates respectively, which may have diluted the feeding pressure and resulted in the lower levels of damage at these seed rates. The high number of volunteers may also explain in part, the lower optimum seed rate at this site. Consequently, it is difficult to draw any firm conclusions about the impact of seed rate in this trial, as it is likely to be confounded by the higher plant numbers. In the other two trials no significant differences in adult feeding damage were seen. As would be expected, plant populations had a clear tendency to increase with seed rate. However, the proportion of plants establishing was either similar between seed rates (Boxworth 2017/18) or decreased with increasing seed rate. This is surprising as it might be expected that plots with smaller plant numbers would lose a greater proportion of plants to CSFB than neighbouring plots with greater plant numbers. As, in the absence of pest damage, plant establishment rate would generally be expected to decrease with increasing seed rate, this

indicates that the proportion of plants lost to CSFB is largely independent of seed rate and suggests that CSFB adults make density-dependent choices about which plants to feed on.

Seed rate had no effect on larval damage or populations, with similar larval numbers found regardless of seed rate or plant population. This is surprising as, assuming that each plot experienced similar levels of adult CSFB pressure and in turn egg-laying, it would be expected that higher larval numbers would be recorded in plots with fewer plants. It has been suggested that larval infestations are regulated in a density-dependent fashion, either through higher levels of mortality in over-infested plants or by larvae dispersing to less infested plants (Thioulouse, 1987). Alternatively, adult CSFB may choose to avoid laying eggs around plants that already have high numbers of eggs around them or have high larval infestations. Increases in larval populations between January and March were recorded at all seed rates except the lowest seed rate, which saw a reduction in larvae per plant. This indicates that CSFB adults may have laid eggs in late winter in areas with higher plant populations. However, a March larval assessment only occurred in one trial, so further work is needed to confirm this effect. One clear effect of seed rate was to increase the number of CSFB larvae per  $m^2$ . As seed rate had little impact on the numbers of CSFB larvae per plant but plant number increased with increasing seed rate, the total number of CSFB larvae per unit area also increased. Therefore, rather than potentially benefiting the crop, increasing seed rate may result in greater problems the following season by increasing the numbers of pupae returning to the soil and, in turn, the number of adult CSFB emerging from the crop. By increasing seed rate from 10 to 120 seeds per  $m^2$  the numbers of CSFB larvae per  $m^2$  was also increased by approximately than 10-fold in some trials. Assuming all larvae develop to adults, this equated to over 24 million CSFB adults per hectare at 120 seed per  $m^2$  in the trial with the highest CSFB larvae pressure (Boxworth 2017/18), compared to approximately 2.5 million per ha at 10 seed per  $m^2$ . Adult CSFB are thought to be able to fly at least two miles (Bonnemaïson, 1965) and so high numbers emerging from an WOSR crop will put nearby crops in the autumn at greater risk. There was also some evidence to suggest that increasing seed rate decreased stem width and this could make plants more susceptible to feeding by CSFB larvae. In turn, this could make plants potentially more prone to lodging if stems became severely weakened by CSFB larval feeding.

Significant reductions in yield were seen at the lowest seed rates, which is expected where these resulted in suboptimal plant populations. In the two Boxworth experiments, optimum seed rates were 86 and 96 seeds/ $m^2$  in 2017/18 and 2018/19 respectively. These are likely to have equated to optimum plant populations of approximately 48 and 60 plants per  $m^2$ . These optimum plant populations are higher than those shown in previous trial work, which range from 29-40 per  $m^2$  (but higher in the presence of a spring drought) (Roques & Berry, 2015). The yield response in the Boxworth 2017/18 trial may have been affected by the very dry June and July 2018 conditions in

the East of England (Met Office, 2020), which may have restricted compensatory branching and thus increased the required plant population for yield. In the 2017/18 trials, the removal of plants for larval assessments from all plots would have further reduced plant populations below optimal levels for the low seed rates (an oversight that was rectified in the 2018/19 trials). The yield response in the Boxworth 2018/19 trial may have been affected by the very dry conditions in September 2018 in the East of England (Met Office, 2020), which would have negatively affected establishment. April 2019 was also particularly dry in the East of England (Met Office, 2020) and this would have restricted branching in WOSR and therefore crops with low plant populations would not have been able to compensate. The optimum seed rate in the High Mowthorpe 2017/18 experiment was much lower at 34 seeds per m<sup>2</sup>, with an optimum plant population of approximately 42 plants per m<sup>2</sup>, which is more in line with the literature. Overall, this suggests that increasing seed rate may be beneficial for reducing losses to CSFB in terms of suboptimal plant populations establishing if conditions are dry at establishment or spring conditions do not promote compensatory growth.

This work suggests that large increases in seed rate had limited benefits in mitigating CSFB damage. Moderate reductions in leaf area lost at the highest seed rates were seen only in situations with very high CSFB pressure (High Mowthorpe 2018/19) or high numbers of volunteers (Mowthorpe 2017/18). Evidence for the impact of leaf area lost on yield during establishment is limited, with Ellis *et al.* (2009) finding no source for the origin of the current adult CSFB leaf area lost treatment thresholds, and this is further investigated in Section 6. Additionally, increasing seed rate did not appear to affect the proportion of plants lost to CSFB. Seed rate should be calculated based on the rate needed to achieve optimal plant populations. To reach optimal plant populations growers need to predict volunteer populations and plant establishment rates (plants as a % of seed sown), which is largely based on soil conditions, cultivation, sowing method, moisture and plants lost to pests (e.g. slugs and CSFB) (Blake *et al.*, 2004; Roques & Berry, 2015). Slight increases in seed rate may be required to compensate for plants lost to CSFB, especially where CSFB pressure is high or where conditions that are poor for establishment (e.g. dry soil or lack of rain) coincide with moderate CSFB pressure. The effect of seed rate on yield appeared to be more closely related to whether optimal plant populations were achieved rather than differences in CSFB damage. In fact, this work suggests that increasing seed rate may actually be detrimental by increasing larvae per unit area and, in turn, pest pressures for the following season. Additionally, increasing seed rate to create an excessively large plant population would result in increased seed costs, lodging risk and produce an over-large canopy that would require careful management. Whether these effects seen at plot scale would also occur at field scale is unknown and would be worth further investigation.

## 5.5. Conclusions

- There was no clear evidence to suggest that WOSR varieties differed in their susceptibility or attractiveness to CSFB.
- Limited evidence suggested that despite experiencing some of the highest levels of leaf loss Amalie and Django were still able to yield better than other varieties that were exposed to less adult CSFB feeding damage. This suggests some level of tolerance to adult feeding damage and may be worthy of further investigation.
- Wembley had some of the highest levels of CSFB larval infestation and yet was still among the highest yielding varieties, suggesting some level of tolerance to larval feeding and may be worthy of further investigation.
- Amalie, Django and Windozz had relatively low adult feeding damage and/or larval populations but ranked well for yield, suggesting potential resistance and may be worthy of further investigation.
- It is difficult to conclude that these trials provide any strong indication of an inherent tolerance or resistance to loss of green leaf area or larval infestation.
- The potential to breed varieties for reduced palatability/attractiveness to CSFB is illustrated by the contrasting levels of adult feeding damage in Amalie, Mentor, Elgar and Windozz, which was correlated with contrasting glucosinolate levels.
- Based on current RL varieties, results suggest that plant breeding is unlikely to provide a potential control option for CSFB in the near future. Nevertheless, plant breeding still has an important role to play as a component of an IPM strategy for CSFB but it may be necessary to screen a wider gene pool to detect evidence of tolerance/resistance to the pest.
- In the short-term, varietal choice may be important for CSFB management in terms of characteristics such as autumn and spring vigour. Although such varietal characteristics were not investigated in this work, their role is worth consideration.
- There was some suggestion that damage from adult CSFB was lower at high seed rates though this was not consistent across the trials.
- Increasing seed rate had little effect on the proportion of plants lost to adult CSFB or larval numbers per plant.
- To ensure optimal plant populations are achieved, small increases in seed rate could be used in high CSFB pressure situations or when moderate CSFB pressure coincides with dry conditions at establishment.
- Increasing seed rate resulted in higher numbers of larvae per unit area, thereby potentially increasing pest pressure in following crops.

## 6. Understanding crop tolerance to adult feeding damage and larval infestation and use this to revise thresholds for adults and larvae

### 6.1. Introduction

Thresholds are pivotal in determining the need for insecticide treatment, limiting the number of applications, and therefore the potential for the development of resistance, and minimising effects on non-target predators and parasites that potentially can keep pest numbers in check. There are thresholds to justify treatment against both the adults and larvae of CSFB. Thresholds to justify treatment against adult CSFB damage are more than 25% leaf area eaten at the cotyledon to two true leaf stage or more than 50% leaf area eaten at the three to four true leaf stage, however the origin of these thresholds is unknown (Ellis *et al.*, 2009). The decision to apply an insecticide is based on a subjective assessment of the level of leaf area lost made by the farmer or agronomist. Deciding whether the treatment threshold has been reached can be difficult as it is easy to overestimate the level of damage without some form of reference material (e.g. Figure 115).



Figure 115. Percent leaf area of rape eaten by flea beetles. Taken from EPPO PP 1/218(1) (2001).

The current threshold for control of CSFB larvae is five larvae per plant. This was based on insecticide trials that showed that, in the absence of an effective treatment, five larvae per plant in the autumn can be expected to reduce yield by 0.34 t/ha (Purvis, 1986). However, it is unknown whether modern OSR varieties differ in their response to larval feeding compared to those used in the Purvis study. More recent work comparing larval numbers in February/March with yield found that five larvae per plant might be expected to reduce yield by 0.46 t/ha (White & Cowlrick, 2017), however this is based on surveys of farm crops rather than randomised, replicated trials.

The need for an insecticide spray against CSFB larvae can be assessed in one of three ways:

1. A plant sample can be taken from the field, the leaf petioles and stems dissected, and the number of larvae counted. This can be a laborious process and care is needed in differentiating between CSFB larvae and the larvae of other stem-boring insects, and so is best undertaken by a certified laboratory. Whilst plants are frequently dissected to look for larvae it is rare that a 25-plant sample is assessed to make an accurate count of numbers per plant.
2. The number of leaf scars (the entry and exit holes left by larvae as they move around the plant) can be used as a less time-consuming alternative to plant dissections to establish whether the larval threshold has been exceeded. Currently, a treatment is justified if 50% of petioles have leaf scarring. This threshold was established by Walters *et al.* (2001) and corresponds to the larval threshold of five larvae per plant (Purvis, 1986).
3. The need for a larval spray can also be determined by monitoring for adult CSFB numbers. Green (2008) showed that the number of adults caught in yellow water traps was significantly correlated with larval infestations. He advocated putting four yellow water traps into a WOSR crop in early September (two in the headland and two in the field along a wheeling) and monitoring the numbers of adult beetles until the end of October. A spray was considered to be justified if catches were greater than a mean of 96 beetles per trap over the monitoring period.

In view of the widespread incidence of CSFB resistance to pyrethroid insecticides there is a need to clarify thresholds for both adults and larvae. This will enable a rational approach to insecticide use more compatible with IPM for CSFB and the demands of the Sustainable Use Directive. There has been limited research on the relationship between CSFB damage by adults and larvae on WOSR yield and yet this is vital in the development of robust thresholds for the pest. Severe CSFB feeding can cause total crop loss and, although this is still the exception and not the norm, it has become more common in many areas of the country. Understanding the relationship between yield loss and pest infestation is inextricably linked with an understanding of crop tolerance to damage; that is the ability of the crop to achieve potential yield even in the presence of the pest. Investigation of crop tolerance to pest attack has been instrumental in the development of robust pest thresholds for both pollen beetle (Ellis & Berry, 2012; Ellis *et al.*, 2017) and wheat bulb fly (Storer *et al.*, 2018).

It is clear that pest damage does not always equate to loss of yield and understanding the threshold beyond which yield is reduced is vital to developing a rational approach to insecticide use. Sprays to control what is effectively cosmetic damage are not sustainable and will only lead to further insecticide resistance. Regrettably, the first question often asked about any pest infestation is, “what can be used to control it?” rather than, “does it need to be controlled?” It is understandable that farmers and agronomists when faced with a potential yield loss initially

consider what chemical will give the best control. However, this does little to advance our knowledge of pest management or our understanding of whether the pest is having a significant impact on yield.

AHDB project 214-0009 (Ellis, 2015), showed that OSR was able to tolerate a much greater loss of leaf area than previously thought. Experiments showed plants that were pruned by having both cotyledons removed yielded as much dry matter at the six-leaf stage as those that were unpruned. The pot experiment was done in a glasshouse/poly tunnel, where conditions would have been more favourable for crop growth than in the field but in spite of this results demonstrated the inherent capacity of OSR to tolerate loss of leaf area and suggests that the current thresholds for adult damage may be too conservative. AHDB Cereals & Oilseeds project 2140005 (Ellis *et al.*, 2017) simulated pigeon damage by completely mowing plots off between December and March. Despite this significant level of defoliation, there was only an average yield reduction of just 0.1 t/ha, which again indicates the inherent tolerance of OSR to pest damage. In CRD funded Project PS2805 'Assessing tolerance to slugs in winter wheat and oilseed rape by simulating pest damage' removal of one cotyledon and/or the first true leaf had no significant effect on yield in any of the six trials, nor was there any indication that low plant populations were more susceptible to simulated slug damage than high plant populations (Defra, 2013). However, loss of the first four true leaves did result in a significant yield reduction (Defra, 2013).

For OSR, the majority of the resources required for seed yield must be captured after flowering (Berry and Spink, 2006) and compensatory branching occurs after the onset of stem extension in the spring. Defoliation of WOSR by mowing the crop in winter has been shown to increase seed yield in some crops (Lunn *et al.*, 2001; Ellis *et al.*, 2017). Yield increases were greatest for early sown crops which had developed large canopies, but late sown crops with small canopies lost yield after defoliation. The optimum canopy size in WOSR is a green area index of 3.5 units by flowering (Berry & Spink, 2009). Previous work has shown that pruning may increase net assimilation rate in rice (Kupkanchanakul & Vergara, 1991). They suggested that if part of the green tissue is removed, the photosynthetic rate of that remaining tissue increases to compensate for the loss. It is possible that a similar mechanism could occur in OSR. If that is the case then reductions in green leaf area before the start of stem extension may not always lead to reductions in seed yield. This objective was designed to analyse further datasets on CSFB damage and yield to determine whether current thresholds are valid and, if necessary, assist in the development of more robust thresholds to rationalise insecticide use against CSFB (Objective 3).



## 6.2. Materials and methods

### 6.2.1. To understand the tolerance of OSR plants to CSFB adult feeding

#### *Historic data*

Data on the tolerance of WOSR to loss of green leaf area as a result of adult CSFB feeding was reviewed. This included data from three historic AHDB and CRD funded projects (Table 17). In addition, data were collated from all experiments in the current study in which % leaf loss was assessed in relation to crop yield.

In AHDB project 2140009 (Ellis, 2015) and CRD project PS2820 (Defra, 2014), a pot experiment was performed where a hole punch and/or scissors were used to remove parts of or entire OSR cotyledons and leaves to simulate pest feeding damage and determine the effect on green leaf area and yield. Yield was determined by oven drying the vegetative parts of the plant at the six true leaf stage. In CRD project PS2805 (Defra, 2013), a total of six field experiments were done in 2010/11 and 2011/12 where OSR cotyledons and leaves were pruned with scissors to mimic slug damage and determine the effect on yield. These experiments included hybrid and conventional varieties, sown at five different seed rates. The combination of these data will help to contribute to the understanding of how much damage an OSR plant can tolerate before it impacts on yield and will be important for reviewing thresholds for CSFB adult feeding damage.

*Table 17. Historic projects used to provide data on the impact of loss of green leaf area in oilseed rape on crop yield.*

<b>Project reference number</b>	<b>Funder</b>	<b>Year</b>	<b>Title</b>	<b>Field or pot experiment (no. of experiments)</b>
PS2805	CRD	2013	Assessing tolerance to slugs in winter wheat and oilseed rape by simulating pest damage	Field (6)
PS2820	CRD	2014	Further investigation of the tolerance of winter wheat and oilseed rape to slugs	Pot (1)
RD-2140009	AHDB	2015	Maximising control of cabbage stem flea beetle (CSFB) without neonicotinoid seed treatments	Pot (1)

In the pot experiments the pruning treatments were specified in terms of the amount of area removed from the cotyledons and leaves and this was not consistent between the two studies. As a result it was important to calculate a measure of cotyledon or leaf area loss that could be applied to both RD-2140009 and PS2820. It was decided to use the % of cotyledon or leaf area lost and that data on the loss of cotyledons should be separated from that on the loss of leaves. This was

because the cotyledons develop first and their loss might be expected to have a bigger impact on yield than loss of leaf area. Also the cotyledons are a different shape and size from the leaves so it could not be assumed that loss of the same amount of area of both would have the same impact on crop yield. In RD-2140009 the amount of cotyledon lost was specified as none, slight (20%), moderate (50%) and severe (100%, Table 18).

*Table 18. Cotyledon and/or leaf pruning treatments used in the pot experiment as part of RD-2140009 together with the calculated % cotyledon/leaf area lost.*

<b>Treatment</b>	<b>Both cotyledons</b>	<b>Leaf 1</b>	<b>Leaf 2</b>	<b>% leaf area/cotyledon lost</b>
1	None	N/A	N/A	0
2	Slight	N/A	N/A	20 cotyledon
3	Moderate	N/A	N/A	50 cotyledon
4	Severe	N/A	N/A	100 cotyledon
5	Moderate	Slight	N/A	20 leaf
6	Moderate	Moderate	N/A	50 leaf
7	Moderate	Severe	N/A	100 leaf
8	Moderate	Slight	Slight	20 leaf
9	Moderate	Slight	Moderate	35 leaf
10	Moderate	Slight	Severe	60 leaf
11	Moderate	Moderate	Slight	35 leaf
12	Moderate	Moderate	Moderate	50 leaf
13	Moderate	Moderate	Severe	75 leaf
14	Moderate	Severe	Slight	60 leaf
15	Moderate	Severe	Moderate	75 leaf
16	Moderate	Severe	Severe	100 leaf

From treatment 5 onwards the cotyledons all had the same treatments so any differences between them would be due to any pruning of leaves 1 or 2. The untreated control (treatment 1), in which there was no pruning, was used to measure yield in the absence of any loss of the cotyledons. The calculation of % leaf area lost was therefore based on just the pruning treatments applied to leaves 1 and 2. Each leaf was pruned as it became fully emerged. When just one leaf is present it is straightforward to calculate the % leaf area lost as 0% (none) 20% (slight) 50% (moderate) or 100% (severe). When two leaves are present the calculation of leaf area lost becomes more complicated: For example, when there are two leaves each leaf effectively contributes 50% of the total leaf area. Therefore in treatment 9 there was slight pruning of leaf 1 and moderate pruning of

leaf 2. This meant 20% of leaf 1 was lost which is the equivalent of 10% of the area of both leaves and 50% of leaf 2 was lost which was equivalent to 25% of both leaves. So in total 35% of both leaves was lost. This rationale was used to calculate the % of cotyledon or leaf area lost in all treatments. In this experiment no follow up treatments were done following slight and severe damage to the cotyledons as it was assumed that after slight pruning of the cotyledons the plants would survive and after severe pruning they would die. In PS2820 either a full leaf or half a leaf was removed so it was straightforward to calculate the % of cotyledon or leaf area lost. (Table 19).

*Table 19. Cotyledon and/or leaf pruning treatments used in the pot experiment as part of PS2820 together with the calculated % cotyledon/leaf area lost.*

<b>Treatment</b>	<b>Leaves pruned</b>	<b>Timing</b>	<b>% leaf/cotyledon area lost</b>
1	None	N/A	
2	One cotyledon	As leaf 1 emerging	50 cotyledon
3	Leaf 1	As leaf 2 emerging	50 leaf
4	Leaf 1 + 2	As leaf 3 emerging	66 leaf
5	Leaf 1 + 2 + 3	As leaf 4 emerging	75 leaf
6	Leaf 1 + 2 + 3 + 4	As leaf 5 emerging	80 leaf
7	Both cotyledons	As leaf 1 emerging	100 cotyledon
8	½ both cotyledons	As leaf 1 emerging	50 cotyledon
9	½ leaf 1	As leaf 2 emerging	25 leaf
10	½ leaf 1 + 2	As leaf 3 emerging	33 leaf
11	½ leaf 1 + 2 + 3	As leaf 4 emerging	37.5 leaf
12	½ leaf 1 + 2 + 3 + 4	As leaf 5 emerging	40 leaf
13	Leaf 1	After leaf 3 emerged	33 leaf
14	Leaf 1 + 2	After leaf 3 emerged	66 leaf
15	Leaf 1 + 2 + 3	After leaf 3 emerged	100 leaf
16	½ leaf 1	After leaf 3 emerged	16 leaf
17	½ leaf 1 + 2	After leaf 3 emerged	33 leaf
18	½ leaf 1 + 2 + 3	After leaf 3 emerged	50 leaf
19	Leaf 1	After leaf 2 emerged	50 leaf
20	½ leaf 1	After leaf 2 emerged	25 leaf
21	Severe shot holing of both cotyledons	As soon as emerged	50 cotyledon

In the field experiments (PS2805) the % cotyledon or leaf area lost was again used to describe the pruning treatments. In this experiment leaf pruning was undertaken to try and simulate slug

damage but as CSFB adults also remove green leaf area it was considered that data could also be used to describe the impact of these pests on yield. Again the data for the cotyledons was kept separate to that for the leaves. The full range of treatments is given in Table 20.

*Table 20. Cotyledon and/or leaf pruning treatments used in the field experiments as part of PS2805 together with the calculated % cotyledon/leaf area lost.*

<b>Treatment</b>	<b>Leaves pruned</b>	<b>% leaves lost</b>
1	None	0
2	Single cotyledon	50% of cotyledons
3	1st leaf as 2nd emerging	50
4	1 <sup>st</sup> & 2 <sup>nd</sup> as 3 <sup>rd</sup> emerging	66
5	1 <sup>st</sup> , 2 <sup>nd</sup> , 3 <sup>rd</sup> & 4 <sup>th</sup> as successive leaf emerging	80

At one site in PS2805 (High Mowthorpe in 2011) bad weather meant that it was not possible to achieve the pruning treatments as per Table 20 so at that site the treatment list was modified (Table 21). In the field experiments each pruning treatment was applied to plots of two varieties, Castille and Excalibur, which were sown at either 10, 20, 40, 80 or 160 seeds per m<sup>2</sup>.

*Table 21. Cotyledon and/or leaf pruning treatments used in the field experiment at High Mowthorpe in 2011 as part of PS2805 together with the calculated % cotyledon/leaf area lost.*

<b>Treatment</b>	<b>Leaves pruned</b>	<b>% leaves lost</b>
1	None	0
2	Single cotyledon	50% of cotyledons
3	1st leaf as 2nd emerging	50
4	Remove 1 <sup>st</sup> as 2 <sup>nd</sup> emerging & 2 <sup>nd</sup> as 3 <sup>rd</sup> as 4 <sup>th</sup> emerging	75
5	1 <sup>st</sup> , 2 <sup>nd</sup> , 3 <sup>rd</sup> & 4 <sup>th</sup> as successive leaf emerging	80

### **Data from other experiments in the current study**

Data were also collected from all experiments in the current project where % leaf loss and yield were assessed. These are listed in Table 22.

*Table 22. Experiments in current study from which data were collated to investigate the impact of loss of leaf area on yield.*

<b>Experiment</b>	<b>Year</b>	<b>Location</b>	<b>County</b>
CSFB larval impact BX17	2017	Boxworth	Cambridgeshire
Variety-seed rate BX18	2018	Boxworth	Cambridgeshire
CSFB larval impact BX18	2018	Boxworth	Cambridgeshire
Variety-seed rate BX19	2019	Boxworth	Cambridgeshire
CSFB larval impact BX19	2019	Boxworth	Cambridgeshire
CSFB larval impact HM17	2017	Huggate	North Yorkshire
CSFB larval impact HM18	2018	Kirby Grindalythe	North Yorkshire
Variety-seed rate HM18	2018	High Mowthorpe	North Yorkshire
CSFB larval impact HM19	2019	Wetwang	North Yorkshire
RL variety trial	2017	Cowlinge	Suffolk

### **Statistical analysis**

Data on the % of cotyledons and leaves lost in relation to crop yield were subjected to regression analyses to determine if there were any obvious relationships between these variables. Where no such relationships were demonstrated the treatment means and their associated yield are presented and comments provided on any trends. Where the slope of the regression line is not significantly different from zero it can be assumed that the loss of leaf area has limited if any impact on crop yield. For data from the experiments in the current study, yield data was adjusted to account for intrinsic differences in yield potential between varieties, and so better identify differences between varieties due adult feeding damage. This was done by adjusting the yield at harvest data by the seed yield given as a percentage of the control as reported in AHDB RL trials for the specific season in question. For example, in 2017/18 RL lists Angus had a seed yield of 106% of the control (AHDB, 2018) so the yield data for Angus in the 2017/18 trial in this work was adjusted down 6%.

#### **6.2.2. To understand the tolerance of OSR plants to CSFB larvae**

##### **Larval impact experiments**

This work was undertaken in all three years of the project with two experiments in each of 2017, 2018 and 2019. The intention was to use experimental treatments to create a range of CSFB larval populations and measure how these affected the yield of the crop. In the first year fleecing was used as a barrier to adult CSFB for differing periods of time to create different levels of egg laying

and ultimately different levels of CSFB larval infestation. The fleece was difficult to work with and easily holed allowing CSFB adults access to the plots. Therefore in years two and three it was replaced by different levels of pyrethroid application (one versus three sprays). Consultation with Steve Foster at Rothamsted Research suggested that although resistance to pyrethroids was widespread in CSFB populations, variable levels of application should result in different levels of larval numbers. In all three years sites with a moderate to high population of CSFB were targeted to provide a wide range of CSFB larval infestations to test.

In 2017, fleecing was used to manipulate CSFB infestation of the experimental plots. The fleece was put in place as soon as the crop has been drilled. The edges of the fleece were buried and weighed down with soil to ensure that it remained in place. Where the host farmer wanted to apply pre-emergence herbicides this was done before the fleece was put in place. There were three fleecing treatments, fleeced for two weeks after drilling, fleeced for eight weeks after drilling and unfleeced. Plots were 12m long and 3m wide with a gap of 1m between plots to allow space for burying the edges of the fleece. The two sites established in 2017 are shown in Table 23.

*Table 23. Experimental sites for manipulation of CSFB larval number by fleecing in 2017.*

<b>Location</b>	<b>Grid reference</b>	<b>OSR variety</b>
Boxworth, Cambridgeshire	TL 34385 64480	Campus
Duggleby, North Yorkshire	SE 8976855969	Barbados

If the host farmer wanted to apply nitrogen after drilling and this was likely to be more than three days after drilling, the fleece was laid and then removed before nitrogen application. It was then replaced after the nitrogen had been applied. Nitrogen applications were broadly comparable between the two sites. Although removing the fleece allowed beetles access to the crop this was only for a short while. Once the fleece was replaced it was still expected that the different fleecing treatments would create differing CSFB larval populations.

Sites were free to receive routine treatments of herbicides, fungicides and nitrogen but no insecticide sprays (such as pyrethroids) which could affect CSFB adults and larvae. Any molluscicide pellets were applied before the fleece was laid. Peach-potato aphid populations were also monitored to avoid TuYV which could confound the potential effects of CSFB larvae on crop yield. If these aphids were present suitable insecticides such as Plenum (pymetrozine) (0.3 kg/ha up to GS30) and Biscaya (thiacloprid) (0.3 l/ha) were applied. These products could only be applied once in the autumn. Plenum was the preferred option as it is primarily recommended for sucking insects and probably less likely to affect CSFB larvae. If necessary sprays to target pollen beetle and seed weevil were applied in the spring.

In 2018 and 2019 fleecing was replaced by one or three applications of pyrethroids to manipulate CSFB larval populations. The locations of the experimental sites are given in Table 24. In 2018 there were three insecticide treatments and in 2019 there were six insecticide treatments (Table 25). In both years plots were 12m long x 3m wide and experiments were laid out as a randomised block design. In 2018 there were five replicates of each of the three treatments and in 2019 there were four replicates of each of the six treatments.

The rate of application of lambda-cyhalothrin in treatment 2 and 3 was three times the approved rate so required an administrative experimental approval (AEA) and crop destruction. ABP 617, which was used in 2019 was also an experimental treatment which required an AEA and crop destruction. All sprays were applied with an OPS sprayer equipped with 02 F110 nozzles to deliver a medium spray quality at 2 bar pressure.

*Table 24. Experimental sites for manipulation of CSFB insecticide treatment in 2018 and 2019.*

<b>Year</b>	<b>Location</b>	<b>Grid reference</b>	<b>OSR variety</b>
2018	Boxworth, Cambridgeshire	TL 33191 64958	Campus
2018	Kirby Grindalythe, North Yorkshire	SE 9236570942	Fencer
2019	Boxworth, Cambridgeshire	TL 33389 64640	Campus
2019	Wetwang, North Yorkshire	SE 9461860499	Fencer

Sites were treated with routine treatments of herbicides, fungicides and nitrogen but no pyrethroid sprays other than those in the treatment list were applied. Molluscicide pellets were applied if required. The sites were monitored for aphids which could confound the potential effects of CSFB larvae on crop yield. If these were found, suitable insecticides were Plenum (pymetrozine) (0.3 kg/ha up to GS30) and Biscaya (thiacloprid) (0.3 l/ha). These could only be applied once in the autumn. Plenum was the preferred option as it is primarily recommended for sucking insects and so probably less likely to affect CSFB larvae. Sprays to target pollen beetle and seed weevil were applied if necessary in the spring.

Table 25. Insecticide treatments used to manipulate CSFB larval numbers in 2018 and 2019.

Insecticide treatment	Product & rate	Timing	Years in which treatments used
1. Untreated	N/A	N/A	2018 & 2019
2. Lambda-cyhalothrin	Hallmark Zeon or alternative @ 225ml/ha	100% emergence	2018 & 2019
3. Lambda-cyhalothrin	Hallmark Zeon or alternative @ 225ml/ha	100% emergence, two weeks later and in mid/end November	2018 & 2019
4. ABP 617	ABP 617 @ 4L/ha	100% emergence, two weeks later and in mid/end November	2019
5. ABP 617	ABP 617 @ 4L/ha + Hallmark Zeon or alternative	100% emergence, two weeks later and in mid/end November	2019
6. ABP 617	ABP 617 @ 4L/ha +Biscaya @ 300ml/ha	100% emergence, two weeks later and in mid/end November	2019

### Assessments

The programme of assessments was the same for all three project years. CSFB adult numbers were monitored by placing two yellow water traps in the trial area at the beginning of the trial. These were at least 20 m apart and 1 m from any tramlines. The location of the traps was clearly marked and a drop of detergent and a Campden tablet added to each. The trap was secured in place with Ringot pegs to prevent it from being knocked over. The traps were emptied at weekly intervals by tipping the contents into a plastic kitchen sieve (approximately 15cm diameter), lined with some muslin or net curtain. At this stage any large insects such as butterflies, bees or wasps were removed to make it easier to examine the final catch. The muslin was then gently folded and placed inside a labelled, plastic screw top container and covered with 70% alcohol to preserve the catch. Traps were then re-set for the next week's trapping. Once in the laboratory the contents of the container was washed into a plastic dish (approximately 25cm long x 15cm wide) and the number of adult CSFB was counted. These assessments were continued until at least the third week of October.

The number of plants was assessed by placing a 0.5 m rod between two rows of crop and recording all those emerged on each side at five points per plot. This was done twice at the 3-4 true leaf stage and when plants were sampled for dissection in November/December. This was done to determine if changes in plant population affected the numbers of CSFB larvae recorded.



Adult CSFB feeding damage was assessed by counting the number of shot holes and estimating the percentage leaf area lost in 50 randomly selected plants per plot at the 3-4 true leaf stage. This was done in the same areas used to assess plant populations.

In November/December 10 plants were randomly selected per plot and returned to the laboratory. The number of leaf-scars (entry/exit holes of larvae) along the petioles and stems was counted on each plant and kept separate for the petioles and stems. This was done to investigate the relationship between number of leaf scars and CSFB larvae. Walters *et al.*, (2001) indicated a highly significant relationship between the percentage of leaves showing CSFB feeding scars and the mean number of larvae per plant during autumn. All leaf petioles and stems were dissected with a sharp scalpel and the total number of cabbage stem flea beetle larvae recorded separately for the petioles and stem.

Each plot was harvested with a combine harvester and sub-samples of seed were taken for determination of moisture content. The yield at 9% moisture content was calculated.

#### **Data from other experiments in the current study**

In addition to the six CSFB larval manipulation experiments, data were also collected from all experiments in the current study where larval numbers and yield were assessed. These are listed in Table 26.

*Table 26. Experiments in current study from which data were collated to investigate the impact of loss of leaf area on yield.*

<b>Experiment</b>	<b>Year</b>	<b>Location</b>	<b>County</b>
RL variety trial	2017	Cowlinge	Suffolk
Defoliation BX17	2017	Boxworth	Cambridgeshire
Variety-seed rate BX18	2018	Boxworth	Cambridgeshire
Variety-seed rate HM18	2018	High Mowthorpe	North Yorkshire
Variety-seed rate BX19	2019	Boxworth	Cambridgeshire
Defoliation BX19	2019	Boxworth	Cambridgeshire

#### **Statistical analysis**

Data on the numbers of CSFB larvae in OSR plants in relation to crop yield were subjected to regression analyses to determine if there were any relationships between these variables. Where no such relationships were demonstrated the treatment means and their associated yield are presented and comments provided on any trends. Where the slope of the regression line is not significantly different from zero it can be assumed that CSFB larval infestation has limited if any impact on crop yield. Data were initially analysed from the larval impact trials where fleecing or

insecticide treatments were used to manipulate CSFB larval numbers. Subsequently these data were combined with all data from experiments in the current study when larval numbers and crop yield were assessed. Individual analyses were also done to investigate whether timing of larval assessment influenced crop yield. The total number of larvae in November/December, January/February and March/April were investigated. Also the number of larvae present in the stems were analysed but only in the spring assessments as none were found in the autumn/winter. For analyses of data from the current study that involved different varieties, yield data was adjusted to account for intrinsic differences in yield potential between varieties, and to better identify differences between varieties due to larval damage. This was done by adjusting the yield at harvest data by the seed yield given as a percentage of the control as reported in AHDB RL trials for the specific season in question. For example, in 2017/18 RL lists Angus had a seed yield of 106% of the control (AHDB, 2018) so the yield data for Angus in the 2017/18 trial in this work was adjusted down 6%.

### 6.3. Results

#### 6.3.1. To understand the tolerance of OSR plants CSFB adult feeding

##### *Historic data - Pot experiments*

Regression analyses provided poor fits between data on % cotyledon or leaf loss and crop yield (dry matter yield (g/plant) at the six true leaf stage). As a result mean values of these variables are provided and comments provided on any trends in the data. In RD- 2140009 there was a small decrease in the dry matter yield of plants at the six true leaf stage when 20% and 50% of the cotyledon area was removed (Table 27). Yield loss increased as the area of cotyledon loss increased. Surprisingly dry matter yield increased when both cotyledons were removed. This result was surprising as it was assumed that following the loss of both cotyledons the plants would die. It does however, suggest some tolerance probably due to compensatory growth in response to the loss of the cotyledons.

*Table 27. Mean cotyledon area lost and associated dry matter yield (g/plant) at the six true leaf stage following various levels of pruning of the cotyledons in RD-2140009.*

<b>% cotyledon area lost</b>	<b>Dry matter yield (g)</b>	<b>% change from control</b>
0	2.58	
20	2.46	-4.8
50	2.27	-12.1
100	3.65	41.6

In the same study dry matter yield also decreased with increasing loss of leaf area (Table 28). However, there was a marked increase in the impact on yield between 50 and 60% leaf area loss.

These data suggest that there may be a threshold level of leaf loss above which the plant will find it difficult to compensate.

*Table 28. Mean leaf area lost and associated dry matter yield (g/plant) at the six true leaf stage following various levels of pruning of the leaves of OSR seedlings in RD-2140009.*

<b>% leaf area lost</b>	<b>Dry matter yield (g)</b>	<b>% change from control</b>
0	2.58	
20	2.48	-4.0
35	2.41	-6.7
50	2.44	-5.5
60	1.76	-31.9
75	1.99	-23.0
100	1.71	-33.8

In PS2820 dry matter yield increased following removal of 50% and 100% of the cotyledon area (Table 29). The result for 100% loss of the cotyledons is similar to that in RD-2140009 although the level of increase is not so great. Interestingly loss of 50% of cotyledon area also increased yield in contrast to the yield reduction in RE-2140009. Across both studies five levels of loss of cotyledon area were investigated and three of these resulted in an increase in dry matter yield.

*Table 29. Mean cotyledon area lost and associated mean dry matter yield (g/plant) at the six true leaf stage following various levels of pruning of the cotyledons in PS2820.*

<b>% cotyledon area lost</b>	<b>Dry matter yield (g)</b>	<b>% change from control</b>
0	3.08	
50	3.64	18.3
100	3.30	7.1

Trends in the data for dry matter yield in relation to loss of leaf area in PS2820 are summarised in Table 30. At up to 33% loss of leaf area dry matter yield increased. At subsequent levels of leaf loss there a trend for yield to decrease particularly at levels above 75% leaf area lost. An exception was at 50% leaf area lost which increased dry matter yield. Removing 37.5%, 40% and 60% of leaf area reduced dry matter yield although the effects were not as dramatic as when leaf loss exceeded 75%.

Table 30. Mean leaf area lost and associated dry matter yield (g/plant) at the six true leaf stage following various levels of pruning of the leaves of OSR seedlings in PS2820.

% leaf area lost	Dry matter yield (g)	% change from control
0	3.08	
16	3.39	10.1
25	3.11	0.9
33	3.17	3.0
37.5	2.86	-7.3
40	2.91	-5.7
50	3.20	3.9
66	3.03	-1.6
75	1.99	-35.4
80	1.73	-43.7
100	2.46	-20.1

In general, the lack of consistent trends in the relationship between loss of cotyledon or leaf area and dry matter yield make it difficult to interpret these data. However, there is a suggestion that plants are able to tolerate some level of leaf loss and particularly of the cotyledons. It is difficult to be precise regarding a level of leaf loss after which yield decreases. At 50% leaf loss, plants produced more dry matter than the control whereas removal of 37.5% and 40% leaf area did decrease yield.

#### **Historic data – Field experiments**

All field data was taken from CRD funded project PS2805. Regression analyses provided poor fits between data on leaf loss and crop yield. As a result mean values of these variables are provided and comments provided on any trends in the data (Tables 32-36). Data was very variable but in general, there was limited reduction in varietal yield until more than 50% leaf area was lost. It should be noted that Catana was only grown once in comparison with six times for both Castille and Excalibur (Table 31).

Table 31. Effect of a range of leaf pruning treatments on mean yield (t/ha) at harvest of three OSR varieties grown in field experiments as part of PS2805. \* no data for that variety-damage combination.

Variety	% leaf area lost				
	0	50	66	75	80
Castille	3.73	3.63	3.37	1.10	2.22
Catana	3.88	4.09	3.69	*	3.53
Excalibur	4.17	4.20	4.08	1.95	3.08

These trends tended to hold across all the years of the study although there was limited data available for loss of 75% of leaf area. Indeed at some sites yield increased following removal of 50% of leaf area in comparison with no leaf loss (e.g. Castille at Rosemaund and Terrington in 2012, Catana at Terrington in 2011, Excalibur at Rosemaund and Terrington in 2012, Table 32).

*Table 32. Effect of a range of leaf pruning treatments on mean yield (t/ha) at harvest of three OSR varieties grown in field experiments at ADAS High Mowthorpe (HM), Rosemaund (RM) and Terrington (TT) in 2011 and 2012 as part of PS2805. \* no data for that variety-site-damage combination.*

Variety	Site	% leaf area lost				
		0	50	66	75	80
Castille	HM11	2.64	2.13	*	1.1	0.51
	HM12	3.42	2.65	2.56	*	1.96
	RM11	3.56	3.53	3	*	1.9
	RM12	3.4	3.54	3.25	*	2.04
	TT11	*	*	*	*	*
	TT12	5.56	6.29	4.66	*	4.61
Catana	HM11	*	*	*	*	*
	HM12	*	*	*	*	*
	RM11	*	*	*	*	*
	RM12	*	*	*	*	*
	TT11	3.88	4.09	3.68	*	3.53
	TT12	*	*	*	*	*
Excalibur	HM11	3.08	2.96	*	1.95	1.25
	HM12	3.6	3.15	3.03	*	2.35
	RM11	5.28	4.46	4.12	*	3.28
	RM12	3.41	3.75	3.39	*	2.67
	TT11	3.82	4.45	4.72	*	3.93
	TT12	5.9	6.47	5.1	*	4.98

When the varieties were grown at five seed rates (Table 33) yield did not decrease markedly until 75% of leaf area was lost. There did not appear to be any great benefit from sowing most varieties at more than 40 seeds per m<sup>2</sup>. This was supported by the calculated optimum plant number which ranged from 13-48 plants per m<sup>2</sup> and averaged 29 plants per m<sup>2</sup>. There was a slight trend for higher seed rates to tolerate more defoliation.

Table 33. Effect of a range of leaf pruning treatments on mean yield (t/ha) of three OSR varieties grown at five seed rates (10-160 seeds per m<sup>2</sup>) in field experiments as part of PS2805. \* no data for that variety-seed rate-damage combination.

Variety	Seed rate	% leaf area lost				
		0	50	66	75	80
Castille	10	3.53	3.60	3.75	1.14	2.00
	20	3.30	3.58	3.3	0.62	1.97
	40	4.15	3.69	3.34	0.96	2.27
	80	3.67	3.66	3.16	1.21	2.5
	160	4.03	3.62	3.29	1.55	2.35
Catana	10	2.19	3.70	2.77	*	1.81
	20	4.34	4.95	3.12	*	3.69
	40	4.20	3.74	4.23	*	4.93
	80	3.94	3.69	4.35	*	3.45
	160	4.73	4.36	3.96	*	3.79
Excalibur	10	4.68	4.53	4.04	1.28	3.18
	20	4.13	4.15	4.34	1.60	2.70
	40	4.13	4.25	4.47	1.83	3.01
	80	4.23	4.02	3.97	2.51	3.35
	160	3.69	4.05	3.60	2.53	3.18

These trends in yields between seed rates and leaf area lost also tended to hold true when data were compared between cropping seasons and sites (Table 34), with yield decreasing markedly at 66-75% leaf area lost, although at Terrington yield was largely unaffected by removal of 80% leaf area lost (at high seed rates in 2011 and several seed rates in 2012). These trends were not strongly affected by variety (Table 35).

Table 34. Effect of a range of leaf pruning treatments on mean yield (t/ha) of OSR grown at five seed rates (10-160 seeds per m<sup>2</sup>) in field experiments at ADAS High Mowthorpe (HM),

Rosemaund (RM) and Terrington (TT) in 2011 and 2012 as part of PS2805. \* no data for that site-seed rate-damage combination.

Site	Seed rate	% leaf area lost				
		0	50	66	75	80
HM11	10	2.60	2.06	*	1.21	0.79
	20	3.01	2.28	*	1.11	0.65
	40	2.77	2.16	*	1.39	0.78
	80	3.02	3.02	*	1.86	1.04
	160	2.89	3.20	*	2.04	1.16
HM12	10	3.37	2.63	2.80	*	1.87
	20	2.70	2.67	2.62	*	1.66
	40	3.79	2.85	2.80	*	2.31
	80	3.98	2.96	2.81	*	2.39
	160	3.71	3.39	2.93	*	2.54
RM11	10	4.62	4.46	2.73	*	2.17
	20	4.01	4.04	3.96	*	2.05
	40	4.95	3.99	3.92	*	2.57
	80	3.88	3.28	3.79	*	3.48
	160	4.65	3.91	3.40	*	2.59
RM12	10	3.70	4.16	4.33	*	2.01
	20	3.68	3.30	3.07	*	2.36
	40	3.66	3.89	2.79	*	2.16
	80	3.33	3.54	3.43	*	2.68
	160	2.69	3.42	2.98	*	2.55
TT11	10	3.02	3.24	3.14	*	2.38
	20	4.03	4.96	4.34	*	3.72
	40	3.91	4.37	4.94	*	4.49
	80	3.80	4.10	4.50	*	3.69
	160	4.48	4.67	4.09	*	4.38
TT12	10	6.63	7.96	5.83	*	6.05
	20	5.36	6.61	5.02	*	4.44
	40	5.94	6.60	5.51	*	4.79
	80	5.63	5.97	3.89	*	4.77
	160	5.10	4.78	4.15	*	3.93

Table 35. Effect of a range of leaf pruning treatments on mean yield (t/ha) of three OSR varieties grown at five seed rates (10-160 seeds/m<sup>2</sup>) at four ADAS sites in field experiments at ADAS High

Mowthorpe, Rosemaund and Terrington in 2011 and 2012 as part of PS2805. \* no data for that site-variety-seed rate-damage combination.

Site	Variety	Seed rate	% leaf area lost				
			0	50	66	75	80
HM11	Castille	10	2.53	1.67	*	1.14	0.9
		20	2.73	1.72	*	0.62	0.37
		40	2.79	1.94	*	0.96	0.3
		80	2.49	2.34	*	1.21	0.4
		160	2.66	2.98	*	1.55	0.65
	Excalibur	10	2.67	2.45	*	1.28	0.7
		20	3.28	2.85	*	1.6	0.92
		40	2.75	2.38	*	1.83	1.27
		80	3.72	3.71	*	2.51	1.67
		160	3.11	3.41	*	2.53	1.68
HM12	Castille	10	3.02	1.99	2.3	*	1.12
		20	2.67	2.49	2.21	*	1.72
		40	3.91	3.02	2.79	*	2.05
		80	3.55	2.45	2.54	*	2.43
		160	3.84	3.33	2.96	*	2.46
	Excalibur	10	3.62	3.28	3.46	*	2.63
		20	2.73	2.85	3.03	*	1.6
		40	3.66	2.68	2.81	*	2.57
		80	4.4	3.47	3.08	*	2.34
		160	3.59	3.45	2.89	*	2.61
RM11	Castille	10	2.59	3.89	2.49	*	1.48
		20	3.05	2.98	3.04	*	1.59
RM11	Castille	40	3.19	3.41	3.19	*	1.94
		80	3.84	4.09	3.41	*	2.65
		160	4.94	3.27	2.86	*	1.84
	Excalibur	10	6.65	5.04	2.97	*	2.86
		20	5.29	5.09	4.88	*	2.5
		40	5.84	4.56	4.65	*	3.41
		80	3.94	1.66	4.17	*	4.3
		160	4.35	4.56	3.93	*	3.35
		160	4.35	4.56	3.93	*	3.35
RM12	Castille	10	3.4	3.84	4.96	*	1.5
		20	3.3	2.92	2.71	*	1.62
<b>Site</b>	<b>Variety</b>	<b>Seed rate</b>	<b>% leaf area lost</b>				



			<b>0</b>	<b>50</b>	<b>66</b>	<b>75</b>	<b>80</b>
RM12	Castille	40	3.6	3.95	2.62	*	2.06
		80	3.32	3.48	2.7	*	2.35
		160	3.43	3.59	3.28	*	2.69
	Excalibur	10	4.01	4.4	3.71	*	2.51
		20	4.06	3.69	3.43	*	3.09
		40	3.71	3.82	2.97	*	2.26
		80	3.33	3.61	4.16	*	3.02
		160	1.95	3.25	2.69	*	2.26
TT11	Catana	10	2.19	3.7	2.77	*	1.81
		20	4.34	4.95	3.12	*	3.69
		40	4.2	3.74	4.23	*	4.93
		80	3.94	3.69	4.35	*	3.45
		160	4.73	4.36	3.96	*	3.79
	Excalibur	10	3.86	2.77	3.51	*	2.95
		20	3.73	4.97	5.57	*	3.75
		40	3.62	5.01	5.64	*	4.06
		80	3.67	4.5	4.65	*	3.92
		160	4.24	4.99	4.23	*	4.97
TT12	Castille	10	5.97	6.66	5.26	*	4.71
		20	4.75	7.79	5.24	*	4.55
		40	6.65	6.13	4.76	*	4.98
		80	5.15	5.95	3.99	*	4.69
		160	5.28	4.94	4.06	*	4.13
	Excalibur	10	7.28	9.26	6.39	*	7.4
		20	5.97	5.43	4.81	*	4.32
		40	5.22	7.06	6.27	*	4.6
		80	6.1	5.99	3.78	*	4.84
		160	4.91	4.62	4.24	*	3.74

Overall data from these field experiments suggested that there is a level of tolerance to loss of green leaf area. Although results suggest that loss of 50% of leaf area has a limited impact on yield it is not possible to confirm this with any degree of certainty due to the variability in the data set. It is probable that other factors such as environmental conditions within seasons determine the extent to which varieties are able to express their tolerance to loss of leaf area.

### **Data from other experiments in the current study**

A series of regressions were performed in order to try and describe the relationship between % leaf area lost and crop yield. The first analysis fitted a single line to the entire data set. This only accounted for 13.3 percent of the variance so was not considered a good fit and will not be presented here. In the second regression analysis a separate line was fitted for each experiment whilst maintaining the same slope for each line. This explained 71.4% of the variance so was considered a reasonable fit. The common slope was -0.045, and so represented a very small decrease in yield with every one per cent increase in leaf area loss. These data are also not presented. In the final regression analysis the best line was fitted for each individual experiment. This increased the % variation accounted for to 73%. The individual slopes of these lines are presented in Table 36 and in Figure 116.

*Table 36. Estimates of the slopes of regression lines for individual experiments in which the relationship between % leaf area lost and crop yield was assessed. The equation of the line is shown for those sites where the slope was significantly different from zero.*

<b>Experiment</b>	<b>Estimated slope of regression line (m)</b>	<b>SE</b>	<b>Probability (P)</b>	<b>Equation of regression line</b>
CSFB larval impact BX17 (Cambridgeshire 2016/17)	0.14	0.068	0.045	$y=5.44 - 0.14x$
Variety-seed rate BX18 (Cambridgeshire 2017/18)	-0.04	0.035	0.256	NS
CSFB larval impact BX18 (Cambridgeshire 2017/18)	0.01	0.036	0.855	NS
Variety-seed rate BX19 (Cambridgeshire 2018/19)	0.08	0.025	0.002	$y=3.42 - 0.08x$
CSFB larval impact BX19 (Cambridgeshire 2018/19)	-0.05	0.025	0.049	$y=3.81 - 0.13x$
CSFB larval impact HM17 (Yorkshire 2016/17)	-0.01	0.081	0.876	NS
CSFB larval impact HM18 (Yorkshire 2017/18)	0.02	0.168	0.928	NS
Variety-seed rate HM18 (Yorkshire 2017/18)	0.01	0.033	0.960	NS
CSFB larval impact HM19 (Yorkshire 2018/19)	-0.67	0.148	<0.001	$y=3.39 - 0.67x$
RL variety trial 2017 Cowlinge (Suffolk)	-0.01	0.049	0.837	NS

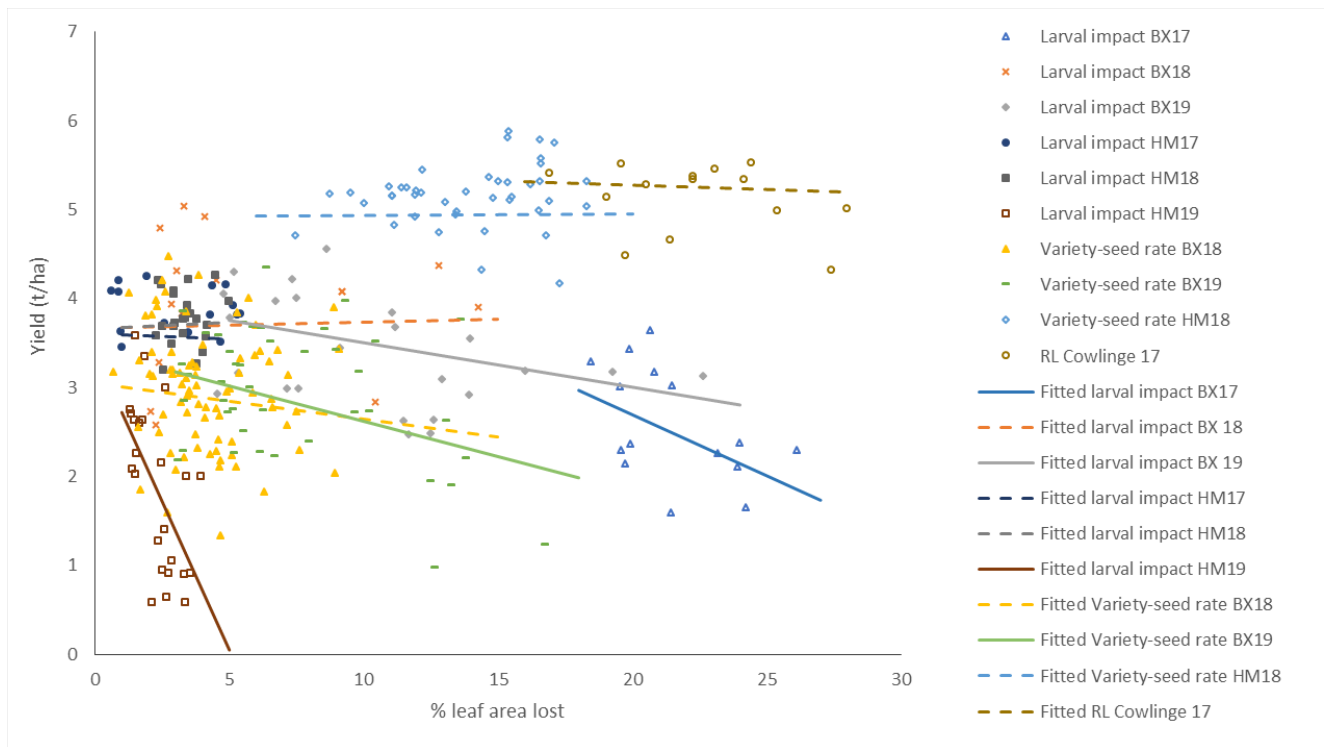


Figure 116. Regression lines for data from experiments as part of the current study which investigated the relationship between % area leaf lost and crop yield (t/ha @ 91% DM). Solid lines indicate significant fitted slopes. Dashed lines indicate non-significant fitted slopes.

The slopes of the lines represent the change in yield (t/ha @91% DM) for every 1% change in loss of leaf area. Only four of the slopes were significantly different from zero and these were for the CSFB larval impact BX17 (Cambridgeshire 2016/17) (slope = 0.14,  $P < 0.05$ ), CSFB larval impact BX19 (Cambridgeshire 2018/19) (slope = -0.05,  $P < 0.05$ ), Variety-seed rate BX19 (Cambridgeshire 2018/19) (slope = 0.08,  $P < 0.01$ ) and CSFB larval impact HM19 (Yorkshire 2018/19) (slope = -0.67,  $P < 0.001$ ) trials. For the remaining six regression lines three showed small positive slopes and three showed small negative slopes. In general, the slopes of the regression lines are close to zero, even where they are significantly different from zero, suggesting that there was limited impact on crop yield of loss of green leaf area. The fact that there are both positive and negative slopes emphasises the lack of a clear relationship between loss of green leaf area and yield. The only outlier is the CSFB larval impact HM19 (Yorkshire 2018/19) trial, which has the largest negative slope of -0.67. This line suggests a loss of 0.67t/ha for every 1% increase in loss of leaf area and would represent a major impact of CSFB adult feeding on the crop. However, results from this site should be treated with caution as it had a very low yield and yet a very low level of leaf loss due to CSFB feeding so the goodness of fit is based on a very small range of data. Consequently it is difficult to extrapolate outside of the available data range. The site established poorly and this may have been due to factors other than CSFB pressure.

Generally the range of leaf area loss due to adult CSFB feeding at each site is quite variable with some sites having a range of just 3% leaf area lost and others about 20%. On balance the data

suggests that the yield loss is negligible over the range of leaf loss (maximum leaf area lost = 28%) in these experiments.

### 6.3.2. To understand the tolerance of OSR plants to CSFB larvae

#### ***Larval impact experiments***

In the larval impact trials numbers of larvae were assessed in the autumn/winter and in the spring. In the autumn/winter these larvae were only recorded in the leaf petioles with none in the stem. Larvae were only found in the stems in the spring but numbers were low. Therefore although total larval numbers were assessed in the spring these data were heavily influenced by the numbers of larvae found in the leaf petioles. Overall larval numbers ranged between 0 and 22 per plant so provided a good range over which to measure their impact on yield.

Estimates of the slopes of regression analyses on the number of CSFB larvae recorded in the autumn/winter were variable with three giving positive slopes (i.e. yield increased as larval number increased) and three giving negative slopes (i.e. yield decreased as larval numbers increased) (Table 37, Figure 117). At those sites where there was a positive slope none differed significantly from zero suggesting that there little or no positive impact of larval feeding on yield.

*Table 37. Estimates of the slopes of regression lines for individual experiments in which the relationship between numbers of CSFB larvae and crop yield in the autumn/winter in the larval manipulation experiments was assessed. The equation of the line is not shown for those sites where the slope was not significantly different from zero.*

<b>Experiment</b>	<b>Estimated slope of regression line (m)</b>	<b>SE</b>	<b>Probability (P)</b>	<b>Equation of regression line</b>
CSFB larval impact BX17 (Cambridgeshire 2016/17)	0.58	0.039	0.142	NS
CSFB larval impact BX18 (Cambridgeshire 2017/18)	0.06	0.127	0.635	NS
CSFB larval impact BX19 (Cambridgeshire 2018/19)	-0.07	0.030	0.032	$y=4.02 - 0.07x$
CSFB larval impact HM17 (Yorkshire 2016/17)	-0.29	0.375	0.440	NS
CSFB larval impact HM18 (Yorkshire 2017/18)	0.35	0.411	0.404	NS
CSFB larval impact HM19 (Yorkshire 2018/19)	-0.74	0.166	<0.001	$y=2.67 - 0.74x$

At the other three sites where the slopes were negative two were significantly different from zero. In the CSFB larval impact BX19 (Cambridgeshire 2018/19) trial there was a small negative slope suggesting that for each additional larva yield would decrease by 0.07 t/ha. In the CSFB larval impact HM19 (Yorkshire 2018/19) trial there was a large negative slope of -0.74 suggesting a loss of 0.74 t/ha for every larva present. However the numbers of larvae at this site were very low (mean 1.1 per plant) so it would be dangerous to extrapolate beyond the range of the data and some caution is required when interpreting the results from this site. Similar concerns were expressed regarding the leaf loss data from this site and it is likely that the poor performance of the crop was affected by some factor other than CSFB attack.

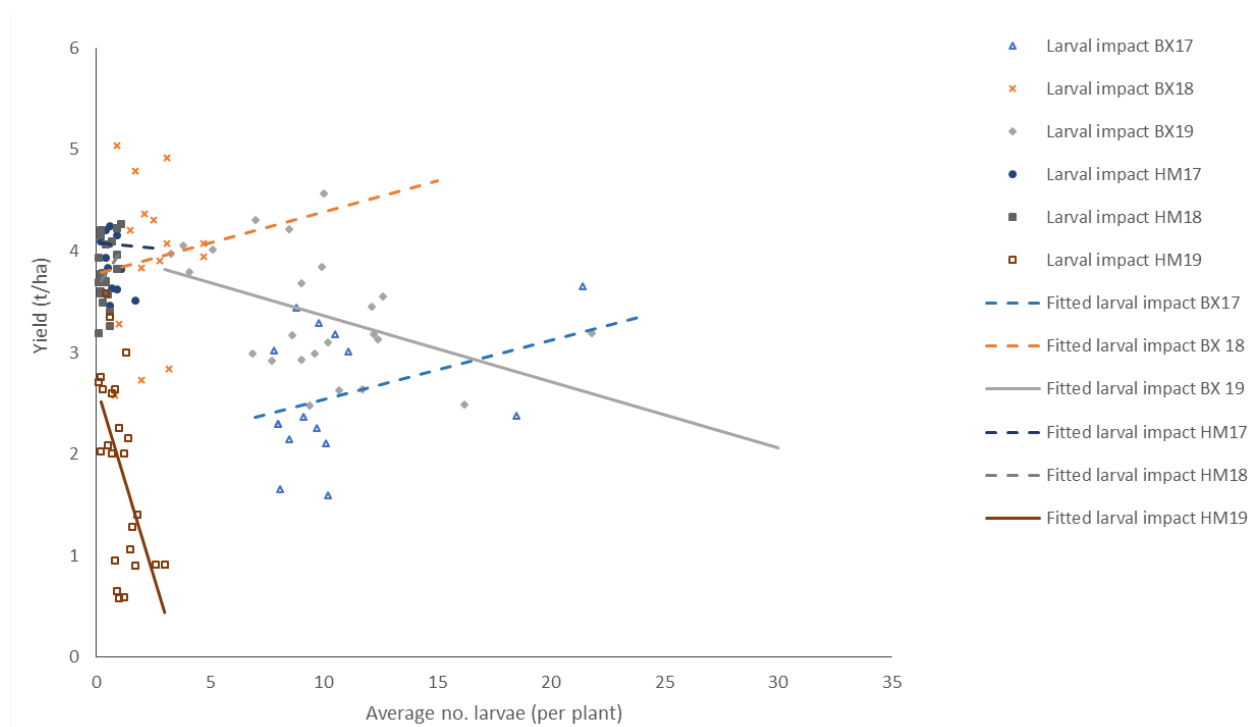


Figure 117. Regression lines for data from larval impact experiments showing relationship between total number of CSFB larvae in the autumn/winter (mean per plant) and OSR yield (t/ha @ 91% DM). Solid lines indicate significant fitted slopes. Dashed lines indicate non-significant fitted slopes.

The numbers of larvae in the stem were recorded in the spring at two sites. However, there was a poor relationship between yield and larval numbers (Table 38). These data are not presented graphically.

Table 38. Estimates of the slopes of regression lines for individual experiments in which the relationship between numbers of CSFB larvae in the stem in the spring and crop yield in the larval

manipulation experiments was assessed. The equation of the line is not shown for those sites where the slope was not significantly different from zero.

<b>Experiment</b>	<b>Estimated slope of regression line (m)</b>	<b>SE</b>	<b>Probability (P)</b>	<b>Equation of regression line</b>
CSFB larval impact BX19 (Cambridgeshire 2018/19)	-0.70	0.111	0.527	NS
CSFB larval impact HM17 (Yorkshire 2016/17)	-0.41	0.746	0.582	NS

Data on the total number of larvae in the spring (combined numbers present in the petioles and stem) were analysed at four sites (Table 39, Figure 118). The regression analysis between larval numbers and yield was a reasonably good fit and accounted for 79.5% of the variation. All lines had a negative slope but only two were significantly different from zero. The greatest slope was at the CSFB larval impact HM19 (Yorkshire 2018/19) trial but results from this site should be treated with caution as previously discussed. In the CSFB larval impact BX19 (Cambridgeshire 2018/19) trial the slope was much smaller suggesting a yield loss of 0.06t/ha for every larva present per plant. This site provides the most reliable estimate of yield loss per larva as numbers of larvae ranged between about five and 30 per plant.

*Table 39. Estimates of the slopes of regression lines for individual experiments in which the relationship between total numbers of CSFB larvae and crop yield in the spring in the larval manipulation experiments was assessed. The equation of the line is not shown for those sites where the slope was not significantly different from zero.*

<b>Experiment</b>	<b>Estimated slope of regression line (m)</b>	<b>SE</b>	<b>Probability (P)</b>	<b>Equation of regression line</b>
CSFB larval impact BX19 (Cambridgeshire 2018/19)	-0.06	0.018	<0.001	$y = 4.47 - 0.06x$
CSFB larval impact HM17 (Yorkshire 2016/17)	-0.01	0.059	0.826	NS
CSFB larval impact HM18 (Yorkshire 2017/18)	-0.02	0.130	0.878	NS
CSFB larval impact HM19 (Yorkshire 2018/19)	-0.52	0.075	<0.001	$y = 3.05 - 0.52x$

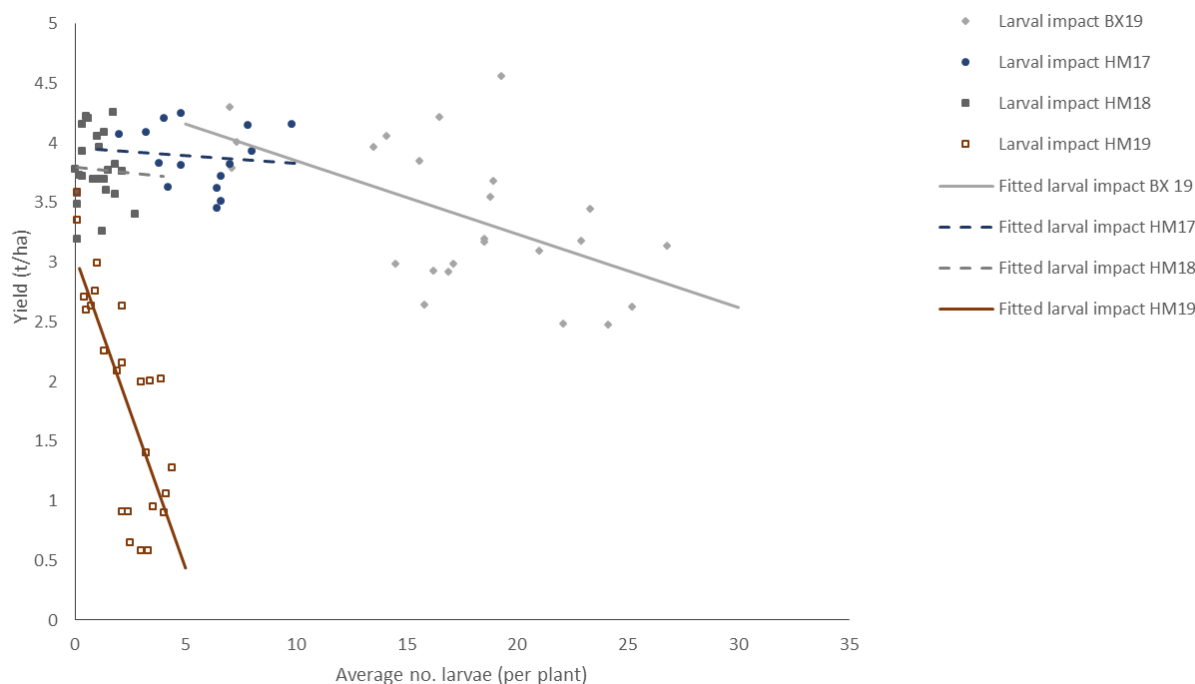


Figure 118. Regression lines for data from larval impact experiments showing relationship between total number of CSFB larvae in the spring (mean per plant) and OSR yield (t/ha @ 91% DM). Solid lines indicate significant fitted slopes. Dashed lines indicate non-significant fitted slopes.

#### Data from all experiments in the current study

These data examine the relationship between CSFB larval numbers and yield over three time periods, November/December, January/February and March April. Data for November/December are taken from nine experiments (Table 40, Figure 119). The analyses showed a reasonable fit accounting for 75.3% of the variation but the slopes were quite variable with only two being significantly different from zero. One of these was for the CSFB larval impact BX19 (Cambridgeshire 2018/19) trial. There was a small negative slope suggesting a loss of 0.07 t/ha for each CSFB larva present. The other site where the slope was significantly different from zero was at the variety-seed rate HM19 (Yorkshire 2018/19) trial and this is an unreliable dataset as already discussed.

Table 40. Estimates of the slopes of regression lines for individual experiments in which the relationship between numbers of CSFB larvae and crop yield in November/December were

assessed. The equation of the line is not shown for those sites where the slope was not significantly different from zero.

<b>Experiment</b>	<b>Estimated slope of regression line (m)</b>	<b>SE</b>	<b>Probability (P)</b>	<b>Equation of regression line</b>
CSFB larval impact BX17 (Cambridgeshire 2016/17)	0.06	0.039	0.136	NS
Defoliation BX17 (Cambridgeshire 2016/17)	-0.02	0.269	0.951	NS
Variety-seed rate BX18 (Cambridgeshire 2017/18)	-0.08	0.059	0.191	NS
CSFB larval impact BX18 (Cambridgeshire 2017/18)	0.06	0.126	0.631	NS
CSFB larval impact BX19 (Cambridgeshire 2018/19)	-0.07	0.030	0.03	$y = 4.02 - 0.07x$
CSFB larval impact HM17 (Yorkshire 2016/17)	-0.29	0.371	0.435	NS
CSFB larval impact HM18 (Yorkshire 2017/18)	-0.06	0.116	0.621	NS
Variety-seed rate HM18 (Yorkshire 2017/18)	0.35	0.407	0.398	NS
Variety-seed rate HM19 (Yorkshire 2018/19)	-0.74	0.165	<0.001	$y = 2.67 - 0.74x$



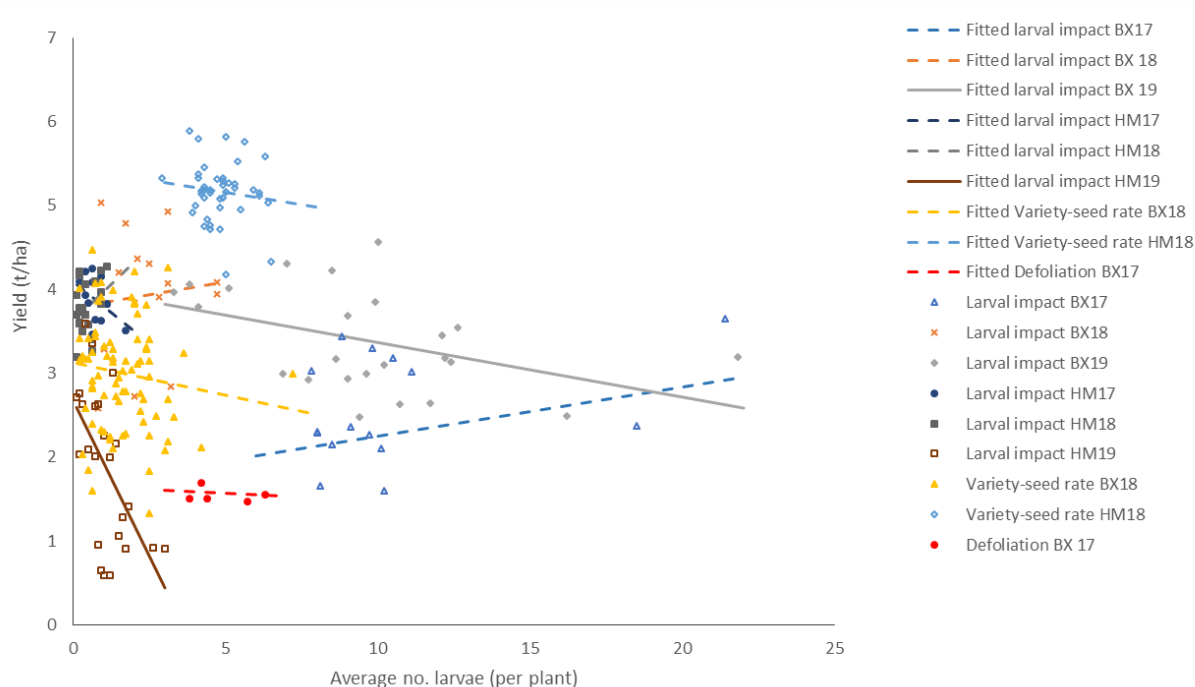
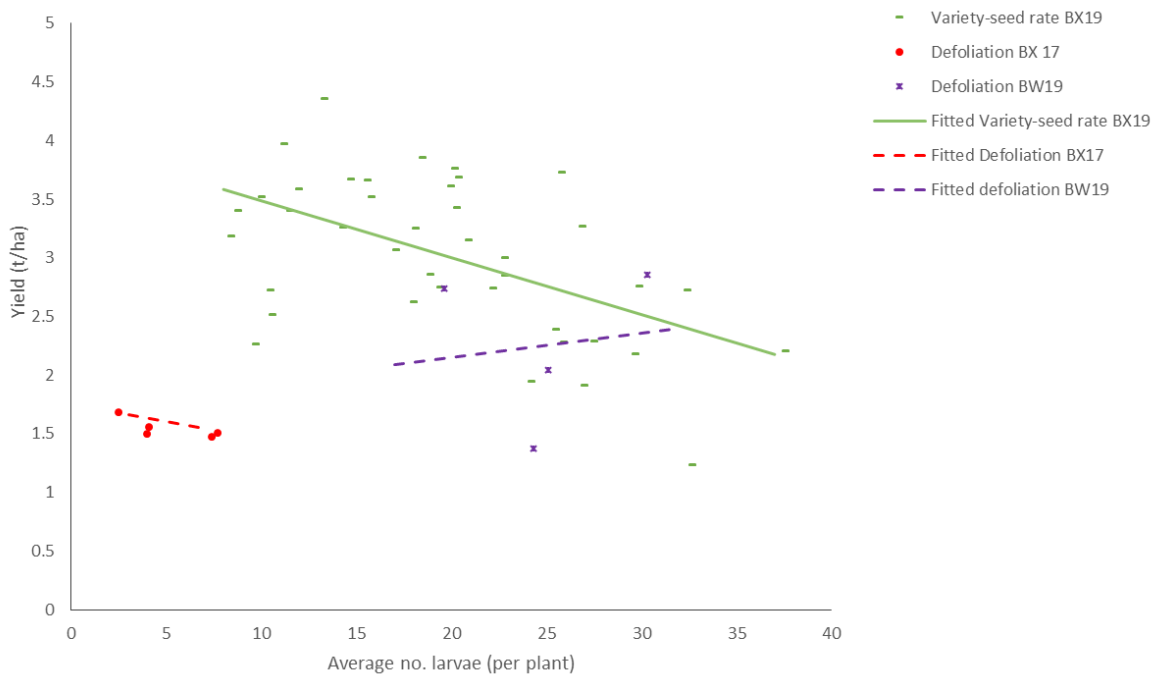


Figure 119. Regression lines for data from larval impact experiments showing relationship between total number of CSFB larvae in November/December (mean per plant) and OSR yield (t/ha @ 91% DM). Solid lines indicate significant fitted slopes. Dashed lines indicate non-significant fitted slopes.

Numbers of CSFB larvae were only assessed at three sites in December/January. The fit was not as good as in November/December and accounted for only 48.1% of the variation (Table 41, Figure 120). At two of the three sites the slope was negative and at the third it was slightly positive. At only one site was the slope significantly different from zero (variety x seed rate Boxworth 2019). At this site for each larva present the estimated yield loss was 0.05 t/ha.

Table 41. Estimates of the slopes of regression lines for individual experiments in which the relationship between numbers of CSFB larvae and crop yield in January/February were assessed. The equation of the line is not shown for those sites where the slope was not significantly different from zero.

Experiment	Estimated slope of regression line (m)	SE	Probability (P)	Equation of regression line
Defoliation BX17 (Cambridgeshire 2016/17)	-0.03	0.124	0.818	NS
Variety-seed rate BX19 (Cambridgeshire 2018/19)	-0.05	0.012	<0.001	$y=3.97-0.05x$
Defoliation BW19 (Cambridgeshire 2018/19)	0.02	0.075	0.783	NS



*Figure 120. Regression lines for data from larval impact experiments showing relationship between total number of CSFB larvae in January/February (mean per plant) and OSR yield (t/ha @ 91% DM). Solid lines indicate significant fitted slopes. Dashed lines indicate non-significant fitted slopes.*

In March/April there was data from seven trials but the slopes of the regression lines were not consistent with five being negative and two positive (Table 42, Figure 121). At only two of the sites were the slopes significantly different from zero. In the CSFB larval impact BX19 trial in 2019 there was a small negative slope suggestion a yield loss of 0.06 t/ha for every CSFB larva present. The only other site where the slope was significantly different from zero was the CSFB larval impact HM19 trial in 2019 and data from this site is not particularly reliable as previously discussed.

*Table 42. Estimates of the slopes of regression lines for individual experiments in which the relationship between numbers of CSFB larvae and crop yield in March/April were assessed. The*

equation of the line is not shown for those sites where the slope was not significantly different from zero.

Experiment	Estimated slope of regression line (m)	SE	Probability (P)	Equation of regression line
Defoliation BX17 (Cambridgeshire 2016/17)	0.02	0.082	0.808	NS
Variety-seed rate BX19 (Cambridgeshire 2018/19)	-0.01	0.011	0.225	NS
CSFB larval impact BX19 (Cambridgeshire 2018/19)	-0.06	0.021	0.004	$y = 4.47 - 0.06x$
Defoliation BW19 (Cambridgeshire 2018/19)	0.08	0.081	0.347	NS
CSFB larval impact HM17 (Yorkshire 2016/17)	-0.01	0.068	0.850	NS
CSFB larval impact HM18 (Yorkshire 2017/18)	-0.02	0.151	0.895	NS
CSFB larval impact HM19 (Yorkshire 2018/19)	-0.52	0.082	<0.001	$y = 3.05 - 0.52x$

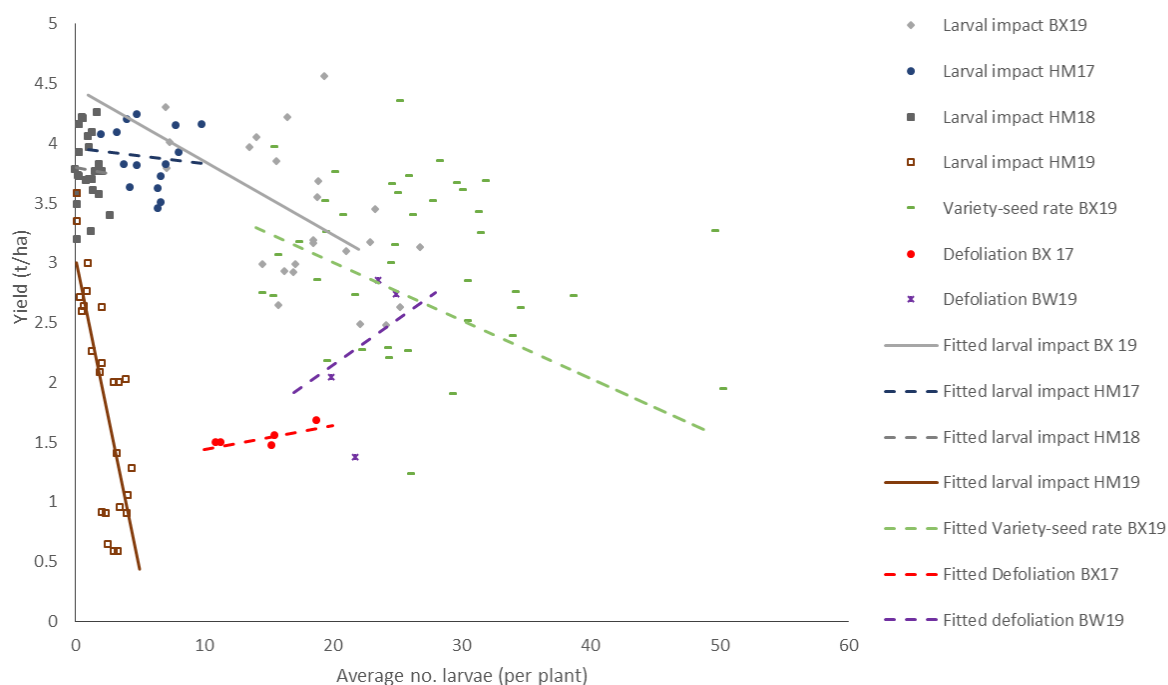


Figure 121. Regression lines for data from larval impact experiments showing relationship between total number of CSFB larvae in March/April (mean per plant) and OSR yield (t/ha @ 91% DM). Solid lines indicate significant fitted slopes. Dashed lines indicate non-significant fitted slopes.

The numbers of larvae in the stems were analysed at five sites and in general numbers were relatively low and never exceeded six per plant. Regression analyses were done on these data

sets and are summarised in Table 43 and Figure 122. There was no consistency in the slope of the regression lines with two being positive and three being negative. None of the slopes was significantly different from zero suggesting that there was minimal impact of larvae in the stem on crop yield.

*Table 43. Estimates of the slopes of regression lines for individual experiments in which the relationship between numbers of CSFB larvae in the stems and crop yield in March/April were assessed. The equation of the line is not shown for those sites where the slope was not significantly different from zero.*

<b>Experiment</b>	<b>Estimated slope of regression line (m)</b>	<b>SE</b>	<b>Probability (P)</b>	<b>Equation of regression line</b>
Defoliation BX17 (Cambridgeshire 2016/17)	0.02	0.587	0.977	NS
Variety-seed rate BX19 (Cambridgeshire 2018/19)	-0.11	0.089	0.231	NS
CSFB larval impact BX19 (Cambridgeshire 2018/19)	-0.07	0.127	0.577	NS
Defoliation BW19 (Cambridgeshire 2018/19)	0.28	0.207	0.185	NS
CSFB larval impact HM17 (Yorkshire 2016/17)	-0.414	0.852	0.628	NS

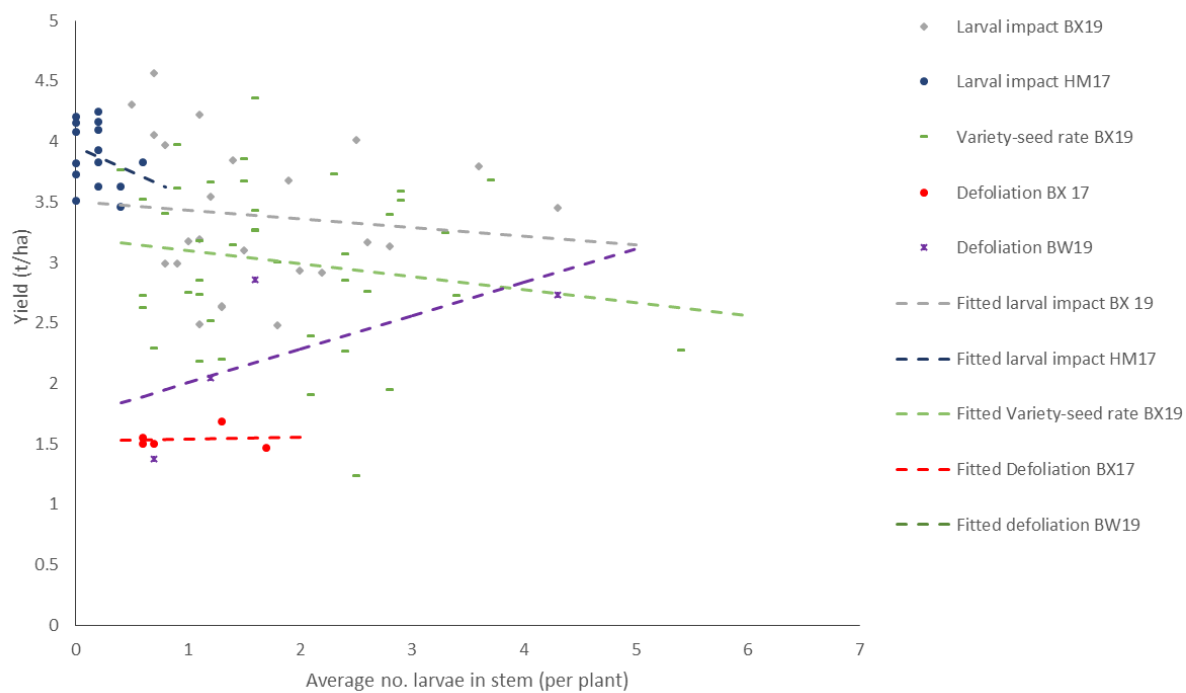


Figure 122. Regression lines for data from larval impact experiments showing relationship between number of CSFB larvae in the stem in March/April (mean per plant) and OSR yield (t/ha @ 91% DM). Dashed lines indicate non-significant fitted slopes.

## 6.4. Discussion

The impact of both the loss of green leaf area as a result of adult CSFB feeding damage and feeding by CSFB larvae either in the leaf petioles or stems on the yield of WOSR was investigated.

### **Effect of loss of green leaf area on yield**

In historic pot and field experiments regression analyses provided poor fits between data on % cotyledon loss or % leaf loss and crop yield (in pot experiments this was expressed as dry matter yield). As a result mean values of these variables were provided and comments provided on any trends in the data. In pot experiments the lack of consistent trends in the relationship between loss of cotyledon or leaf area and dry matter yield made it difficult to interpret these data. However, there is a suggestion that plants are able to tolerate some level of leaf loss and particularly of the cotyledons. In some instances yield was increased following the removal of both cotyledons. Removal of up to 33% of leaf area of pot grown OSR also seemed to have limited effect on yield. In RD-214009 yield loss was most noticeable after 60% loss of leaf area and in PS2820 75% loss of green area appeared to be the threshold. However, this is a significant level of leaf loss and so might be expected to have some impact on yield. It should also be remembered that yield was assessed in these pot experiments at the six true leaf stage. Therefore the crop had a significant amount of growing time before the production of pods and seed and potentially could have compensated further for the loss of cotyledon or leaf area.

Although datasets for historic field experiments were also very variable, they tended to support the conclusions from the pot experiments that there was a degree of tolerance to loss of green leaf area. Overall data from these field experiments suggest that loss of 50% of leaf area has a limited impact on yield, with marked reductions in yield not seen until 66-75% of leaf area had been removed. There also appeared to be little benefit from sowing more than 40 seeds per m<sup>2</sup> in terms of yield per se, although at some sites higher seed rates appeared better able to tolerate leaf area loss. This result is supported by the AHDB Oilseed rape growth guide (AHDB, 2020b) which suggests that the target plant population for WOSR is 25-40 plants per m<sup>2</sup>. The guide refers to a target plant population rather than a seed rate as % establishment can vary (e.g. between soil types).

Regression analyses were possible on datasets taken from 10 experiments which are part of the current study. The % loss of leaf area was regressed against crop yield and analyses provided a good fit accounting for 73% of the variation. Out of 10 experiments only four provided regression lines that were significantly different from zero. Therefore in six out of 10 experiments there was no clear effect of loss of green leaf area and crop yield. Where the slopes of the regression lines were significantly different from zero all were negative indicating that crop yield decreased with increasing level of leaf loss. The largest negative slope of -0.67 was recorded at the CSFB larval impact experiment in Yorkshire in 2019. This suggests a loss of 0.67t/ha for every 1% increase in loss of leaf area and would be a major impact of CSFB adult feeding on the crop. However, this site had a very low yield and less than 5% leaf area lost so the goodness of fit of the regression line is being based on a very small range of data. Consequently it is difficult to extrapolate outside of the available data range and results from this site should be treated with caution. The site established poorly and this may have been due to factors such as lack of moisture in addition to CSFB pressure. At the remaining three sites where there was a negative slope to the regression line, which ranged between 0.05-0.14t/h for every one per cent loss of leaf area. Overall, in the experiments done in this project the range of leaf area lost due to adult CSFB feeding was quite variable with some sites having a range just 3% leaf area lost and others about 20%. It is also difficult to separate the impact of leaf area loss and subsequent larval damage on yield at these trials. On balance the data suggests that the yield loss is negligible over the range of leaf loss (maximum of 28%) in these experiments. These results support the results from the pot experiments and historic field experiments where the level of leaf area lost was much greater.

Current thresholds against adult CSFB suggest that treatment is justified between the cotyledon and two true leaf stage if 25% of leaf area is lost. Between the three to four leaf stage treatment is justified if 50% of green leaf area is lost. Results from this project generally support the latter threshold (although even this may be too conservative) but suggest that the crop is more tolerant of leaf loss between the cotyledon and two leaf stage than previously thought.

Whilst results suggest a level of tolerance to leaf area lost it should be borne in mind that the timing of beetle infestation is critical. There are numerous examples of crops failing to establish due to severe CSFB adult pressure. If the beetles migrate early into an emerging crop the ability to compensate for feeding damage is greatly reduced. If beetles feed on newly emerging plants and possibly even cotyledons that have yet to emerge it is likely that the plant will be killed. Further study on the factors that initiate CSFB adult migration will therefore be crucial if farmers and agronomists are to take advantage of the inherent ability of the rape crop to tolerate loss of green leaf area. Once the crop is above the ground then it will have a good chance of being able to tolerate all but the most severe CSFB infestation.

### ***Effect of CSFB larval infestation on yield***

Dissection of OSR plants from autumn through to the spring showed that CSFB larval numbers tended to increase. This suggests that larvae continue to hatch at any time from autumn until the following spring as indicated by observations of the range of sizes of those recovered. The majority of larvae were also present in the leaf petioles even in the spring rather than moving to the stem. This was also particularly noticeable in experiments to investigate defoliation as a means of controlling CSFB larvae (see Section 8).

Regression analyses were used to investigate the relationship between larval numbers and crop yield. In total 36 analyses were done on total CSFB larval numbers per plant and numbers in the stems (most were on total numbers per plant by default as so few were found in the stems) and also at various timings between autumn and spring. In only nine out of 36 analyses (25%) were the slopes of the regression lines significantly different from zero. So in 75% of cases the presence of CSFB larvae had limited if any impact on crop yield, however at several sites the range in larval numbers was small making impacts on yield difficult to determine. Where statistical differences were confirmed the slopes of the regression lines were always negative so crop yield decreased with increasing number of CSFB larvae. The largest negative slope was always associated with the variety-seed rate experiment in Yorkshire in 2019 (-0.52 - -0.74 t/ha per CSFB larva). This would represent a decrease in yield of between 0.52-0.74 t/ha for each increase of one CSFB larva per plant and could therefore account for a significant effect on the crop. However, this site had a very low yield and larval numbers were never above five/plant. Extrapolating beyond this data range would be statistically unsound so results from this site should be treated with caution. Similar concerns were expressed when looking at the relationship between leaf area and yield at this site. If results from Yorkshire in 2019 are discarded then only 4/35 (11%) regression analyses showed negative slopes significantly different from zero and 31/35 (89%) had slopes that showed no significant difference from zero. This result suggests that in the majority of experiments in this study the presence of CSFB larvae had limited impact on yield and crops have some inherent

tolerance to feeding by CSFB larvae. It may be that the relationship between larval load and yield involves other factors, for example Williams & Garden (1961) found a strong relationship between larval mines per inch of plant height and yield.

Where a negative slope of the regression line was seen yield decreased by between 0.05 and 0.07 t/ha for every increase of one CSFB larva per plant. This is similar to previous work that found yield decreased by 0.07 and 0.05 t/ha for each increase in CSFB larva (Purvis, 1986; White & Cowrick, 2017 respectively), suggesting that modern varieties are no more or less tolerant to larval feeding. Similar relationships were found between larvae and yield in autumn, winter and spring, suggesting that treatments should continue to be based on autumn larval numbers. The yield impacts identified here suggest that if 10 larvae per plant were present they might be expected to reduce yield by between 0.5-0.7 t/ha which at a price of £300/tonne would be equivalent to £150-210/ha. Clearly it would be worth taking steps to protect the crop against such a level of yield loss. The current autumn threshold for CSFB larvae is five per plant and this might be expected to reduce yield by £75-105/ha.

The mean number of larvae in the stems in the spring (larvae were never recorded in the stems in the autumn) never exceeded six per plant across all sites at which they were assessed. At none of these sites were the slopes of the regression lines significantly different from zero suggesting that at numbers up to six per plant there was limited if any impact of CSFB larvae in the stem on yield. Despite the relatively low numbers in the stem this finding is interesting as it has been suggested that feeding in the stem has a greater impact on yield than that in the petioles.

From observations of CSFB larvae dissected from OSR plants in the spring it was noticeable that there was a large variation in larval size. It seems likely that larvae continue to hatch over winter and into the spring when temperatures allow. Depending on their size and when they hatch larvae are likely to have varying effects on crop yield. Taking account of this will be important in determining whether control measures are required. For example, if the majority of larvae in a crop result from eggs that hatch in the spring it is debatable whether they will have such a significant impact on the crop as larvae that hatched the previous autumn. Purvis (1986) suggests that spring hatching larvae have a smaller damage potential than autumn hatching larvae due to being at smaller instar stages. This suggests that the autumn assessment of CSFB larval numbers is more important than assessments the following spring and should carry more weight when deciding on the need for control measures, a finding similar to that by Purvis (1986). As a result of widespread resistance to pyrethroid insecticides in CSFB populations across the UK there are limited options for chemical control of the pest. Providing intelligence on CSFB larval populations in the autumn would help in predicting potential yield loss and whether chemical or alternative control options would be cost effective. More work is required to clarify the relative importance of winter and spring



larval populations of CSFB larvae in order to predict better the need for control measures against the pest.

In general, research has shown that OSR has an inherent tolerance to loss of green leaf area due to feeding by adult CSFB and to feeding by their larvae within the leaf petioles and plant stems. However, the degree to which this tolerance is expressed is likely to be dependent upon a number of other factors such as environmental conditions, the timing of adult CSFB migration in relation to crop growth, crop condition and how the size of CSFB larvae is affected by the timing of egg hatch. These areas are worthy of further research and will help to contribute to the development of a sustainable IPM strategy for the pest.

## **6.5. Conclusions**

### **6.5.1. Adult CSFB feeding damage**

- Historic pot and field experiments demonstrate that OSR plants are able to tolerate some level of leaf loss and particularly of the cotyledons. Biomass was substantially reduced at 60 to 75% loss of green leaf area and could be considered the threshold.
- Data from historic field experiments tended to support the conclusions from the pot experiments that there was a degree of tolerance to loss of green leaf area, with marked yield loss not seen until 66-75% leaf area had been lost.
- Regression analyses of leaf area lost data from experiments in the current project showed that in only three of 10 experiments yield decreased with increasing loss of green leaf area. On balance the data suggests that the yield loss in these field experiments was negligible over the range of leaf loss (maximum leaf area lost was 28%).
- Data investigating the relationship between leaf area loss and yield generally supports the current three to four leaf stage threshold. If anything the data suggest that the crop is more tolerant of leaf loss between the cotyledon and two leaf stage than previously thought and possibly at the three to four leaf stage too.
- Whilst results suggest a level of tolerance to leaf area lost it should be borne in mind that the timing of beetle infestation is critical. Crops can be lost if beetle migration occurs as OSR seedlings are just emerging.

### **6.5.2. Damage from CSFB larvae**

- Regression analyses found only 4/36 (11%) experimental datasets showed evidence that OSR yield decreased with increasing numbers of CSFB larvae. This shows a high potential for crops to tolerate feeding damage but work is needed to identify the factors that govern this tolerance.

- Yield decreased by between 0.05 and 0.07 t/ha for every increase of one CSFB larva per plant where a statistically significant slope of the regression line was demonstrated. This is in line with previous studies. Based on this relationship, at a price of £300/tonne, 10 larvae per plant might be expected to decrease yield by about £150-210/ha.
- The presence of a mean of up to six larvae per plant in the stems in the spring does not appear to result in any additional yield loss to that already described. It is unknown what impact higher stem larval populations would have as six larvae per stem was the maximum recorded in these experiments.
- Yield impacts per larva were similar regardless of assessment timing, suggesting that autumn larval assessments remain the most important in terms of treatment decisions. Larvae that invade plants in the autumn are likely to have a greater impact on yield than larvae that invade later because plants are at an earlier growth stage when invaded and the larvae remain in the plants for longer.
- Dissection of plants showed that CSFB larval numbers tended to increase between autumn and spring. High levels of late winter and spring larval invasion on-farm have also been reported in recent years. However, there is uncertainty about the importance that late larval invasions have in terms of their impact on yield. As plants will be at a later growth stage when later invasions occur they may be better able to tolerate the feeding and so it is possible that such late invasions have less impact. It is likely that the size of larvae in the later winter and spring is important in determining their effect on yield. Further work to investigate the relationship between yield and larval size or invasion date is worthwhile.
- The majority of larvae were present in the leaf petioles with very few in the stem even in the spring.

## 7. Using volunteer oilseed rape as a trap crop

### 7.1. Introduction

Trap cropping is a method of reducing pest damage by attracting pests away from a sensitive crop and towards a trap crop. The trap crop is usually a plant stand (sown or otherwise) that is more attractive to the pest than the sensitive crop. This approach has been successfully implemented to reduce damage in commercial crops in a number of countries including Estonia to reduce pollen beetle (*Brassicogethes aeneus*) damage in WOSR, and in the United States to reduce silverleaf whitefly damage (*Bemisia argentifolii*) in beans and Lygus bug damage in cotton (Shelton & Badenes-Perez, 2006; Sarkar *et al.*, 2018).

Trap crop borders consisting of non-OSR Brassicaceae have been shown to reduce CSFB infestation in the neighbouring WOSR crop (Barari *et al.*, 2005). However, drilling these is costly, they require careful management and land is lost to the trap crop that could otherwise be sown with WOSR. A less expensive alternative, requiring minimal management input and not affecting the area that can be sown with WOSR, is to delay control of volunteer OSR (vOSR). Dense carpets of vOSR often appear in August following WOSR harvest (Figure 123) but are usually destroyed by growers in mid- to late August in preparation for the following crop.



Figure 123. A yellow water trap in a thick carpet of volunteer OSR in a field following OSR in mid-August.

Adult CSFB and other flea beetles (*Phyllotreta* spp.) are attracted to glucosinolate breakdown products called isothiocyanates, which are given off by the crop as plant volatiles (Bartlett *et al.*, 1992; Pivnick *et al.*, 1992; Tóth *et al.*, 2003). CSFB have sensitive chemosensory cells on their antennae that respond to chemicals given off by the host plant (Isidoro *et al.*, 1998), and it is likely that they use these to locate crops from some distance. Pivnick *et al.* (1992) suggest that, for

*Phyllotreta* spp., high densities of OSR plants are likely to be attractive due to the large amounts of plant volatiles given off, and it is possible that the same applies to CSFB. If so, a field of densely populated vOSR is likely to be more attractive to CSFB than a sown field where the crop has yet to emerge or is less densely populated than the area of vOSR.

Delaying the destruction of vOSR until late September (or later) may reduce CSFB pressure in WOSR crops in nearby fields by i) being more attractive to migrating CSFB and ii) discouraging CSFB (that emerged in the previous crop) from leaving the vOSR. Additionally, as CSFB wing muscles are thought to gradually atrophy once the beetles have arrived in a crop and mated (Bonnemaison, 1965), adults may have limited ability to move into WOSR crops in nearby fields when the vOSR is destroyed. Any eggs or larvae present in the vOSR at this time are very likely to die as they will not be able to complete their life cycle without finding another host. The aim of this objective is to investigate the potential to use vOSR as a trap crop for CSFB (Objective 4).

## **7.2. Materials and methods**

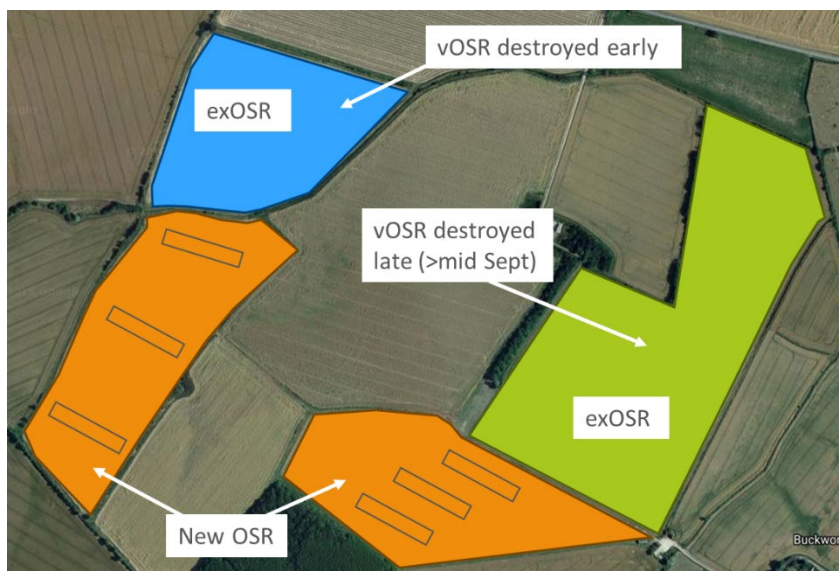
### **7.2.1. Trial design**

This work investigated two treatments. In treatment 1 vOSR was destroyed early (early August to early September depending on the site) and in treatment 2 the vOSR was destroyed late (mid- to late September depending on the site). Due to the mobility of CSFB adults and the aim of using moderately sized areas of vOSR to attract them, this work is not suited to small plot experimentation so was studied on a field scale.

Each trial involved two pairs of adjacent fields, either on the same farm or neighbouring farms. In each pair, one field was coming out of WOSR ('exOSR' field) and the other field was going into WOSR ('new OSR') (Figure 124). The exOSR fields act both as a source of CSFB, as beetles will have emerged from these crops, and as a source of vOSR, which may attract migrating CSFB or deter those already present from leaving. Farms (or neighbouring farms) were selected that had experienced similar levels of CSFB pressure in their exOSR fields at establishment the previous year. Each vOSR destruction treatment was applied in separate exOSR fields by the host farmers (i.e. vOSR was destroyed early in the exOSR field in one of the pairs of adjacent fields, and the vOSR was destroyed late in the exOSR field in the other pair of adjacent fields) (Figure 124). A number of factors were used to select sites for the trials:

- The new OSR fields should be drilled on dates as close to each other as possible, so that these crops were at similar developmental stages at the time of CSFB infestation.
- The new OSR in treatment 2 (late destruction timing) should have emerged by the time the vOSR was destroyed in the adjacent exOSR field, to ensure that the vOSR had the potential to draw CSFB away from the new crop.

- The new OSR crops had the same seed rates and WOSR variety, to account for any effect variety or seed rate might have on CSFB migration.
- The exOSR fields had no new WOSR crops adjacent to them other than the 'new OSR' trial field, to minimise the effect of other, nearby new crops of WOSR attracting CSFB away from the trial fields.
- The same insecticide regime be applied to the new OSR fields while the trial was underway.



*Figure 124. Example layout of the vOSR trap crop trials. Orange fields are newly sown WOSR fields (“new OSR”). Rectangular boxes within these are assessment areas. Blue and green fields had WOSR crops that were harvested in the summer prior to the start of the trial, and contained vOSR. Volunteers are destroyed early in the blue field and late in the green field.*

### 7.2.2. Trial sites

In 2017/18, two trials were located at two separate farms in Cambridgeshire. In 2018/19, four trials were located at farms in Cambridgeshire, Hertfordshire and Suffolk. Site details are given in Table 44. Finding suitable farms that met all the requirements for these trials was challenging so there were some exceptions to the trial design and site requirements described above. At site 2 in 2017/18, only a small area of vOSR (2 ha) was left in treatment 2 rather than a whole field of vOSR. At site 1 in 2018/19, a single new field of WOSR crop was used as the new OSR field instead of separate fields. This field was large (37.5 ha) with an area of woodland separating each side adjacent to the fields containing the vOSR so acting as a natural barrier between the two vOSR destruction treatments. At site 3 in 2018/19, the new OSR field in treatment 1 received two pyrethroid applications prior to the first plant population assessment compared to one application in treatment 2. However, the new OSR field in treatment 2 received an additional pyrethroid prior to the larval assessment. Given that pyrethroid resistance is common in Hertfordshire, it is likely that the different spray regimes at site 3 had little effect on the results. At site 4 in 2018/19, a second

field of WOSR was adjacent to the exOSR field in treatment 1 and so may have acted to attract CSFB away from the new OSR field in this treatment, however it was relatively small (1.5 ha) so it was felt that any effect would be minor. The timing of sprays was also slightly different at this site, with the new OSR field in treatment 1 receiving a pyrethroid application one week after that in treatment 2. It is unlikely that this had impact on the damage or plant population assessments as these occurred after both sprays, however it may have affected early assessments of CSFB adults caught in water traps. Any other differences (e.g. seed rate and variety) are shown in Table 44.

### **7.2.3. Assessments**

A total of nine yellow water traps (YWTs) (round, 26 cm diameter, 8.5 cm deep) (Flora trap, Ringot Ltd, France) were placed on the ground in each new OSR field in three assessment areas; 'near' to the exOSR field (approximately 10 m from the field margin), in the 'middle' of field and 'furthest' from exOSR field (approximately 10 m from the field margin) (Figure 125). Each assessment area was approximately 50 m wide x 10 m deep. Three YWTs were also placed on the ground in the exOSR fields, about 20 m from the field edge and parallel to those in the new OSR field (Figure 125 & Figure 126). All YWTs were placed at least 20 m apart and 15 m from the field edge. Adult CSFB caught in the traps (Fig. 4) were counted and traps emptied weekly until at least the end of October in the new OSR fields and until approximately four weeks after the vOSR were destroyed in the exOSR fields. In 2018/19, raised YWTs were included in the trials. Ground-based YWTs will catch both flying and hopping CSFB but raised traps are likely to catch only flying CSFB. Part of the rationale for this work was that CSFB are thought to gradually lose their ability to fly once they are in the crop (Bonnemaïson, 1965) so the use of raised traps allowed this to be investigated, as well as provide some insight into autumn flight activity. Two raised YWTs were placed in each of the near and middle assessments areas at least 3-4 m from any tramlines in the new OSR fields at each site (Figure 125). These traps were the same as the ground-based traps but raised 1 m above soil level on poles.

Table 44. Site details for new OSR fields at each trial. \* Extrovert drilled around headlands. Treatment 1 = vOSR destroyed early. Treatment 2 = vOSR destroyed late. \*\* Calculated based on a seed rate of 3.4 kg/ha (as reported by the host farmer) assuming thousand grain weight = 5. † Approximate area.

Year	Site no.	Treatment	Location	Grid ref.	OSR variety	Seed rate	Drill date	vOSR control date	Area of vOSR in exOSR field (ha) †
2017/18	1	1	Great Paxton, Cambridgeshire	TL216642	Campus	4.8 kg/ha	26/8	30/8	14.5
2017/18	1	2	Great Paxton, Cambridgeshire	TL225636	Campus	4.8 kg/ha	25/8	21/9	12.5
2017/18	2	1	Buckworth, Cambridgeshire	TL136772	Campus	5.5 kg/ha	3/9	17/8	7
2017/18	2	2	Buckworth, Cambridgeshire	TL141768	Campus	5.5 kg/ha	4/9	9/10	2
2018/19	1	1	Eltisley, Cambridgeshire	TL230605	DK Expansion*	50 per m <sup>2</sup>	20/8	30/8	57.5
2018/19	1	2	Eltisley, Cambridgeshire	TL230605	DK Expansion*	50 per m <sup>2</sup>	20/8	17-24/9	82
2018/19	2	1	Caldecote, Hertfordshire	TL234375	Nikita	4.5 kg/ha	23/8	25/8	22.5
2018/19	2	2	Caldecote, Hertfordshire	TL233370	Nikita	4.5 kg/ha	23/8	25/9	3.5
2018/19	3	1	Kimpton, Hertfordshire	TL148185	DK Imperial CL	60 per m <sup>2</sup>	15/8	3/9	15.5
2018/19	3	2	Kimpton, Hertfordshire	TL168189	DK Exalte	68 per m <sup>2</sup> **	11/8	24/9	8
2018/19	4	1	Bentley, Suffolk	TM116389	Elgar	3.5 kg/ha	28/8	3/9	11.5
2018/19	4	2	Bentley, Suffolk	TM116379	Elgar	3.7 kg/ha	24/8	13/9	16



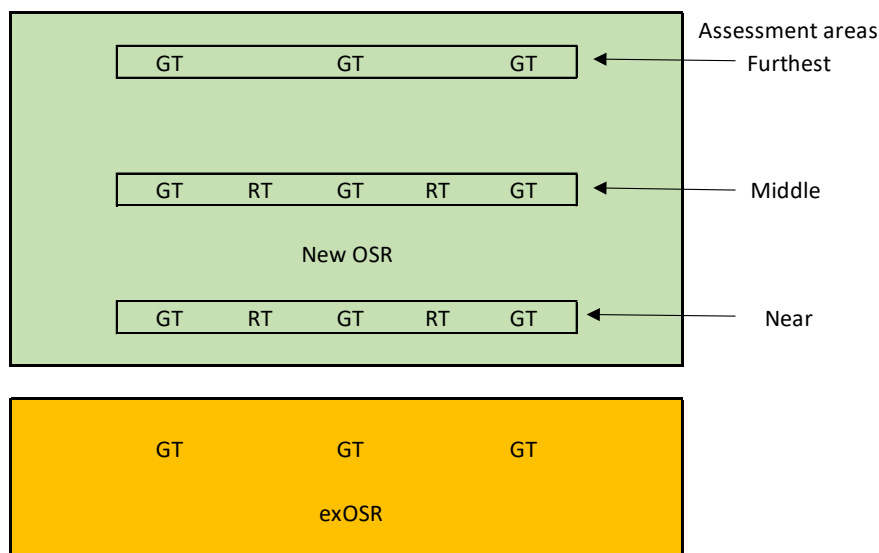


Figure 125. Layout of assessment areas and trap positions in each pair of fields at each site. GT = ground-based yellow water trap. RT = raised yellow water trap. New OSR = field going into WOSR. ExOSR = field coming out of WOSR.



Figure 126. Yellow water traps in the vOSR in an exOSR field (left). CSFB caught in yellow water trap (right).

Plant populations and CSFB feeding damage were assessed twice during crop establishment in the new OSR fields. These assessments occurred at about the two true leaf stage (range BBCH 12-14) and six true leaf stage (range BBCH 14-18) in each assessment area. Plant populations were assessed by counting plants along each side of 0.5 m rod at five randomly selected locations in each assessment area and using the row width to calculate plants per m<sup>2</sup>. CSFB feeding damage was assessed by estimating the percentage leaf area lost in ten plants at five randomly selected locations (50 plants in total) per assessment area. In 2018/19, assessments of larval populations were done to determine whether reductions in adult CSFB pressure due to delayed vOSR destruction resulted in subsequent reductions in larval pressure. At each site, ten plants (stems and petioles) were randomly selected from each assessment area in the new OSR fields in



December. These were then returned to the laboratory and all leaf petioles and stems were dissected with a sharp scalpel. The number of CSFB larvae in the stems and petioles were recorded separately. Due to limitations in resources, this was done at sites 2 and 3 only.

#### 7.2.4. Statistical analysis

Differences between treatments were analysed by site using analysis of variance. Significant differences between treatments were identified using Duncan's multiple range test indices. Due to traps being knocked over or going missing, data was missing for some weeks at some sites. Where this occurred, Genstat estimated values for missing weeks based on the difference between treatment 1 and 2 in the other weeks. Too little data was available for analysis of number of adult CSFB caught in raised traps. Bar charts are presented as summaries using the standard error of the difference between means as an indication of data variability.

### 7.3. Results

#### 7.3.1. 2017/18 trials

##### Site 1

Adult CSFB were caught from the week commencing (w/c) 21 August (when the traps were first put out) until w/c 6 November (Figure 127). Peak activity was observed w/c 11 September when an average of 40 beetles were caught per trap in the exOSR field in treatment 1. Peak activity in the new OSR fields occurred w/c 25 September when an average of 32 beetles were caught per trap in treatment 1.

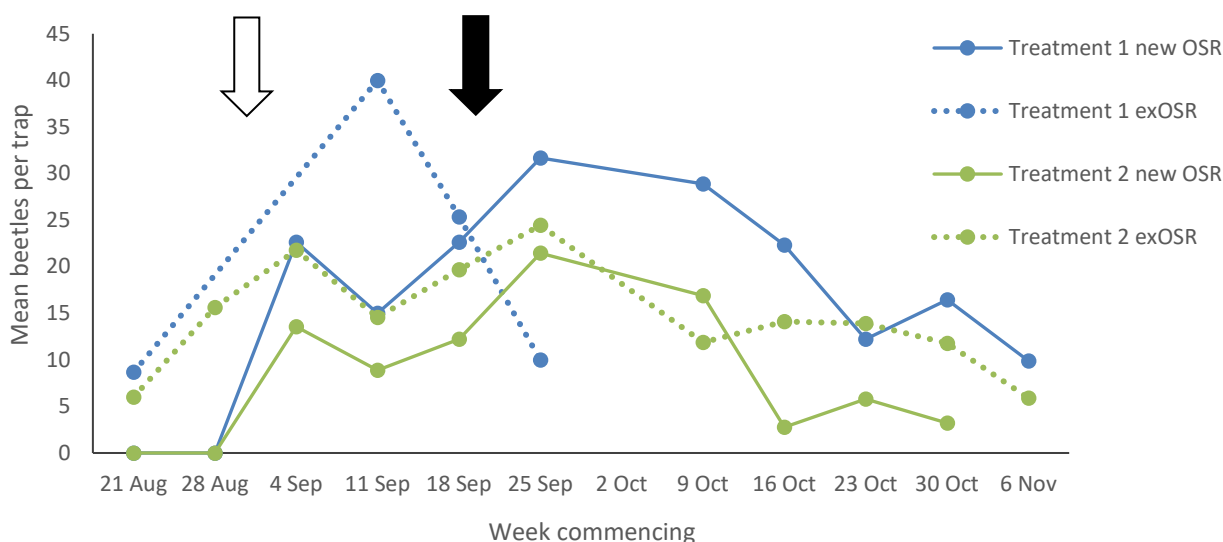
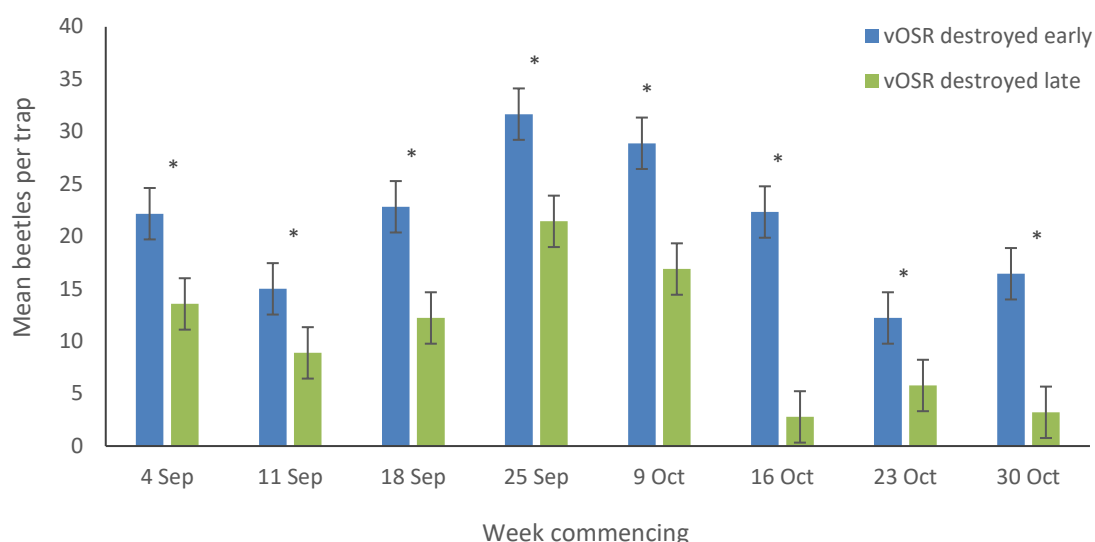


Figure 127. Mean number of CSFB adults caught in ground level yellow water traps in each field throughout the trial at site 1 in 2017/18. White arrow indicates when vOSR was controlled in treatment 1 (30 August) and the black arrow indicates when vOSR was controlled in treatment 2 (21 September).

There was no significant difference in the number of CSFB caught in the exOSR fields prior to the plants in the new OSR fields emerging (mean beetles per trap = 13 and 14 in treatments 1 and 2 respectively). Consequently, any differences recorded between the treatments are likely to be due to the effect of delaying control of vOSR rather than differences in CSFB numbers in the nearest source of the pest.

In the new OSR fields, CSFB numbers were significantly lower where the adjacent exOSR field had its volunteers removed late (treatment 2) than where they were removed early (treatment 1) ( $F = 3.1$ ,  $df = 94$ ,  $P = 0.006$ ), with significant reductions seen in each week of the trial (Figure 128). Reductions ranged from 32% (w/c 25 September) to 88% (w/c 16 October). Where volunteers were removed late (treatment 2) reductions in adult numbers were similar across the field, with 56% fewer adults in the area closest to the exOSR field compared to the same area in the field where the volunteers were removed early (treatment 1). The reductions for the area in the middle of the field and that furthest from the exOSR field were 43% and 52% respectively.



*Figure 128. Mean numbers of adult CSFB caught in ground level yellow water traps in new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 1 in 2017/18. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.*

Feeding damage was generally lower where vOSR was destroyed late (treatment 2) than where it was destroyed early (treatment 1). These differences were statistically significant in the areas of the fields nearest and furthest from the exOSR at BBCH 12 (38% and 48% less damage in each area respectively) ( $F = 5.1$ ,  $df = 586$ ,  $P = 0.007$ ) and nearest to the exOSR field at BBCH 17 (48% reduction;  $F = 5.1$ ,  $df = 586$ ,  $P = 0.007$ ) (Figure 129).

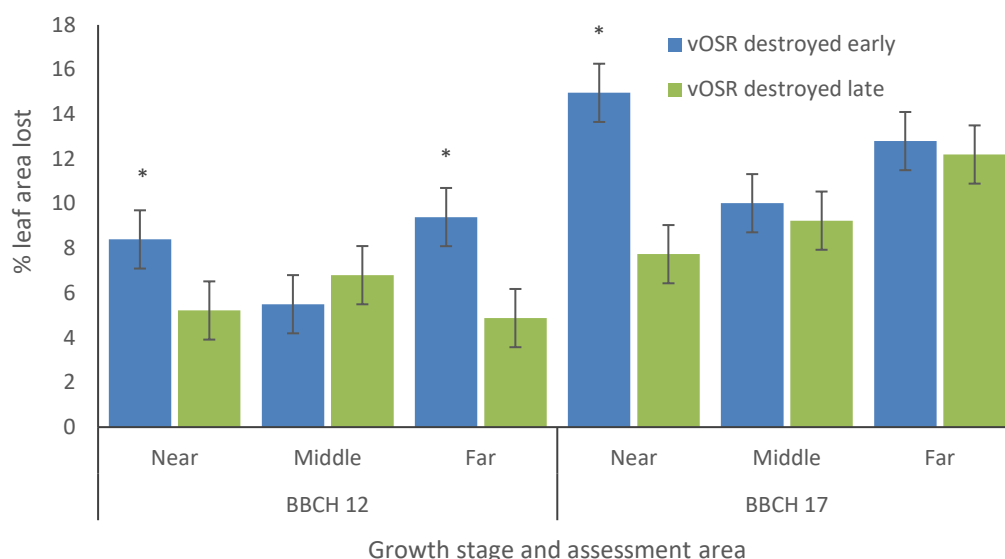


Figure 129. Mean CSFB feeding damage at BBCH 12 and BBCH 17 in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 1 in 2017/18. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

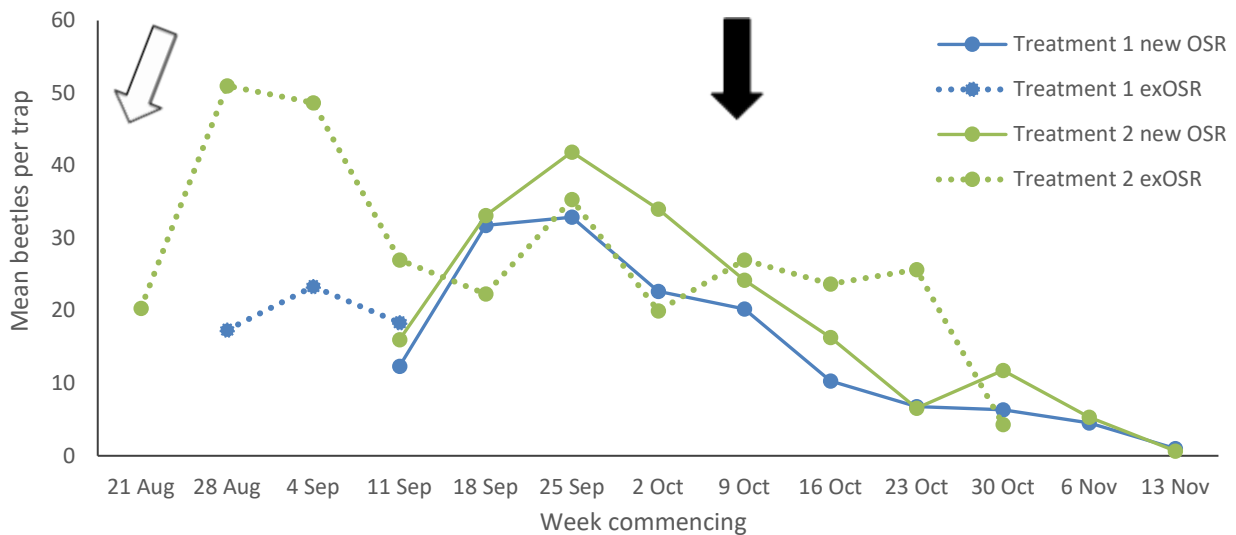
Overall plant populations were significantly higher in the new OSR field that was adjacent to the exOSR field in which the volunteers were destroyed late (treatment 2) (mean plant population = 49 per m<sup>2</sup>) than where the volunteers were destroyed early (treatment 1) (mean plant population = 39 per m<sup>2</sup>) (F = 12.7, df = 48, P < 0.001). However, differences in plant populations between the treatments varied with growth stage and area of the field (Table 45), ranging from a 52% increase in the middle of the field at BBCH 12 where the volunteers were destroyed late (treatment 2) to a 6% increase in the area furthest from exOSR field at BBCH 17 where the volunteers were destroyed early (treatment 1).

Table 45. Mean plants per m<sup>2</sup> in each area of the field (in relation to the exOSR field) and two growth stages of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 1 in 2017/18. Values in brackets indicate standard error of the mean. All values rounded to nearest integer.

Treatment	Growth stage	BBCH 12			BBCH 17		
	Area	Near	Middle	Far	Near	Middle	Far
1 (vOSR destroyed early)		36 (7)	40 (4)	39 (7)	37 (3)	40 (3)	40 (5)
2 (vOSR destroyed late)		50 (5)	61 (4)	46 (7)	45 (4)	54 (6)	38 (3)

## Site 2

Adult CSFB were caught from w/c 21 August (when the traps were first put out) until w/c 13 November (Figure 130). Peak activity was observed w/c 28 August when an average of 51 beetles were caught per trap in the exOSR field due to have its volunteers removed late (treatment 2). Peak activity in the new OSR fields occurred in w/c 25 September when an average of 42 beetles were caught per trap in the field adjacent to the exOSR that had its volunteers removed late (treatment 2).



*Figure 130. Mean number of CSFB adults caught in ground level yellow water traps in each field throughout the trial at site 2 in 2017/18. White arrow indicates when vOSR was controlled in treatment 1 (17 August) and the black arrow indicates when vOSR was controlled in treatment 2 (9 October).*

Comparison of numbers of CSFB in the exOSR fields showed that significantly more were caught where the volunteers were due to be removed late (treatment 2) than where they were due to be removed early (treatment 1) in w/c 28 August (mean beetles per trap = 17 and 51 in treatment 1 and 2 respectively) and 4 September (mean beetles per trap = 23 and 49 in treatment 1 and 2 respectively) ( $F = 20.9$ ,  $df = 18$ ,  $P < 0.001$ ) (Figure 130). This suggests that the new OSR field in treatment 2 is likely to have experienced higher CSFB pressure due to the greater number of beetles in the nearest source of CSFB.

For the new OSR fields, there was no significant effect on CSFB numbers of removing vOSR early or late, except in specific areas of the field in w/c 25 September and 2 and 9 October. On these dates beetle numbers were significantly higher where vOSR was destroyed late (treatment 2) than where it was destroyed early (treatment 1) in the middle (w/c 25 September) and near assessment areas (w/c 2 and 9 October) (Figure 131;  $F = 3.5$ ,  $df = 18, 117$ ,  $P < 0.001$ ).

At BBCH 12, feeding damage was significantly lower where vOSR was destroyed early (treatment 1) than where it was destroyed late (treatment 2) in the areas of the field nearest to the exOSR field (60% reduction) and the middle of the field (38% reduction). In contrast feeding damage in the area furthest from the exOSR was significantly lower where vOSR was destroyed late (treatment 2) than where it was destroyed early (treatment 1) (50% reduction;  $F = 26.6$ ,  $df = 2, 588$ ,  $P < 0.001$ ; Figure 132). At BBCH 17, there were no significant differences between the treatments, although damage was generally lowest where vOSR was destroyed early (treatment 1, Figure 132).

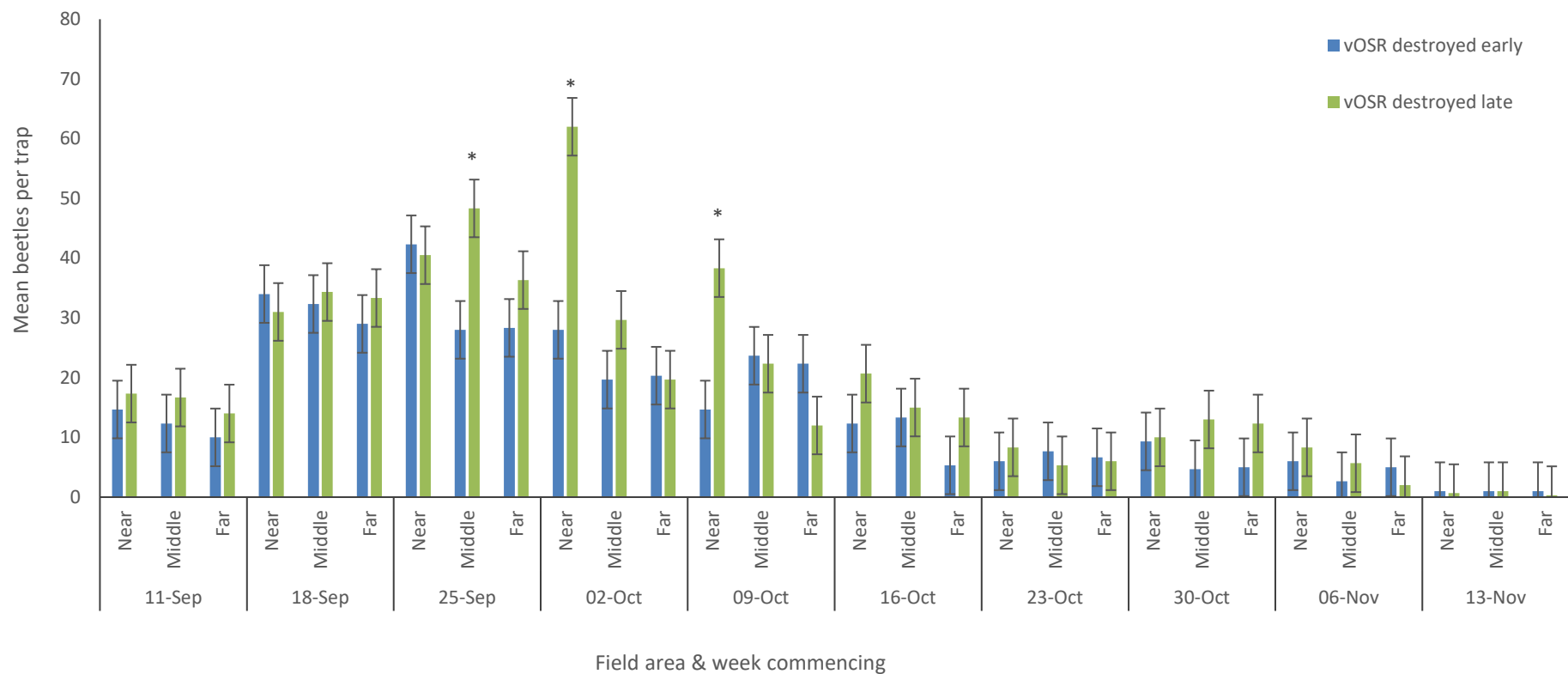


Figure 131. Mean numbers of adult CSFB caught in ground level yellow water traps in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 2 in 2017/18. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

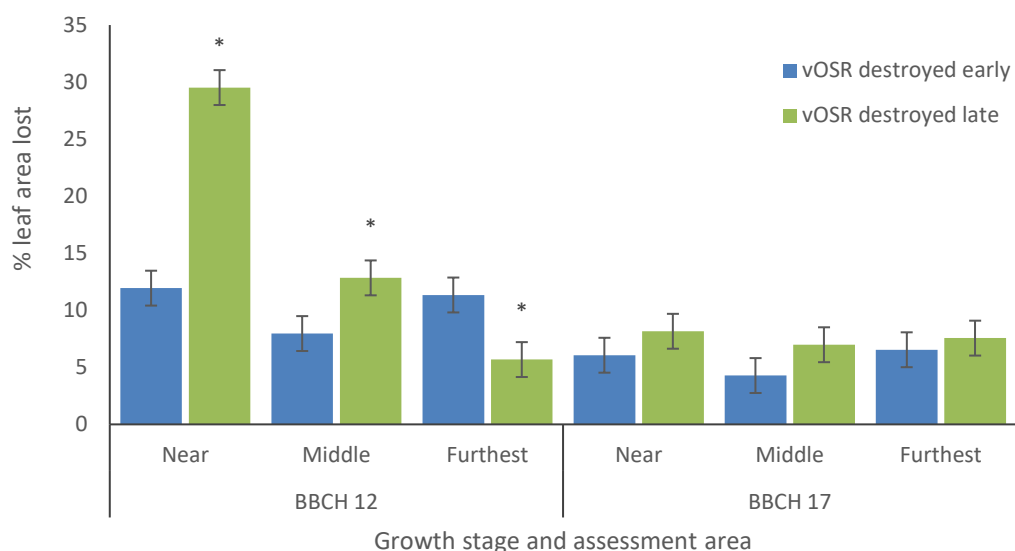


Figure 132. Mean CSFB feeding damage at BBCH 12 and BBCH 17 in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 2 in 2017/18. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

There were no significant differences in plant populations between the treatments at site 2 (mean plant population = 134 and 140 per m<sup>2</sup> in the early and late destroyed vOSR treatments respectively).

### 7.3.2. 2018/19 trials

#### Site 1

Adult CSFB were caught from w/c 3 September (when the traps were first put out) and continued to be trapped until w/c 5 November (Figure 133). Peak activity was observed w/c 3 September when an average of 26 beetles were caught per trap in the exOSR field in which vOSR was destroyed early (treatment 1). Peak activity in the new OSR fields occurred in w/c 22 October when an average of 14 beetles were caught per trap in the field adjacent to that in which the vOSR was destroyed late (treatment 2). Beetles were caught in raised traps at lower numbers than in ground-based traps, but the pattern of catch data broadly follows that for ground-based traps (Figure 134).

Comparison of numbers of CSFB in the exOSR fields showed that there was a significantly greater number of CSFB caught where the vOSR was destroyed early (treatment 1) compared to where it was destroyed late (treatment 2) in w/c 3 September (mean beetles per trap = 27 and 5 in treatment 1 and 2 respectively) and where the vOSR was destroyed late (treatment 2) compared to where it was destroyed early (treatment 1) in w/c 17 September (mean beetles per trap = 4 and 12 in treatment 1 and 2 respectively) ( $F = 10.2$ ,  $df = 28$ ,  $P < 0.001$ ). The high number of beetles caught in where the vOSR was destroyed early (treatment 1) may have been due to the

disturbance caused by the destruction of vOSR in this field, which occurred on 25 August. Given the fluctuation in beetle numbers, overall the CSFB pressure in the exOSR fields were similar.

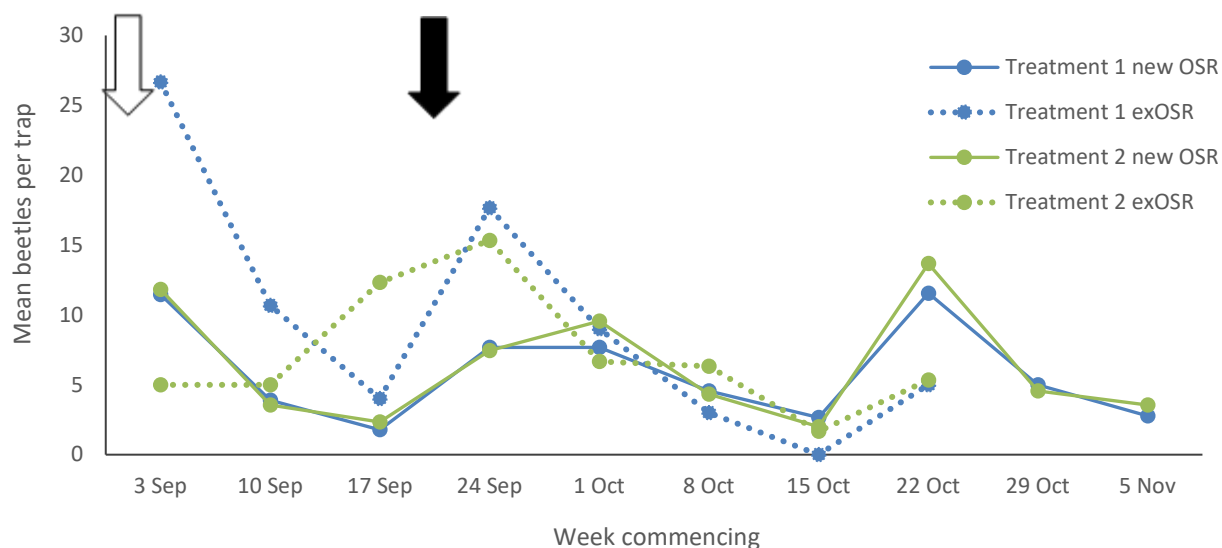


Figure 133. Mean number of CSFB adults caught in ground level yellow water traps in each field throughout the trial at site 1 in 2018/19. White arrow indicates when vOSR was controlled in treatment 1 (30 August) and the black arrow indicates when vOSR was controlled in treatment 2 (17-24 September).

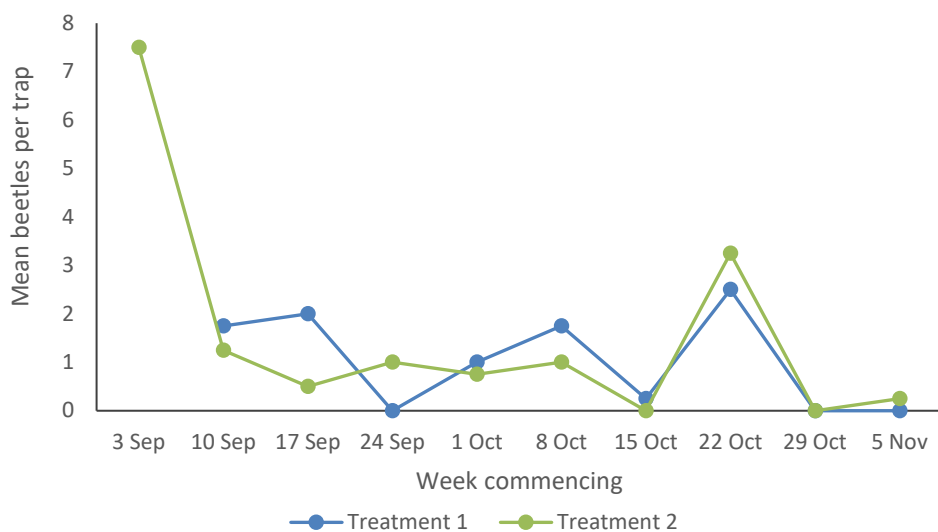
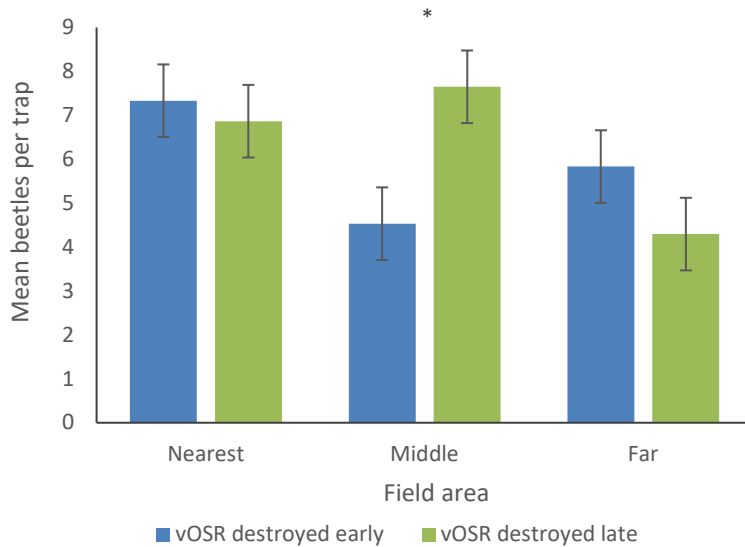


Figure 134. Mean number of CSFB adults caught in raised yellow water traps throughout the trial in each field of new OSR adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 1 in 2018/19.

In the new OSR fields, mean beetles per ground-based trap were generally similar between the treatments when numbers were averaged across the assessment areas (Figure 133). However, significant differences between treatments were found when considering the assessment areas, with significantly higher numbers of beetles in the middle of the field where the vOSR was destroyed late (treatment 2) than where it was destroyed early (treatment 1) (Figure 135;  $F = 8.7$ ,



df = 117,  $P < 0.001$ ). Differences between assessment areas within each treatment were also found, with significantly higher beetle numbers in the area of the field nearest to the exOSR field (mean beetles per trap = 7) than the middle of the field (mean beetles per trap = 5) where the vOSR was destroyed early (treatment 1) (Figure 135;  $F = 8.7$ ,  $df = 117$ ,  $P < 0.001$ ). Whereas where the vOSR was destroyed late (treatment 2) beetle numbers were significantly higher in the middle of the field (mean beetles per trap = 8) than the area furthest from the exOSR field (mean beetles per trap = 4) (Figure 135;  $F = 8.7$ ,  $df = 117$ ,  $P < 0.001$ ).



*Figure 135. Mean numbers of adult CSFB caught in ground level yellow water traps in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 1 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.*

Feeding damage was generally lower where vOSR was destroyed late (treatment 2) than where it was destroyed early (treatment 1), with significantly less damage observed in the middle of the field (76% less damage) and the area furthest from the exOSR field (57% less damage) (Figure 136;  $F = 76.9$ ,  $df = 588$ ,  $P < 0.001$ ).

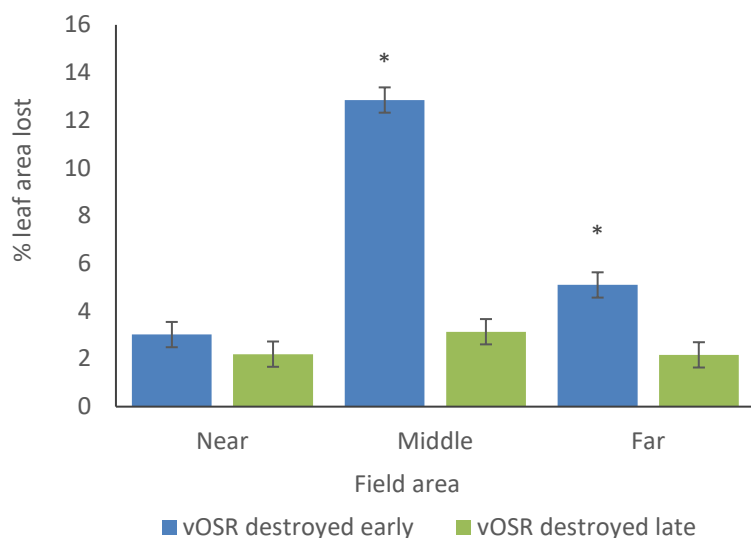


Figure 136. Mean CSFB feeding damage in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 1 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

Plant populations differed with growth stage and area of the field (Table 46), but overall plant populations were significantly higher in the new OSR field adjacent to where vOSR was destroyed late (treatment 2, mean plant population = 37 per m<sup>2</sup>) than where it was destroyed early (treatment 1) (mean plant population = 34 per m<sup>2</sup>) (F = 4.4, df = 48, P = 0.041).

Table 46. Mean plants per m<sup>2</sup> in each area of the field (Near, Middle & Far in relation to the exOSR field) and two growth stages in new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 1 in 2018/19. Values in brackets indicate standard error of the mean. All values rounded to nearest integer.

Treatment	Growth stage	BBCH 14			BBCH 16		
	Area	Near	Middle	Far	Near	Middle	Far
1 (vOSR destroyed early)		33 (2)	34 (3)	32 (3)	38 (2)	34 (3)	33 (2)
2 (vOSR destroyed late)		42 (2)	33 (2)	33 (2)	38 (2)	37 (1)	38 (2)

### Site 2

Adult CSFB were caught from the w/c 27 August (when the traps were first put out) and continued to be caught until w/c 3 December (Figure 137). Peak activity in ground-based traps was observed w/c 10 September when an average of 217 beetles were caught per trap in the exOSR field in which vOSR was destroyed early (treatment 1). Peak activity in the new OSR fields occurred in w/c 3 September when an average of 52 beetles were caught per trap the field adjacent to the exOSR where vOSR was destroyed early (treatment 1). In raised traps, lower numbers of beetles were

caught than in ground-based traps and the peak activity occurred later, in w/c 8 October (Figure 138). Very few beetles were caught in raised traps from the end of October.

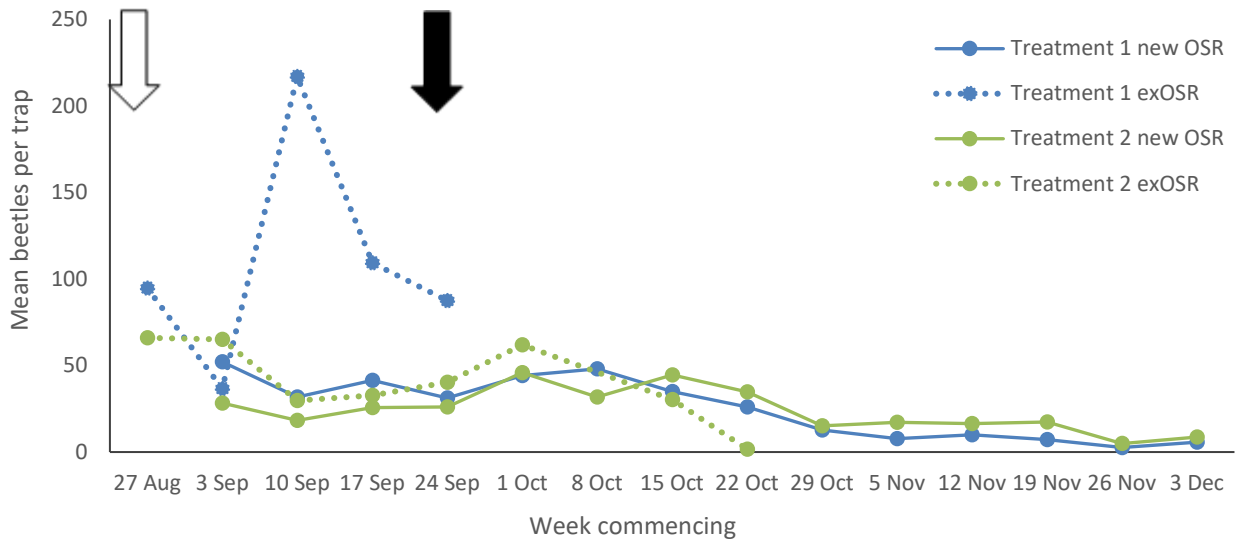


Figure 137. Mean number of CSFB adults caught in ground level yellow water traps in each field throughout the trial at site 2 in 2018/19. White arrow indicates when vOSR was controlled in treatment 1 (28 August) and the black arrow indicates when vOSR was controlled in treatment 2 (25 September).

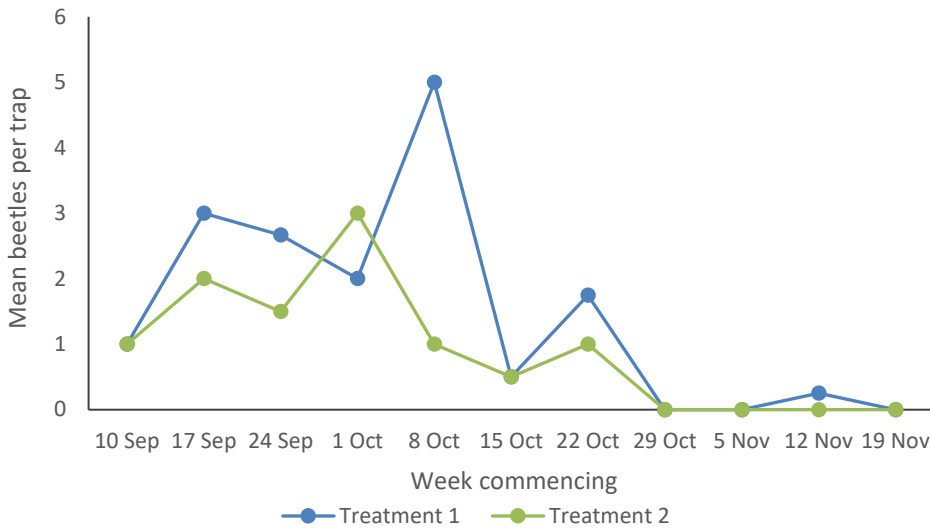


Figure 138. Mean number of CSFB adults caught in raised yellow water traps throughout the trial in each field of new OSR adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 2 in 2018/19.

Numbers of CSFB in the exOSR fields were significantly different only in w/c 10 September, with a mean of 217 beetles per trap where vOSR was destroyed early (treatment 1) compared to 30 per trap where it was destroyed late (treatment 2,  $F = 4.5$ ,  $df = 19$ ,  $P = 0.01$ ). The effect this would have on the relative CSFB pressure in the new OSR fields is difficult to determine as, although the

difference was temporary, it may have been sufficiently large enough to affect pest pressure in the neighbouring field.

In the new OSR fields, beetle numbers were generally highest where vOSR was destroyed early (treatment 1, vOSR destroyed 28 August) until early October, after which numbers tended to be highest where vOSR was destroyed late (treatment 2, vOSR destroyed 25 September, Figure 139). The only significant differences between the treatments occurred in specific assessment areas on specific weeks, with mean beetles per ground level trap significantly higher where vOSR was destroyed early (treatment 1) compared to where it was destroyed late (treatment 2) in w/c 3 September in the near and middle assessment areas, w/c 10 September in the near area, w/c 17 September in the middle and far areas and w/c 8 October in the near area (Figure 139;  $F = 4.0$ ,  $df = 161$ ,  $P < 0.001$ ). In contrast beetle numbers were significantly higher where vOSR was destroyed late (treatment 2) compared to where it was destroyed early (treatment 1) in w/c 15 October in the far area and w/c 22 October in the near area (Figure 139;  $F = 4.0$ ,  $df = 161$ ,  $P < 0.001$ ).

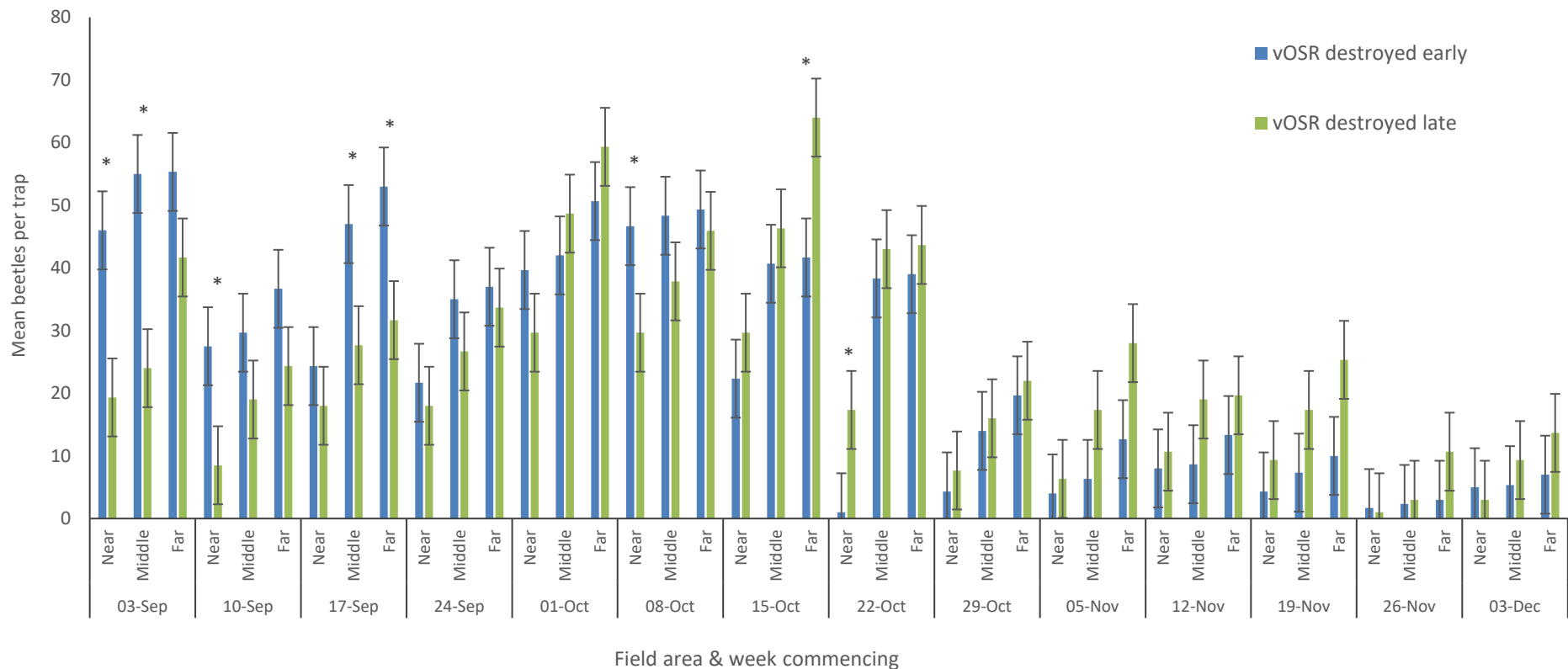
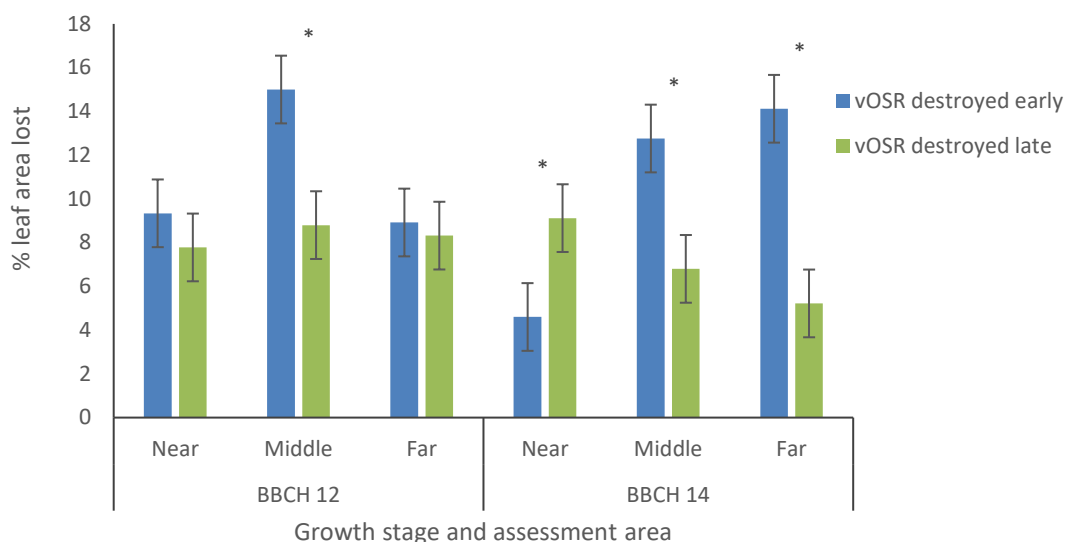


Figure 139. Mean numbers of adult CSFB caught in ground level yellow water traps in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 2 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

CSFB feeding damage was generally higher where the vOSR was destroyed early (treatment 1) compared to where it was destroyed late (treatment 2) (Figure 140), with significantly greater damage at BBCH 12 in the middle assessment area (70% greater) and BBCH 14 in the middle (88% greater) and far (170% greater) areas ( $F = 10.9$ ,  $df = 588$ ,  $P < 0.001$ ). In contrast damage was significantly greater where the vOSR was destroyed late (treatment 2) at BBCH 14 in the near area (98% greater) (Figure 140;  $F = 10.9$ ,  $df = 588$ ,  $P < 0.001$ ).



*Figure 140. Mean CSFB feeding damage in each assessment area (Near, Middle & Far) and at each growth stage of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 2 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.*

There were no significant differences in plant populations between treatments at this site, although there was general trend for greatest plant numbers where vOSR was destroyed early (treatment 1, Table 47). Analysis showed a significant effect of treatment on overall larval numbers (but with no interaction with field area) ( $F = 62.0$ ,  $df = 54$ ,  $P < 0.001$ ), with the mean larvae per plant significantly lower where vOSR was destroyed late (treatment 2) (mean larvae per plant = 4) compared with where it was destroyed early (treatment 1, mean larvae per plant = 13), a 69% decrease. Larval numbers by area are shown in Table 48.

*Table 47. Mean plants per m<sup>2</sup> in each area of the field (Near, Middle & Far in relation to the exOSR field) and at two growth stages in new OSR crops adjacent to a field in which vOSR had been*

destroyed early (treatment 1) or late (treatment 2) at site 2 in 2018/19. Values in brackets indicate standard error of the mean. All values rounded to nearest integer.

Treatment	Growth stage	BBCH 12			BBCH 14		
		Area	Near	Middle	Far	Near	Middle
1 (vOSR destroyed early)		71 (10)	75 (8)	77 (10)	65 (6)	75 (7)	67 (7)
2 (vOSR destroyed late)		75 (6)	63 (6)	67 (9)	61 (7)	61 (4)	55 (3)

Table 48. Mean CSFB larvae per plant in each area of the field (Near, Middle & Far in relation to the exOSR field) in December in new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 2 in 2018/19.

Treatment	Area		
	Near	Middle	Far
1 (vOSR destroyed early)	14	12	14
2 (vOSR destroyed late)	2	7	5

### Site 3

Adult CSFB were caught in ground-based traps from w/c 27 August (when the traps were first put out) until w/c 12 November (Figure 141). Peak activity was observed w/c 8 October when an average of 58 beetles were caught per trap in the exOSR field in which vOSR was destroyed late (treatment 2). Peak activity in the new OSR fields occurred in w/c 1 October when an average of 56 beetles were caught per trap in the field adjacent to that in which the vOSR was destroyed early (treatment 1). In raised traps, beetles were caught from w/c 3 September until w/c 12 November (Figure 142). Other than w/c 3 September and 8 October, very low numbers were caught in the raised traps.

Numbers of CSFB in the exOSR fields were similar except for two weeks when beetle numbers were significantly higher where vOSR was destroyed late (treatment 2) compared to where it was destroyed early (treatment 1). This happened in w/c 2 September, with a mean of 24 beetles per trap in treatment 2 compared to 9 per trap in treatment 1, and w/c 1 October, with a mean of 35 beetles per trap in treatment 2 compared to 14 per trap in treatment 1 ( $F = 4.5$ ,  $df = 23$ ,  $P = 0.005$ ). This indicates the CSFB pressure may have been slightly higher in the exOSR field neighbouring the new OSR crop where the vOSR was destroyed late (treatment 2) at the beginning of the trial. An increase in beetle numbers caught in the ground-based traps in this field in early October probably resulted from the mechanical disturbance caused by destruction of the vOSR increasing beetle activity in the days before the traps were emptied.

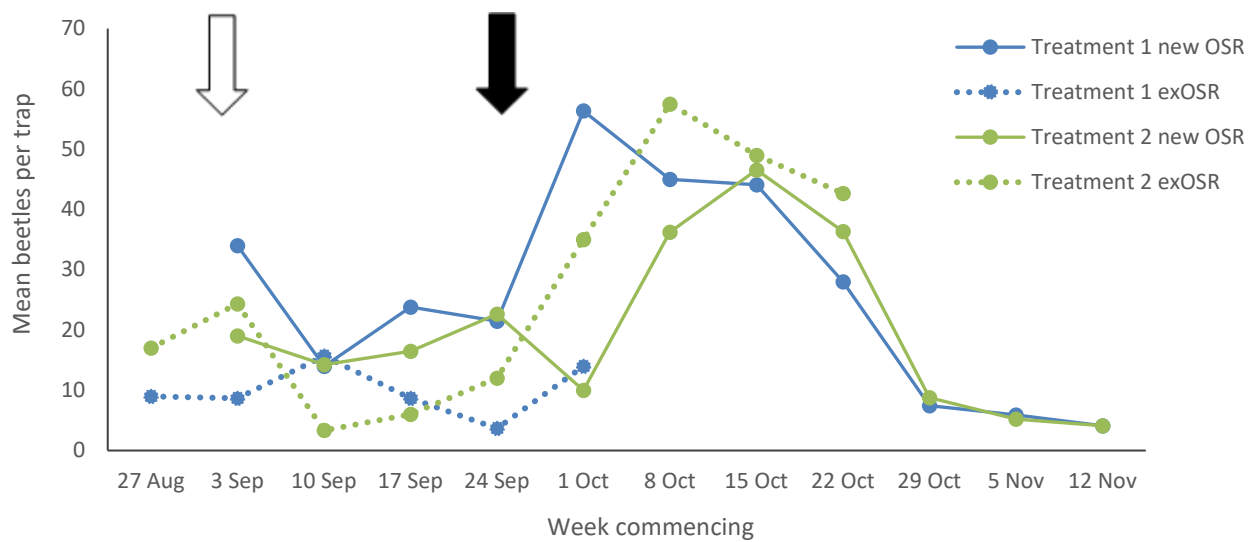


Figure 141. Mean number of CSFB adults caught in ground-level yellow water traps in each field throughout the trial at site 3 in 2018/19. White arrow indicates when vOSR was controlled in treatment 1 (3 September) and the black arrow indicates when vOSR was controlled in treatment 2 (24 September).

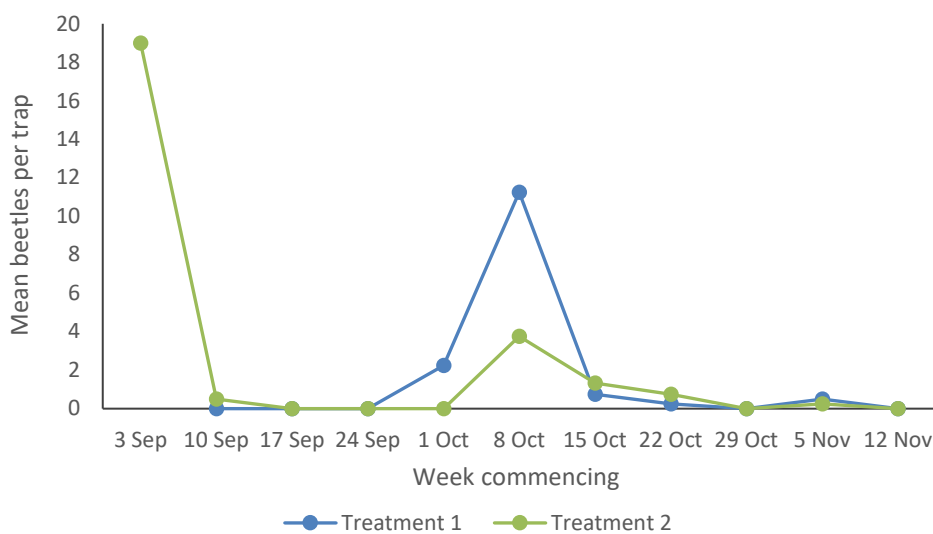


Figure 142. Mean number of CSFB adults caught in raised yellow water traps throughout the trial in each field of new OSR adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 3 in 2018/19.

Beetle numbers caught in ground level traps in new OSR crops were generally lower where vOSR was destroyed late (treatment 2) than where it was destroyed early (treatment 1). These differences were significant in w/c 3 September (44% lower) and 1 October (82% lower) (Figure 143;  $F = 11.6$ ,  $df = 128$ ,  $P < 0.001$ ). Significant differences in beetle numbers in specific assessment areas were also found between the treatments, with lower numbers in treatment 2 compared to treatment 1 in the area nearest to the exOSR field (47% lower) and the middle of the field (23% lower) (Figure 144;  $F = 8.3$ ,  $df = 128$ ,  $P < 0.001$ ).



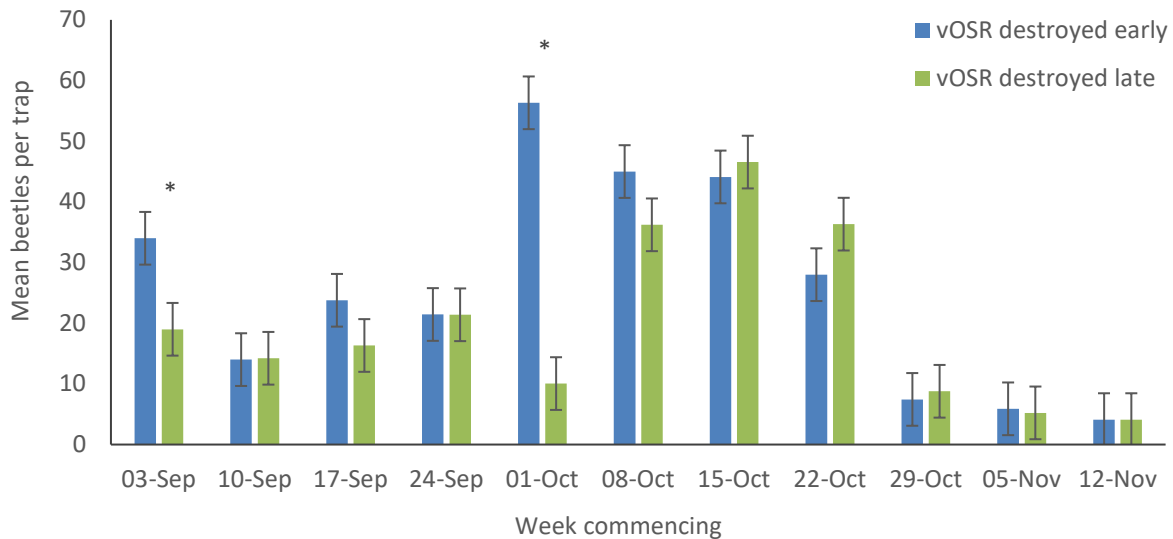


Figure 143. Mean numbers of adult CSFB caught in ground level yellow water traps in new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 3 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

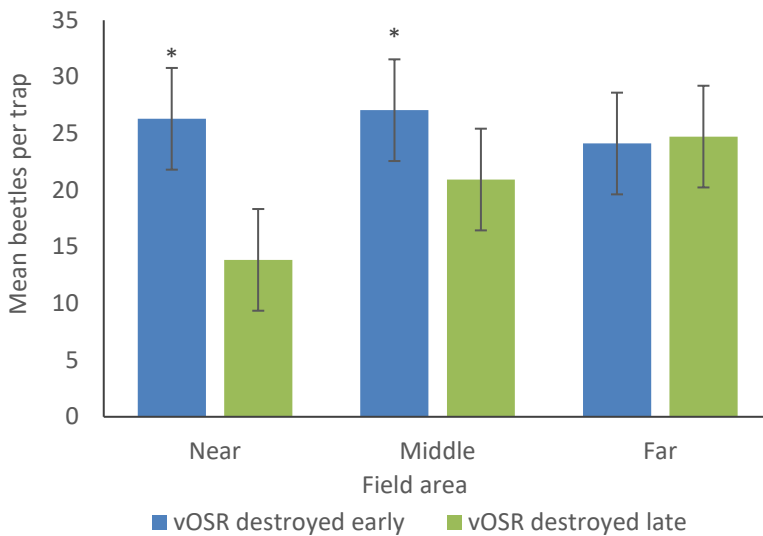
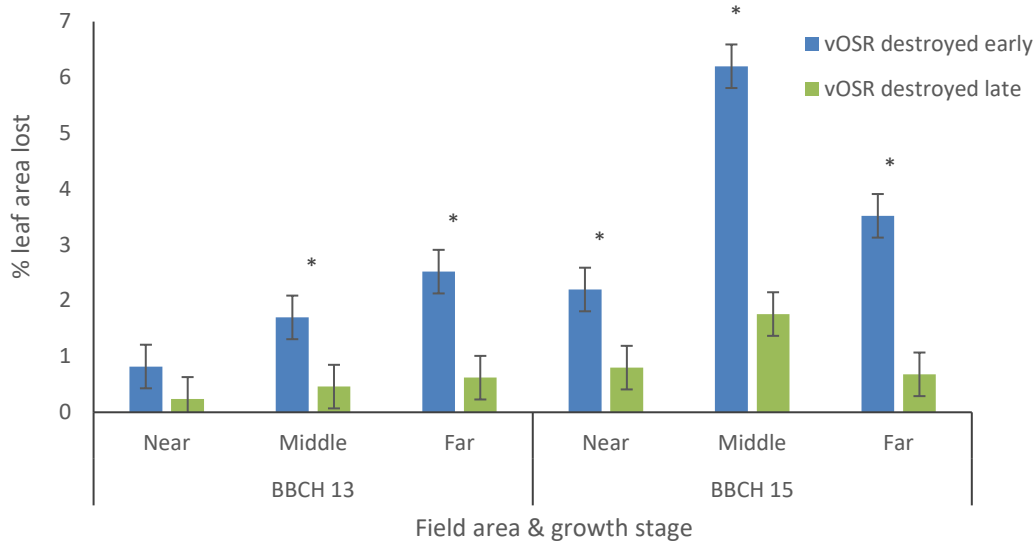


Figure 144. Mean numbers of adult CSFB caught in ground level yellow water traps in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 3 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

Adult feeding damage was consistently lower where vOSR was destroyed late (treatment 2) (Figure 145), with significant reductions compared to where vOSR was destroyed early (treatment 1) in the middle (73% lower) and far (75% lower) assessment areas at BBCH 13, and all areas at BBCH 15 (near, middle and far areas = 64%, 72% and 81% lower respectively) ( $F = 5.9$ ,  $df = 588$ ,  $P = 0.003$ ).



**Figure 145.** Mean CSFB feeding damage in each assessment area (Near, Middle & Far) and at each growth stage of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 3 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

Analysis showed a significant effect of treatment and field area (but not assessment date) on plant populations, with significantly higher populations where vOSR was destroyed late (treatment 2) than where it was destroyed early (treatment 1) in the middle of the field (45% increase) and the area furthest from the exOSR field (53% increase) (Figure 146;  $F = 5.1$ ,  $df = 48$ ,  $P = 0.01$ ).

Analysis showed a significant effect of treatment on overall larval numbers (but with no interaction with field area) ( $F = 29.8$ ,  $df = 54$ ,  $P < 0.001$ ), with mean larvae per plant significantly lower where vOSR was destroyed late (treatment 2, larvae per plant = 8) than where it was destroyed early (treatment 1, larvae per plant = 19), a 58% decrease. Analysis also showed that larval numbers differed across the fields, with larvae per plant significantly higher in the area of the field closest to the exOSR field than the other areas in both treatments ( $F = 6.8$ ,  $df = 54$ ,  $P = 0.002$ ). Larval numbers by area are shown in Table 49.

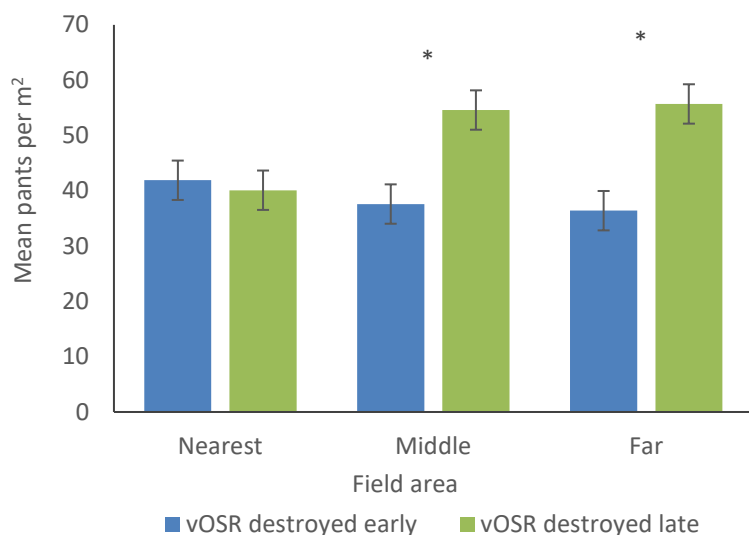


Figure 146. Mean plants per m<sup>2</sup> in each area of the field (Near, Middle & Far in relation to the exOSR field) (averaged across assessment dates) in new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 3 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

Table 49. Mean CSFB larvae per plant in each area of the field (in relation to the exOSR field) in December in new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 3 in 2018/19.

Treatment	Area		
	Near	Middle	Far
1 (vOSR destroyed early)	25	17	16
2 (vOSR destroyed late)	13	7	5

#### Site 4

Adult CSFB were caught in ground-based traps from w/c 27 August (when the traps were first put out) until w/c 5 November (Figure 147). Peak activity was observed w/c 15 October when an average of 29 beetles were caught per trap in the new OSR field adjacent to the exOSR where vOSR was destroyed early (treatment 2). In raised traps, adult CSFB were caught from w/c 3 September until w/c 5 November, with peak catches occurring in w/c 3 September and 8 and 15 October (Figure 148).

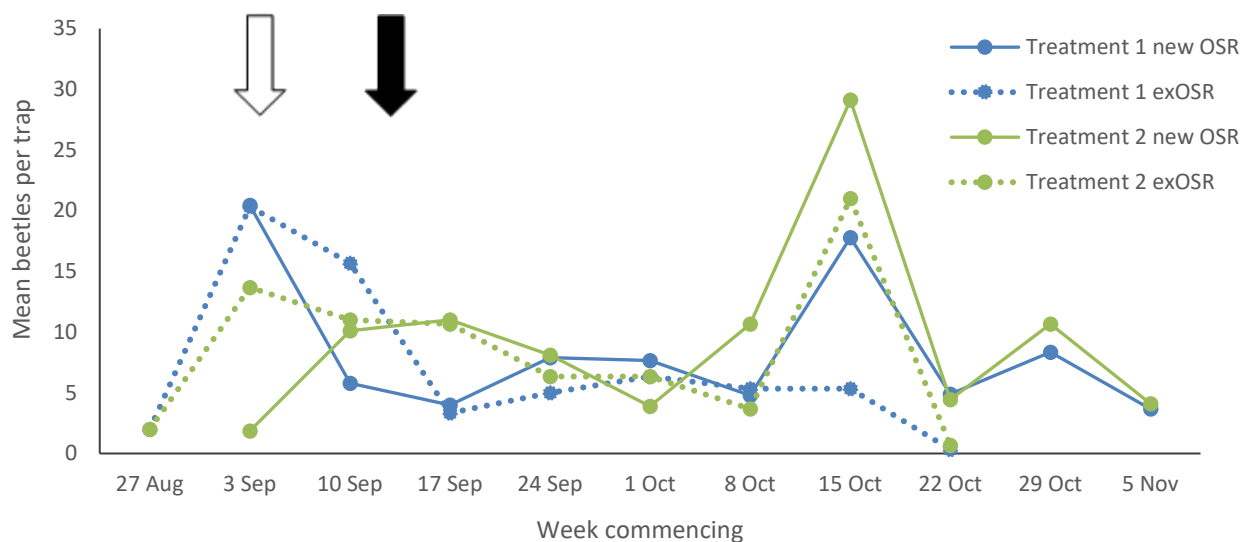


Figure 147. Mean number of CSFB adults caught in ground-level yellow water traps in each field throughout the trial at site 4 in 2018/19. White arrow indicates when vOSR was controlled in treatment 1 (3 September) and the black arrow indicates when vOSR was controlled in treatment 2 (13 September).

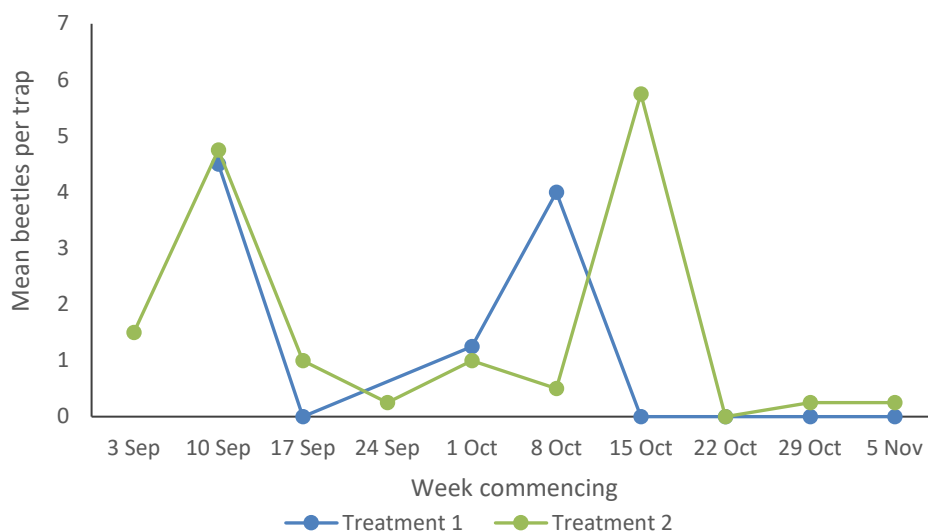


Figure 148. Mean number of CSFB adults caught raised yellow water traps throughout the trial in each field of new OSR adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 4 in 2018/19.

Numbers of CSFB in the exOSR fields were similar except for w/c 3 September when beetle numbers were significantly higher where vOSR was destroyed early (treatment 1), with a mean of 20 beetles per trap compared to 14 per trap where vOSR was destroyed late (treatment 2), and w/c 17 September and 15 October when numbers were significantly higher where vOSR was destroyed late (treatment 2), with a mean of 11 and 21 beetles per trap compared to 3 and 5 per trap where vOSR was destroyed early (treatment 1) for those weeks respectively ( $F = 6.8$ ,  $df = 36$ ,  $P < 0.001$ ). This indicates that CSFB pressure may have been slightly higher in the exOSR field

where vOSR was destroyed early (treatment 1) than where it was destroyed late at the beginning of the trial. Increased numbers caught in the ground-based traps in the exOSR field where vOSR was destroyed late (treatment 2) in mid-September and early October is likely due to the presence of vOSR in this field continuing to attract migrating CSFB.

In the new OSR crops, beetle numbers caught in ground level traps were generally similar between the treatments (Figure 149), except in all assessment areas in w/c 3 September where mean numbers of beetles per trap were significantly lower where vOSR was destroyed late (treatment 2) compared to where it was destroyed early (near, middle and far areas = 83%, 91% and 97% lower respectively). In contrast beetle numbers were significantly lower where vOSR was destroyed early (treatment 1) than where it was destroyed late (treatment 2) in the middle assessment area in w/c 15 October (62% lower) ( $F = 1.8$ ,  $df = 120$ ,  $P = 0.034$ ).

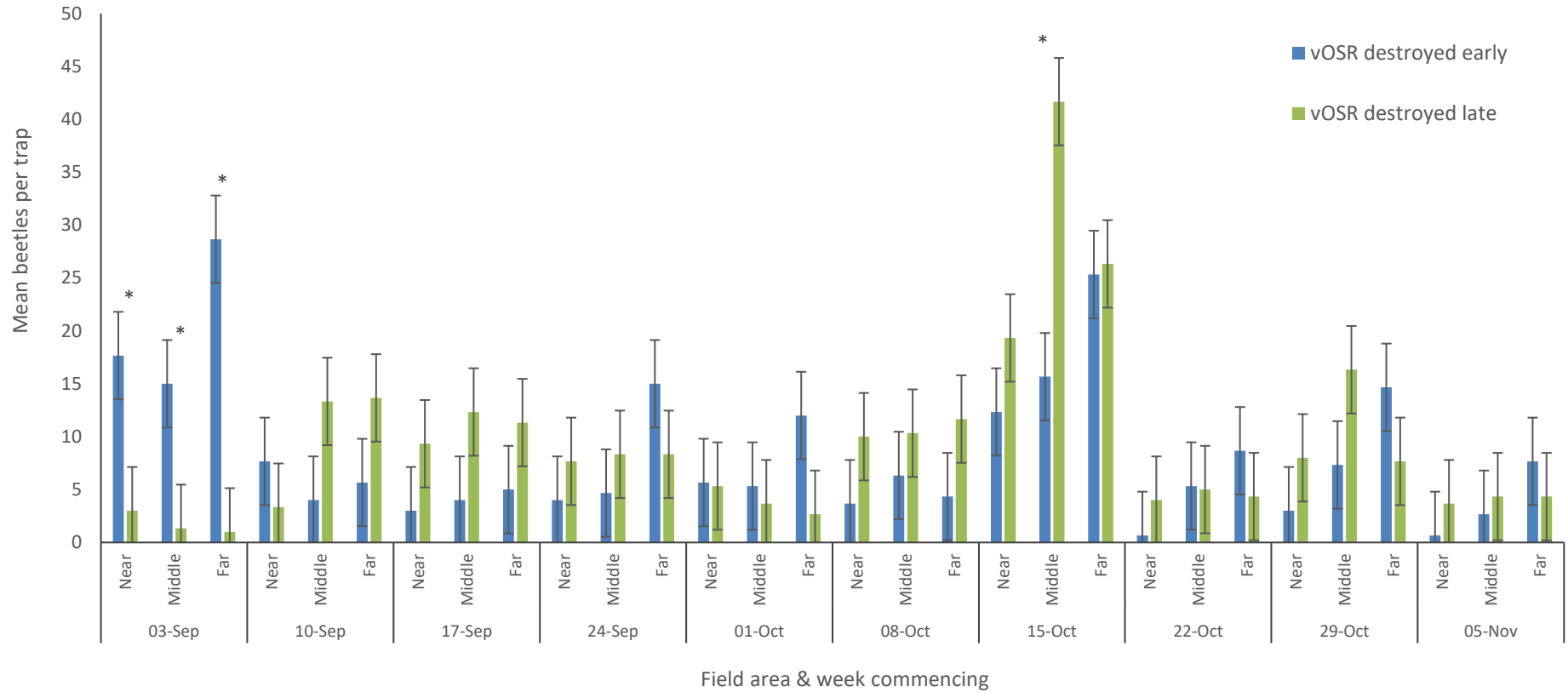
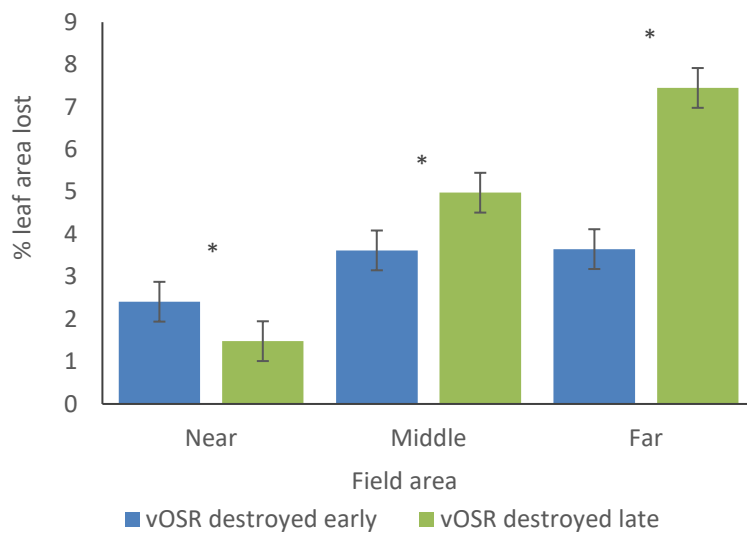


Figure 149. Mean numbers of adult CSFB caught in ground level yellow water traps in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 4 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

Adult feeding damage varied with assessment area (Figure 150), with significant reductions in damage observed in the new OSR adjacent to where vOSR was destroyed late (treatment 2) compared where it was destroyed early (treatment 1) in the area of the field nearest to the exOSR field (39% lower). In contrast damage was significantly higher in treatment 2 compared to treatment 1 in the middle of the field (38% higher) and the area furthest from the exOSR field (104% higher) ( $F = 6.9$ ,  $df = 588$ ,  $P < 0.001$ ).



*Figure 150. Mean CSFB feeding damage in each assessment area (Near, Middle & Far) of new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 4 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.*

Analysis showed a significant effect of treatment and field area (but not assessment date) on plant populations, with significantly lower populations where vOSR was destroyed late (treatment 2) than where they were destroyed early (treatment 1) in the area of the field nearest to the exOSR field (21% lower) (Figure 151;  $F = 5.1$ ,  $df = 48$ ,  $P = 0.01$ ). These differences were not evident in other field areas.

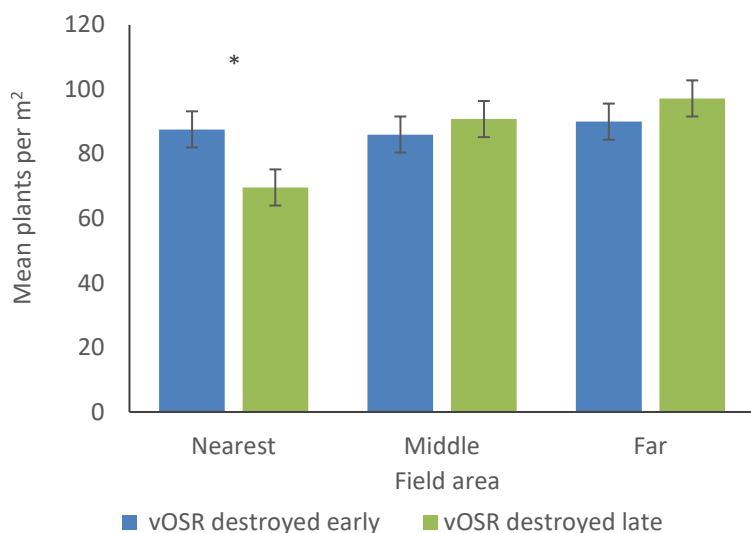


Figure 151. Mean plants per m<sup>2</sup> in each area of the field (Near, Middle & Far in relation to the exOSR field) (averaged across assessment dates) in new OSR crops adjacent to a field in which vOSR had been destroyed early (treatment 1) or late (treatment 2) at site 4 in 2018/19. Bars indicate the SED. Asterisks indicate where significant differences between treatments were observed.

#### 7.4. Discussion

Reducing adult CSFB pressure is most important during the early stages of crop establishment, and this was achieved at most sites by simply delaying the removal of vOSR in a nearby field. This approach tended to result in lower levels of adult CSFB infestation, although the effect varied between sites. At site 1 in 2017/18, significant reductions in beetle numbers (up to 88%) were seen every week in the crop adjacent to late destroyed vOSR compared to a nearby crop adjacent to a field in which the vOSR had been removed early. It is notable that these reductions continued for several weeks after the vOSR had been controlled in the adjacent field in mid-September. At sites 2 and 3 in 2018/19, the late removal of vOSR resulted in consistent reductions in adult CSFB in September and early October, with significant reductions in some weeks (up to 82% lower). At site 4 in 2018/19, beetle numbers were significantly lower at the start of September in the field adjacent to late destroyed vOSR.

Increases in beetle numbers in the fields adjacent to late destroyed vOSR were observed at several sites in October (e.g. site 2 in 2017/18, sites 2 and 4 in 2018/19). This may be due to these beetles being disturbed by the destruction of vOSR in the neighbouring field. However, it could be argued that such late migrations of beetles would have a minimal effect on crops as they would be beyond the four true leaf stage at which they become less susceptible to feeding damage. OSR is highly tolerant to loss of leaf area following establishment (Ellis, 2015) and the current treatment threshold indicates that beyond BBCH 14 treatments are not necessary unless damage is occurring at a greater rate than the crop is growing (AHDB, 2016).



The reductions in adult CSFB infestations in crops adjacent to areas of late destroyed vOSR were mirrored by reductions in feeding damage at the majority of sites. Often there was a significant reduction in leaf area lost at both crop growth stages surveyed (up to 76% less damage) with the only exceptions being at site 2 in 2017/18 and site 4 in 2018/19, where impacts on leaf area lost were minimal or varied. Concurrent with reductions in leaf area lost were increases in WOSR plant populations in fields adjacent to late controlled vOSR. Delaying destruction of vOSR resulted in significant increases in plants per m<sup>2</sup> in half of the trials (up to 56% increase) and had little effect in the other trials, although at site 4 in 2018/19 a significant reduction in one part of the field was recorded. The reason for this reduction at site 4 in 2018/19 is difficult to ascertain as CSFB numbers and damage were lower in this treatment, and in any case remaining plant populations were likely high enough to yield well. Delaying destruction of vOSR was also shown to significantly reduce larval populations at both sites at which this was assessed (up to 69% less larvae).

These results indicate that, on balance, delaying destruction of vOSR provides benefits to establishing WOSR in nearby fields by reducing adult CSFB numbers and damage, increasing plant populations and reducing CSFB larval numbers. However, at some sites little or no benefit of delaying destruction of vOSR was observed although, equally, there was little detrimental effect. These sites may be instructive in highlighting the situations in which vOSR may not act as an effective trap crop. For instance, at site 2 in 2017/18, only a small area of vOSR (2 ha) was left in the ground until late September rather than the whole field. Given that the number of plants may be important in determining the attractiveness of a crop to flea beetles (Pivnick *et al.*, 1992), it is possible that the relatively small area of vOSR at this site may not have been sufficiently attractive to distract migrating CSFB from moving into the neighbouring newly emerging WOSR crop. It is also worth noting that the number of adult CSFB were significantly higher in the field of vOSR that was controlled late compared to the vOSR that was destroyed early, especially in the early weeks of the trial. This may have resulted in the WOSR in the adjacent field experiencing a higher CSFB pressure.

Site 2 in 2018/19 was another trial in which delaying removal of vOSR produced mixed results. Although beetle numbers were reduced until early October, numbers increased after this. However, this late movement of CSFB adults appeared to have had no effect on larval numbers in December, with populations significantly lower in the delayed vOSR destruction treatment. This suggests that either the majority of egg-laying occurred in the vOSR in the delayed vOSR destruction field or that egg hatch was delayed due to oviposition occurring in cooler conditions. If the latter was to occur, then it is worth noting that the impact of late hatching eggs (and so late larval invasion) on yield is poorly understood. It is reasonable to expect that early hatching larvae would have a greater impact on plant development than late hatching larvae because invasion

occurs when the plants are younger and potentially more susceptible, and the pest spends more time feeding within the plants.

The other trial at which there were fewer benefits of delaying destruction of vOSR was site 4 in 2018/19. At this site, the vOSR in the late destruction treatment was removed relatively early (13 September), meaning that these volunteers would have acted as a trap crop for only a short period. Despite this, prior to 13 September, CSFB infestation was significantly lower in the adjacent newly emerging WOSR crop (up to 97% lower) suggesting that the vOSR acted as an effective trap crop while it was in situ. This illustrates the importance of delaying the removal of volunteers for as long as possible to benefit from their presence as a trap crop. Ideally volunteers should be left until the bulk of CSFB migration is complete.

This work gives some insight into adult CSFB activity in late summer and autumn. At all trial sites, CSFB pressure was high, with a minimum of 26 beetles caught per trap in a single week and a maximum of 217. Interestingly, at most sites peak trap catches occurred in fields containing vOSR rather than those containing the WOSR crop. This is likely due to the CSFB populations in these fields comprising individuals that emerged from the previous WOSR crop as well as CSFB that have migrated in from other fields, potentially attracted to the vOSR. More surprising is the fact that CSFB continued to be caught in ground-based traps in the vOSR fields several weeks after the vOSR had been destroyed, with peak ground-based trap catches even occurring after the destruction of the vOSR at some sites. This may be due to increased beetle activity following the physical disturbance caused by destruction of the volunteers. It is also possible that this is due to adult CSFB emerging late from aestivation, although it is thought that the bulk of such emergence appears to occur in August (Alford, 1979). Additionally, given that these fields no longer contained a food source, at least in suitable quantities, the continued presence of adult CSFB in the vOSR fields may also indicate that many are unable to leave due to the deterioration of their wing muscles during egg production (Bonnemaison, 1965). However, as beetle catches increased in ground-based traps in several of the new WOSR crops following late destruction of vOSR in adjacent fields (e.g. sites 2-4 in 2018/19), as well as peaks in catches in raised traps in October, suggest that at least a proportion of CSFB remain able to fly in October.

The conditions that influence flight activity and migration in CSFB are poorly understood. It has been suggested that a minimum of 16°C is required for flight (Ebbe-Nyman, 1952) and peak migration has been linked to daily maximum temperatures above 20°C (Sivčev *et al.*, 2016) or a fall and rise in daily maximum temperature above 20°C (Ballanger, 1984). The only UK study with relevant data also suggests a relationship between temperature and adult activity, with activity peaking when conditions were warmest and reducing as temperatures cooled (Alford, 1979), although this work only gave weekly average temperatures so the relationship between daily mean

or maximum cannot be determined. Relating ground-level trap catch data to average and maximum daily temperature data for each site in this work found that initial catch data was associated with conditions above 16°C and that temperatures below 8°C were associated with fewer catch numbers. However, the latter could also be related to reduced activity due to physiological changes and increased mortality as autumn progresses. Relating raised trap data to average and maximum daily temperature data for each site found that catches tended to stop below 16°C and that most peaks in flight activity (as inferred from raised trap catches) occurred when peak temperatures increased rather than average temperatures, suggesting that flight activity needs only short periods of warm conditions to occur. Equally, there were some sites at which peak temperatures were above 16°C for several days but little flight activity was recorded, though this tended to occur later in September so it is possible that this was partially due to the loss of wing muscles observed by Bonnemaïson (1965).

Much of the work ADAS has conducted monitoring adult CSFB activity over the last twenty years has used ground-based traps, usually not placed in the field until late August or early September. There are two issues with this approach. Firstly, ground-based traps cannot discern between hopping and flying beetles and so provide little information on flight activity, and secondly that CSFB activity often starts earlier than these dates and so trap catches provide little information on the conditions that initiate activity in late summer. The introduction of raised traps in these trials in 2018/19 was done to provide further information on flight activity. These revealed peaks of flight activity in early and mid-September at three sites (especially w/c 3 September) and further peaks in mid-October at three sites (especially w/c 8 October). Traps were not put out early enough at any of the sites to identify the start of adult activity following aestivation in the summer.

These trials also presented an opportunity to investigate how the distribution of adult beetles and their damage varied within fields. Many pests are unevenly distributed in crops, for example peach-potato aphid (*Myzus persicae*) and brassica pod midge (*Dasineura brassicae*), and this has also been anecdotally reported for CSFB. The spatial incidence of CSFB varied at most sites but the pattern differed between sites. At some sites, incidence increased at crop edges (e.g. feeding damage at site 1 in 2017/18), which may reflect CSFB migrating from these areas. This effect was also associated with the area of the field closest to the adjacent field of vOSR (e.g. feeding damage and adult incidence at site 2 in 2017/18 and adult incidence at site 1 in 2018/19), which likely reflects CSFB moving across from the vOSR. At other sites, pressure was greatest in the middle of the field (e.g. feeding damage at site 3 in 2018/19). The cross-site variation of within field spatial distribution of CSFB is likely due to a number of factors, including direction of migration, the presence of natural enemies and cross-field differences in microclimate, crop condition and crop growth stage.

In general, leaving volunteers in the ground appears to be an effective means reducing of CSFB pressure in nearby sown WOSR, especially during early crop establishment. There are minimal associated costs for the grower and the approach essentially involves doing nothing for several weeks. To realise the benefit of vOSR as a trap crop it is crucial to leave the volunteers in situ until the bulk of CSFB migration has occurred and at least until mid-September. The benefits are likely to increase the longer the volunteers are left, although further research would be required to confirm this. An additional benefit of delaying removal of vOSR is a general reduction in CSFB populations, as any eggs or larvae present in the field when the vOSR is destroyed will die due to the lack of a host. Destroying vOSR too early may actually increase CSFB pressure in nearby sown WOSR by acting to attract large populations of the pest to the area, which then fly into nearby sown WOSR when their food source of vOSR is removed. Clearly, which crop follows the vOSR and the practicality of accessing fields to remove the volunteers in late September/ October may affect decisions on the timing of destroying the volunteers. Host farmers in this work removed volunteers by either cultivating or applying a herbicide. None reported any major issues with either approach, although it was noted that wetter conditions would likely make late control more difficult, particularly using cultivation. It is also worth considering the impact of delayed volunteer control on disease pressure, as volunteers could provide a source of inoculum of diseases such as phoma leaf spot and stem canker, thereby potentially increasing disease severity in nearby crops in the autumn. Further research is needed to determine any impacts on disease pressure.

There were sites in which using vOSR as a trap crop was less beneficial, and this may be due to factors such as the area of vOSR left in the ground and the timing of vOSR removal (both discussed above). A further factor affecting the effectiveness of vOSR as a trap crop may be the growth stage of the volunteers in relation to that of emerging WOSR nearby. CSFB adults have been found to be attracted to young OSR plants more than older plants in laboratory studies (S. Cook, pers. comm.), a finding in line with anecdotal reports from growers who have redrilled poorly established parts of fields and noticed that trap catches in the redrilled areas increase as the crop emerges. If so, this presents the possibility that vOSR emergence could be manipulated (e.g. through cultivation) to maximum its attractiveness to CSFB. Given that these trials show that delaying control of vOSR can reduce CSFB pressure in neighbouring crops, identifying the optimal position of the area of vOSR on farm is worth consideration. This work used fields of vOSR adjacent to fields of sown WOSR, primarily to be give the approach the most robust test possible and to have greater confidence in attributing differences in CSFB pressures to timing of the destruction of vOSR. It may be that situating new WOSR crops further away from the vOSR trap crop may further reduce damage by making it more difficult for the CSFB left in the vOSR field to move to the new WOSR crops when the volunteers are destroyed. This should be balanced against any reduction in the relative attractiveness of the vOSR by moving it further away and is worth further investigation.

Overall, this work shows that utilising vOSR as a trap crop may form a useful component of an integrated pest management strategy. Of course, other brassicas are also attractive to CSFB and so brassica cover crops could also act as a trap crop (Lambdon *et al.*, 1998; Barari *et al.*, 2005) and, as long as these are removed before the CSFB larvae complete their life cycle (around March), would likely provide the same benefits in reducing CSFB populations.

## **7.5. Conclusions**

- Volunteer OSR can be utilised as a trap crop by delaying its destruction until after the bulk of CSFB migration is complete.
- Using this approach resulted in significant reductions in adult CSFB infestation (up to 88%) and damage (up to 76%), significant increases in plant population (up to 56%), and significant reductions in CSFB larvae (up to 69%) in newly sown WOSR in adjacent fields.
- Benefits were not seen at all sites, possibly due to an insufficient area of vOSR being left in the ground and the timing of vOSR removal.
- Adult CSFB activity continued into November and flight activity into October. A better understanding of the conditions that promote flight would help time control measures.
- To improve the effectiveness of using vOSR as a trap crop, the impact of vOSR area, vOSR removal date, relative attractiveness of different growth stages of vOSR and the emerging WOSR crop, and location of vOSR on the farm are worth further investigation.

## **8. Defoliation of WOSR to control CSFB larvae**

### **8.1. Introduction**

The utilisation of dual-purpose crops, especially wheat and oilseed rape (OSR) grown for forage and grain production in sheep-grazing systems, was reviewed by Dove & Kirkegaard (2014). When sown early and grazed in winter before stem elongation, late-maturing wheat and OSR crops can be grazed with little impact on yield. Maintaining yield depends upon the timing and extent of defoliation in relation to plant development and the seasonal conditions for recovery and regrowth (Kirkegaard *et al.*, 2012). In south-east Australia, trials showed that WOSR could provide 3 - 6.8 t dry matter/ha for forage in the winter and recover to produce 2.5-4.9 t/ha grain yield (Sprague *et al.*, 2015). As previously discussed, AHDB Cereals & Oilseeds Project 2140005 'Validation of an integrated pest management strategy for pollen beetle to minimise the development of insecticide resistance' showed that OSR crops mown off during early winter are able to regrow and still yield well (Ellis *et al.*, 2017).

Defoliation, either through grazing by farm animals or topping, also offers an opportunity to control CSFB larvae and reduce yield losses caused by the pest. The majority of CSFB larvae remain in the petioles until spring (White, 2016), meaning that defoliation in the winter need only remove petioles rather than the stem. Larval control would occur either by killing them directly (e.g. through ingestion by sheep or the action of a flail) or indirectly (e.g. by exposing them to natural enemies or inclement weather). Defoliation could be particularly valuable where high larval populations and insecticide resistance are recorded. The previous research in Australia and Canada (Kirkegaard *et al.*, 2012; Dove & Kirkegaard, 2014; Sprague *et al.*, 2015) suggests that this technique is worthy of further investigation in UK crops and has the potential to significantly reduce CSFB infestations particularly in regions in which the pest is most damaging and where there are no other chemical control options due pyrethroid resistance. The aim of this objective is to investigate defoliation as a potential cultural control option for CSFB larvae (Objective 4).

### **8.2. Materials and methods**

#### **8.2.1. Defoliation as a potential control option for CSFB larvae**

One experiment per year was done in each of two years (2016/17 and 2018/19) to determine the effects of defoliation on CSFB larval infestation. There were four treatments in 2017 (Table 50) and five treatments in 2019 (Table 51). A rolling treatment was included in 2019 to investigate whether it was possible to kill larvae by squashing them within the plants and also whether the plants would recover from such a treatment. In both years the experimental plots were 24 m long x 3 m wide and were arranged in a fully randomised block design. In 2017 there were 16 plots with four treatments replicated four times and in 2019 there were 25 plots with five treatments replicated five

times. Each site received routine herbicide, fungicide and fertiliser treatments but no insecticide treatments that could have affected CSFB larvae.

Table 50. Defoliation treatment list 2017. Actual dates in brackets.

Treatment no.	Defoliation treatment
1	No defoliation
2	Defoliation in December [9/12/16]
3	Defoliation in January (approx. one month after T2) [10/1/17]
4	Defoliation just after the start of stem elongation (early March) [10/3/17]

Table 51. Defoliation treatment list 2019. Actual dates in brackets.

Treatment no.	Defoliation treatment
1	No defoliation
2	Defoliation in December [4/12/18]
3	Defoliation in February [15/2/19]
4	Defoliation in December and removal of debris [4/12/18]
5	Rolling [24/1/19]

Plots in treatments 2, 3 and 4 in both years were defoliated using a 110 cm wide Wessex flail attached to a Ford 1210 tractor. The flail was set to cut at a height of approx. 5 cm (Figure 152). Plant debris was left in the field after defoliation in all treatments in 2017 (Figure 153). In 2019, debris in treatment 4 was removed using a rake, bagged and disposed of away from the crop (Figure 153). The height of crop was measured after defoliation to indicate the extent to which plants were cut back. Plots in treatment 5 in 2019 were rolled using a 72 L water filled garden roller which could be pulled along the length of the plot. Each section of the plot was rolled only once. The advantage of using a water filled roller was that it could be taken to the field empty and filled on site.

Timings of treatments were designed to coincide with particular stages of the CSFB life cycle and crop development. CSFB larval invasion can start in October but the number of larvae tends to build up rapidly from December (Alford, 1979). The December defoliation treatments were intended to coincide with this increase in larval numbers. In recent years, further larval invasion has been recorded throughout the winter and early spring (Collins, 2017) so January to March defoliations were investigated to assess the impact on these late-hatching larvae. Previous work has suggested that defoliation after stem extension results in a yield penalty (Kierkegaard *et al.*, 2012) so treatment 4 in 2017 (March defoliation) was timed to occur after the crop reached this stage to confirm this effect. Treatment 5 plots were rolled after a frost when the roller caused least damage



to the soil. As the intention was to squash the larvae within the plants it was considered that the best chances of achieving this were when the ground was hard following a frost. Both trials were located in Cambridgeshire (Table 52).



Figure 152. Undefoliated plants (left) and defoliated plants (right) immediately the defoliation on 9 December in the 2016/17 field trial.



Figure 153. Left: Plot defoliated on 10 March (treatment 4) in the 2016/17 field trial. Neighbouring plot to the left had been defoliated on 9 December (treatment 2). Photo taken on 10 March (immediately after the March defoliation). Right: Plot defoliated on 4 December with debris removed (treatment 4) in the 2018/19 trial. Plot to the left had not been defoliated (treatment 1). Plot to the right has been defoliated on 4 December and the debris left (treatment 2). Photo taken on 4 December (immediately after defoliation).

Table 52. Experimental sites for defoliation experiments in 2017 and 2019.

Year	Location	Grid reference	OSR variety
2016/17	Boxworth, Cambridgeshire	TL343644	Campus
2018/19	Boxworth, Cambridgeshire	TL331646	Campus



## **Assessments**

CSFB larval numbers were assessed by collecting ten randomly selected plants (stems and petioles) per plot. These were then returned to the laboratory and all leaf petioles and stems were dissected with a sharp scalpel. The number of CSFB larvae in the stems and petioles were recorded separately. In 2017, these assessments were done in the untreated control plots just before each defoliation treatment, in treatment 2 on 9 December, in treatment 3 on 9 January, in treatment 4 on 9 March and a final assessment in all plots on 24 March (approximately two weeks after T4). The final assessment was timed to occur early enough to ensure CSFB larval numbers were assessed before they left the plants to pupate. Ideally plants for dissection were collected on the same day as the defoliation treatment. These samples allowed comparisons of larval numbers between the untreated control and the defoliated plots prior to each defoliation to help with interpretation of larval counts at the final assessment.

In 2019, larval numbers were assessed by collecting ten randomly selected plants (stems and petioles) per plot and returned to the laboratory. The number of larvae in the petioles and stems was counted on each plant. Larval numbers were assessed immediately before each defoliation treatment, in treatments 2 and 4 on 5 December, in treatment 3 on 15 February, in treatment 5 on 24 January, and in treatment 1 on 19 February. A final larval assessment occurred in all treatments on 8 March. These samples allowed the change in larval number to be tracked through the winter and for larval numbers to be compared between the treatments before larvae left the plants to pupate.

In 2017, green area index (GAI) was assessed in every plot prior to each defoliation treatment and at the same time as the final larval assessment on 24 March. This was done by taking a photograph directly overhead, looking straight down onto the plants and using the BASF GAI calculator (<https://www.agricentre.basf.co.uk/en/Services/Online-Tools/OSR-GAI-Online/>). These assessments allowed the impact of defoliation on green leaf area to be determined. GAI was not assessed in 2019. Each plot was harvested with a combine harvester and seed samples taken samples for determination of moisture content. The yield in tonnes/ha adjusted to 9% moisture was calculated.

In the 2019 trial, additional assessments to investigate the impact of defoliation on phoma were included. This was because ADAS pathologists suggested that defoliation may also affect phoma disease severity. Smaller plants are usually considered to be more at risk from phoma leaf spot due to their smaller size and the shorter distance between any remaining leaf material and the stem. On 4 December the incidence (number of plants affected) and severity (% leaf area affected) of phoma leaf spot was recorded on 25 randomly selected plants from each plot. On 18 July (at BBCH 80-85, start of pod fill) plants were assessed for the presence of phoma stem canker. This

involved sampling 25 plants from each plot and scoring stem canker and phoma stem lesions separately using a 0-4 index where 0 = no infection, 1 = <50% stem girdled, 2 = >50% girdled, 3 = >50% girdled, stem base weakened, 4 = plant prematurely ripened or dead.

### **Weather data**

Temperature data were sourced from MetMake within the IRRIGUIDE tool (Silgram *et al.*, 2007), interpolated by the MetMake tool for each site (using the grid reference) from reported weather data recorded at Met Office weather stations. Altitudes for each site were required as input data for MetMake. Altitude data were sourced from FreeMapTools (<https://www.freemaptools.com/elevation-finder.htm>). The grid references for each site were converted to 6 figures.

### **Statistical analysis**

All data in both 2016/17 and 2018/19 were subjected to analysis of variance. Significant differences between treatments were identified using Duncan's multiple range test indices. The numbers of CSFB larvae present in plots before defoliation were compared with numbers present in the same plots two weeks after the final defoliation treatment in March. This provided data on how numbers changed over the duration of the experiment. CSFB larval numbers were also compared across all defoliation treatments at the final assessment date in March. In the 2016/17 trial, CSFB larval numbers immediately prior to defoliation were compared between the undefoliated plots and those plots due for defoliation. Data on GAI, phoma, stem canker and crop yield were also analysed.

#### **8.2.2. Investigating whether CSFB larvae can re-invade OSR plants from defoliated OSR debris**

If OSR plants are defoliated to control CSFB larvae and the plant debris not removed there is potential for larvae to leave the debris and re-invade the defoliated plants. This would clearly defeat the object of defoliating the plants for CSFB larval control. As a result, an experiment was done in 2017/18 to test whether larvae are able to re-invade the plants. It was subsequently suggested that the ability of larvae to re-invade the plants is dependent upon soil moisture with re-invasion being more likely if the soil is moist (B. Ulber, pers. comm.). Therefore, a second experiment was done in 2018/19 to test whether soil moisture has any effect on larval re-invasion. In both years these were pot experiments and were done in an unheated polythene tunnel at ADAS Boxworth.

In October 2017, a total of 30, five litre pots (5 litre) were filled with soil (sterilised Kettering loam and lime free grit 3-6mm in a 4:1 ratio plus 2kg/m<sup>3</sup> Osmacote mini) to a depth of approximately 2

cm below the rim. The pots were filled at least 2-3 days before sowing to ensure they were well watered ahead of sowing. Seven OSR non-insecticide treated seeds (cv Catana) were sown 2 cm deep in each pot in mid-October. Pots were watered as necessary. It was originally intended to take debris from plots in the field at each defoliation timing and add the debris to specific pots. However, as the field trial was postponed in 2017/18 due to inclement weather, 110 randomly selected WOSR plants that had been naturally infested with CSFB were instead collected from the field on 30 January. Larval numbers were immediately assessed in the stems and petioles of 20 of these using plant dissection. This allowed the larval populations in the field to be determined. The remaining plants were cut in half horizontally to mimic defoliation and the pieces from three plants were placed around the base of each pot (Figure 155). On 9 March, the OSR plants within all the pots were removed and dissected. The number of scars on the petioles and stems and numbers of larvae within petioles and stems were recorded after plant dissection. This allowed larvae per plant in the pot-grown OSR to be compared with larvae per plant from the field-sourced plants.



*Figure 154. Left: Pot-grown OSR in early January 2018 prior to plant pieces added from field-sourced, CSFB-infested OSR. Right: Pot-grown OSR in early March several weeks after addition of pieces of field-sourced, CSFB-infested OSR.*

In October 2018, a total of 16, five litre pots were filled with soil (sterilised Kettering loam and lime free grit 3-6mm in a 4:1 ratio plus 2kg/m<sup>3</sup> Osmacote mini) to a depth of approximately 2 cm below the rim. The pots were filled at least 2-3 days before sowing to ensure they were well watered ahead of sowing. Ten OSR non-insecticide treated seeds (cv Campus) were sown 2 cm deep in each pot in early October. The pots were watered as necessary until they had emerged. Once they had established, plants in each pot were thinned to leave four in total. A total of eight pots was allocated to each of two treatments (Table 53) to create a fully randomised 2 x 2 factorial plus

control design. Pots were clearly labelled. Different coloured labels were used to identify the two watering regimes.

*Table 53. Treatment list for re-invasion experiment using two watering regimes in 2018/19. Actual dates in brackets.*

<b>Treatment no.</b>	<b>Pot treatment</b>	<b>Watering regime</b>
1	Inoculated with rape from December defoliation [4/12/18]	Regular watering
2	Inoculated with rape from December defoliation [4/12/18]	Minimal watering

On 4 December, approximately 100 plants were collected from discard plots in the defoliation field trial. All but 20 plants were cut in half horizontally to mimic mowing and the pieces were placed around the base of each pot. The number of pieces placed per plot depended on the number of pot-grown OSR in each pot, with two pieces (equivalent to one plant) placed per pot-grown OSR. This was done to avoid adjusting the larval counts at the final assessment, which had to be done for the 2017/18 trial (see *Statistical analysis* section below). For the remaining 20 plants, larval numbers were immediately assessed in the stems and petioles using plant dissection. This allowed the larval populations in the infested, field-sourced material to be determined.

Pots were watered regularly or infrequently to create the two watering regimes. The watering regimes only began once plant pieces were added to the pots. Before this, pots were watered as necessary to ensure that the OSR seedlings survived. The two watering regimes were defined as below:

- Regular watering: The pots were checked each weekday to determine whether watering was needed. The aim was to ensure that the pots were always moist at the soil surface.
- Minimal watering: The OSR seedlings were only watered sufficiently to keep them alive. Provisionally it was decided that this should be fortnightly but pots were checked regularly to see if more frequent watering was needed.

On 19 February, all pot-grown OSR were dissected with a sharp scalpel and the number of CSFB larvae within the following size categories; small (<3 mm), medium (3-5 mm) and large (>5 mm) were counted per plant. Measuring larvae meant that it was possible to determine if their size had any impact on their ability to invade the pot grown plants.

### ***Statistical analysis***

In 2017/18, the numbers of petiole and stem scars and the number of larvae in each pot of OSR were adjusted so that they could be compared with the equivalent numbers in the field-sourced plants. This was done because the number of plants differed between pots whereas the number of pieces of field-sourced plants used to inoculate the pots was constant at six pieces (i.e. three plants) per pot. For pot grown plants the data was adjusted by totaling the number of scars and larvae in

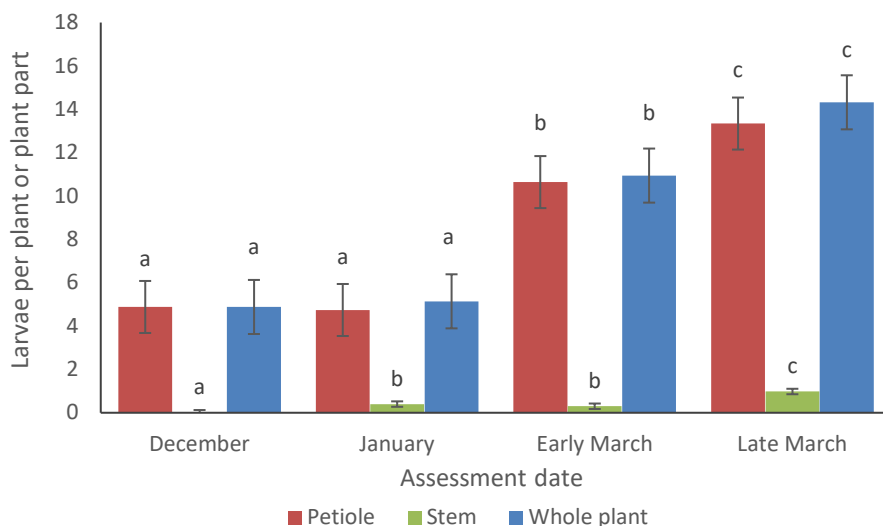
each pot and dividing these values by the number of plants in the pot, giving a mean number of scars or larvae per plant for each pot. This value was then divided by three to make the data comparable with that for the field-sourced plants. In 2018/19, this adjustment was unnecessary as the pots were infested with field-sourced plants in proportion to the number of plants in the pot. The differences in number of larvae per plant between the field and pot OSR were compared using Fisher's *t*-test. Bar charts are presented as summaries using the standard error of the difference between means as an indication of data variability.

### 8.3. Results

#### 8.3.1. Defoliation as a potential control option for CSFB larvae

##### **2016/17 field trial**

Numbers of CSFB larvae in the petioles, stems and total larval numbers are shown in Table 54. Many more larvae were recovered from the leaf petioles than from the stems at all assessment dates (e.g. 92% of all larvae recorded at the final assessment in late March were found in the petioles compared to 8% in the stems). Larval numbers in all areas of plants in the untreated control increased significantly throughout the assessment period ( $P < 0.001$ , Table 55). On 9 December 2016, the untreated control had 4.9 larvae per plant and on 24 March 2017 numbers had increased to 14.3 larvae per plant, an increase of 193% (Figure 155). Numbers in the stem were much lower and increased from zero to 1.0 per plant in the untreated control over the same time period.



**Figure 155.** Mean larvae per plant or plant part (stems and petioles) in untreated control plots showing increase larval number throughout winter and early spring. Letters indicate significant differences between assessment dates for each plant part separately. Bars followed by the same letter are not significantly different ( $P=0.05$ ). Bars indicate the SED.

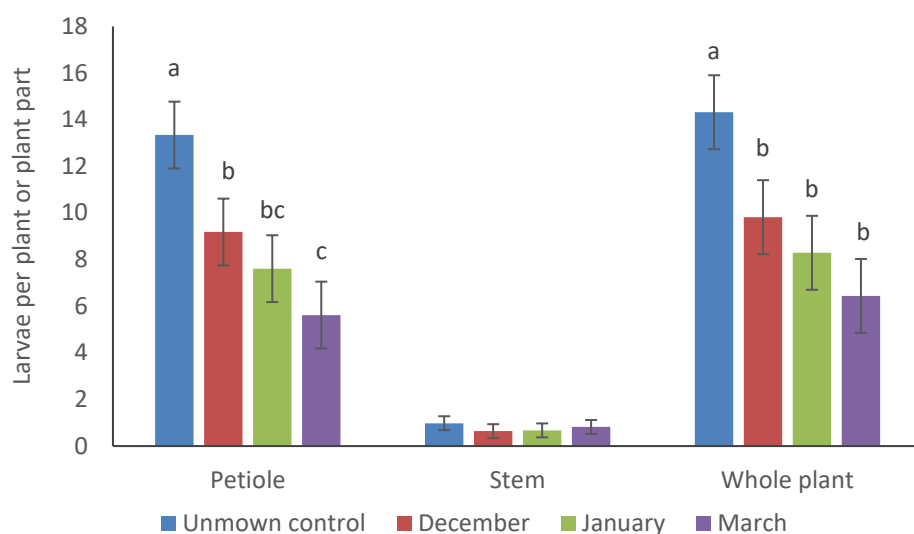
Table 54. Number of CSFB larvae in leaf petioles, stems and total larvae (mean per plant) in untreated control plots throughout treatment period (December 2016-March 2017) and numbers in plots immediately prior to defoliation in December, January and March (\* no assessment done).

Treatment	Timing of assessment											
	December defoliation			January defoliation			March defoliation			March defoliation + two weeks		
	No. in petiole	No. in stem	Total	No. in petiole	No. in stem	Total	No. in petiole	No. in stem	Total	No. in petiole	No. in stem	Total
1.Untreated control	4.9	0	4.9	4.7	0.4	5.1	10.6	0.3	10.9	13.3	1.0	14.3
2.December defoliation	4.6	0	4.6	*	*	*	*	*	*	9.2	0.6	9.8
3. January defoliation	*	*	*	5.4	0.4	5.8	*	*	*	7.6	0.7	8.3
4. March defoliation	*	*	*	*	*	*	10.8	0.2	11.0	5.6	0.8	6.4

Table 55. Comparison of mean numbers of CSFB larvae in leaf petioles, stems and total larvae (mean/plant) in untreated control plots throughout treatment period (December 2016-March 2017) and mean numbers in plots immediately prior to defoliation on 9 December (T1), 9 January (T2) and 9 March (T3) compared with numbers in the untreated control on 24 March 2017 (T4) (\* no assessment done). P values indicate significant differences in larval numbers over time within individual defoliation treatments.

Treatment	Timing of assessment											
	Number in petiole				Number in stems				Number in whole plant			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
1. Untreated control	4.9	4.7	10.6	13.3	0	0.4	0.3	1.0	4.9	5.1	10.9	14.3
Probability	P<0.001				P<0.001				P<0.001			
SED (147 df)	1.20				0.13				1.25			
2. December defoliation	4.6	*	*	9.2	0	*	*	0.6	4.6	*	*	9.8
Probability	P<0.001				P<0.001				P<0.001			
SED (49 df)	0.77				0.13				0.80			
3. January defoliation	*	5.4	*	7.6	*	0.4	*	0.7	*	5.8	*	8.3
Probability	P<0.05				P=0.104				P=0.01			
SED (49 df)	0.87				0.16				0.94			
4. March defoliation	*	*	10.8	5.6	*	*	0.2	0.8	*	*	11.0	6.4
Probability	P<0.001				P<0.001				P<0.001			
SED (49 df)	1.16				0.20				1.21			

There were no significant differences between larval numbers prior to each defoliation in the untreated control plots and the plots due to be defoliated ( $P \geq 0.05$ ). Consequently, any differences recorded at the final larval assessment were due to the impact of the defoliation treatments. At the final assessment date larval numbers were significantly lower in the defoliation treatments than the untreated control plots in the petioles ( $F = 10.5$ ,  $df = 12$ ,  $P = 0.001$ ) and the whole plant (petiole and stem numbers combined;  $F = 9.0$ ,  $df = 12$ ,  $P = 0.002$ ) but not in the stems (Figure 156). The later the defoliation the greater was the impact on larval numbers. Defoliating in December reduced larval numbers by 31% in comparison with the untreated control. January defoliation decreased larval numbers by 42% and March defoliation by 55%.



**Figure 156.** Mean numbers of CSFB larvae in OSR petioles, stems and whole plants on 24 March 2017 (two weeks after the March defoliation treatment). Letters indicate significant differences between treatments for each plant part separately. Bars followed by the same letter or by no letter are not significantly different ( $P=0.05$ ). Bars indicate the SED.

Comparison of larval numbers before defoliation and afterwards at the final assessment show that larval numbers were significantly higher in the petioles ( $F = 34.6$ ,  $df = 1$ ,  $P < 0.001$ ), stems ( $F = 25.4$ ,  $df = 49$ ,  $P < 0.001$ ) and whole plants ( $F = 42.4$ ,  $df = 49$ ,  $P < 0.001$ ) in the December treatment, significantly higher in the petioles ( $F = 6.7$ ,  $df = 48$ ,  $P = 0.013$ ) and whole plants ( $F = 7.2$ ,  $df = 48$ ,  $P = 0.01$ ) in the January treatment, and significantly lower in the petioles ( $F = 20.3$ ,  $df = 49$ ,  $P < 0.001$ ), stems ( $F = 9.4$ ,  $df = 49$ ,  $P = 0.003$ ) and whole plants ( $F = 14.4$ ,  $df = 49$ ,  $P < 0.001$ ) in the March treatment (Table 55, Figure 157). Overall the mean larvae per plant increased by 112% and 42% between the defoliations and the final assessment in the December and January defoliation treatments respectively, and decreased by 48% in the March defoliation treatment. These changes are in contrast to the untreated control, where larval numbers increased 193% over the same period.



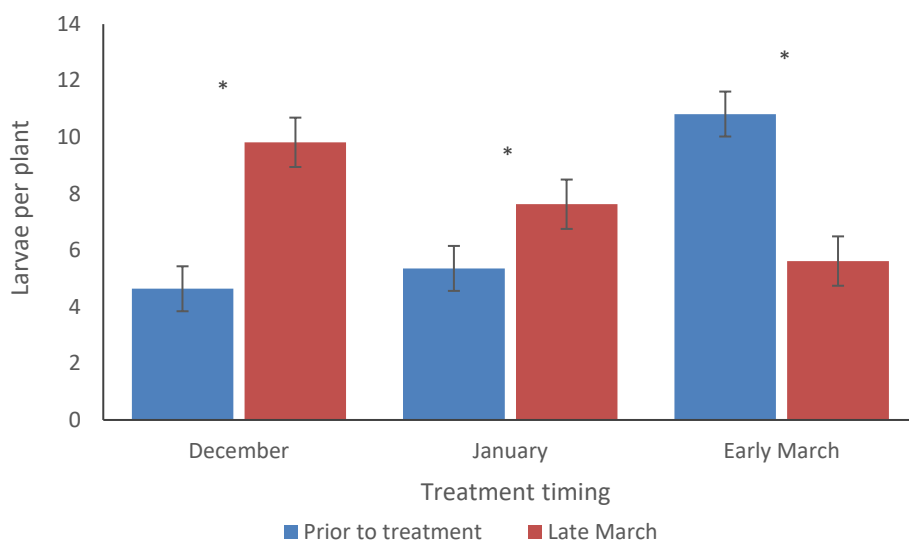


Figure 157. Mean numbers of CSFB larvae in OSR plants immediately before each defoliation treatment and at the final assessment in late March. \* indicates significant differences between assessment dates. Bars indicate the SED.

There was no significant difference in GAI between the treatments immediately prior to each treatment and at the final assessment on 24 March (Table 56). There was no significant difference in yield at harvest between treatments (Figure 158), although there was a trend for both December and January defoliation to increase yield. December defoliation increased yield by 14% and January defoliation by 7% in comparison with the untreated control. In comparison defoliation in March decreased yield by 12%.

Table 56. Mean green area index (GAI) in each treatment prior to each defoliation timing (T1 = December defoliation, T2 = January defoliation and T3 = March defoliation) and at the final assessment in late March. SED = standard errors of differences of means.

Treatment	Mean GAI			
	9 December	10 January	10 March	24 March
1. Untreated control	0.31	0.87	0.33	0.59
2. December defoliation	0.30	0.84	0.35	0.58
3. January defoliation	0.34	0.9	0.33	0.60
4. March defoliation	0.39	0.84	0.4	0.41
SED (12 df)	0.03	0.1	0.04	0.07

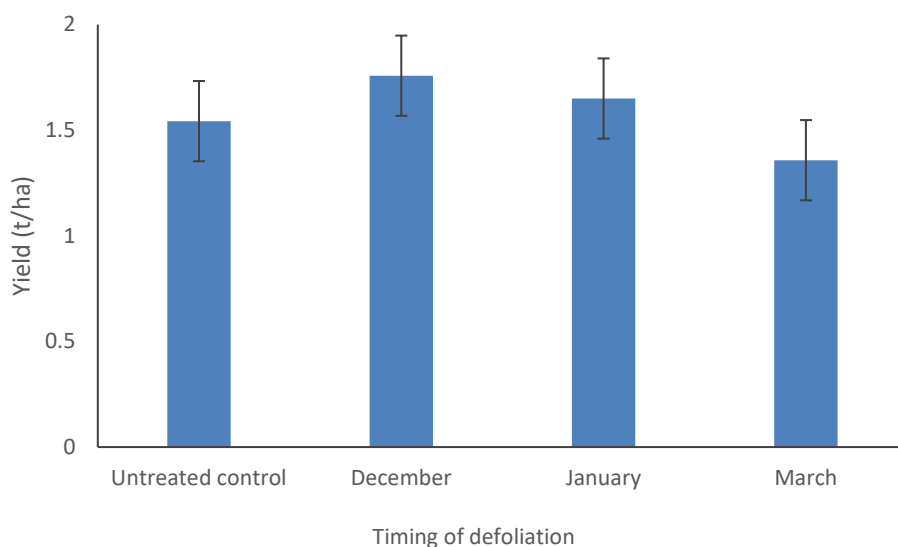


Figure 158. Mean yield of OSR (t/ha @91% DM) in July 2017 following defoliation to control CSFB larvae at different timings. Bars indicate the SED.

### 2018/19 field trial

As in 2017, larval numbers in the petioles were much higher than in the stems. For example, 87% of all larvae recorded at the final assessment on 8 March were found in the petioles compared to 13% in the stems. Larval numbers in the petioles and whole plants increased from December to February but decreased in March, while numbers in the stems increased throughout the assessment period (Figure 159). Overall, this trial found larvae per plant to increase 163% between December and January and 203% between December and February. Note these values are not taken from the same plots, rather plots prior to each treatment timing, and so cannot be statistically analysed.

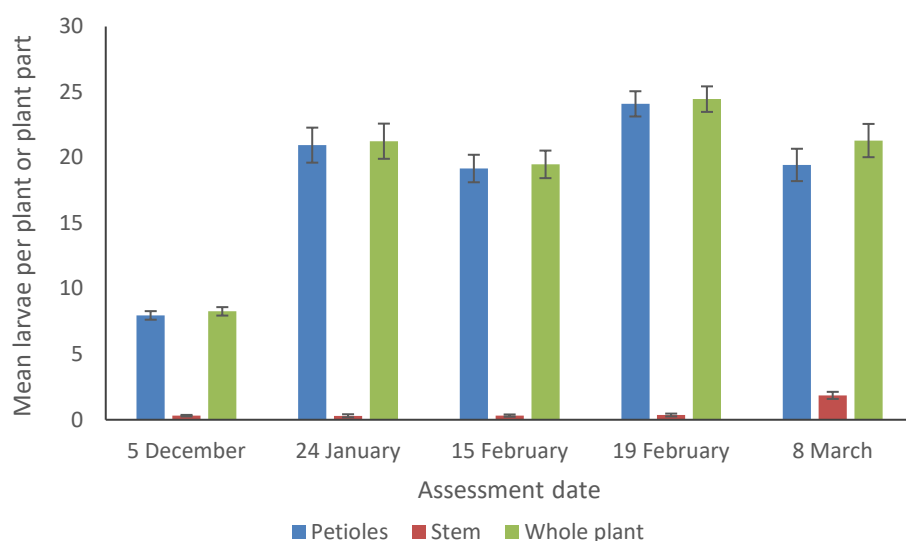


Figure 159. Mean larval numbers per plant or plant part at each assessment date. Plants assessed from treatments 2 and 4 in December, treatment 5 in January, treatment 3 on 15 February and treatment 1 on 19 February and 8 March. Bars indicate the SED.

At the final assessment on 8 March, numbers of CSFB larvae in the leaf petioles and the whole plants differed significantly between treatments (petioles:  $F = 7.0$ ,  $df = 16$ ,  $P = 0.002$ ; whole plant:  $F = 6.6$ ,  $df = 16$ ,  $P = 0.003$ ) but numbers of larvae in the stems were not significantly different (Table 57). Larval numbers in the petioles were significantly lower than the untreated control in the December defoliation followed by debris removal and February defoliation treatments (Figure 160). Larvae per plant was significantly lower than the untreated control in the February defoliation treatment only (Figure 160). February defoliation was most effective and reduced larval numbers by 55%. Defoliation in December reduced numbers by 23% when the plant debris was left in situ and 26% when the plant debris was removed. Therefore, removal of the plant debris did not significantly improve larval control. All defoliation treatments had significantly lower numbers of larvae than plots that were rolled ( $P < 0.05$ ). Rolling appeared to have little impact on larval numbers. Although larval numbers in the stems were much lower than in the petioles and did not differ significantly between treatments, there was a trend to find lowest numbers where plots were defoliated in February.

Table 57. Mean number of CSFB larvae in all treatments on 8 March 2019 (T1 = untreated control, T2 = defoliation in December, T3 = defoliation in February, T4 = defoliation in December and remove debris, T5 = rolling).

Larval numbers	T1	T2	T3	T4	T5
Petioles	19.4	13.9	8.5	12.9	20.8
Stems	1.9	2.5	1.2	2.8	3.3
Total	21.3	16.4	9.7	15.7	24.1

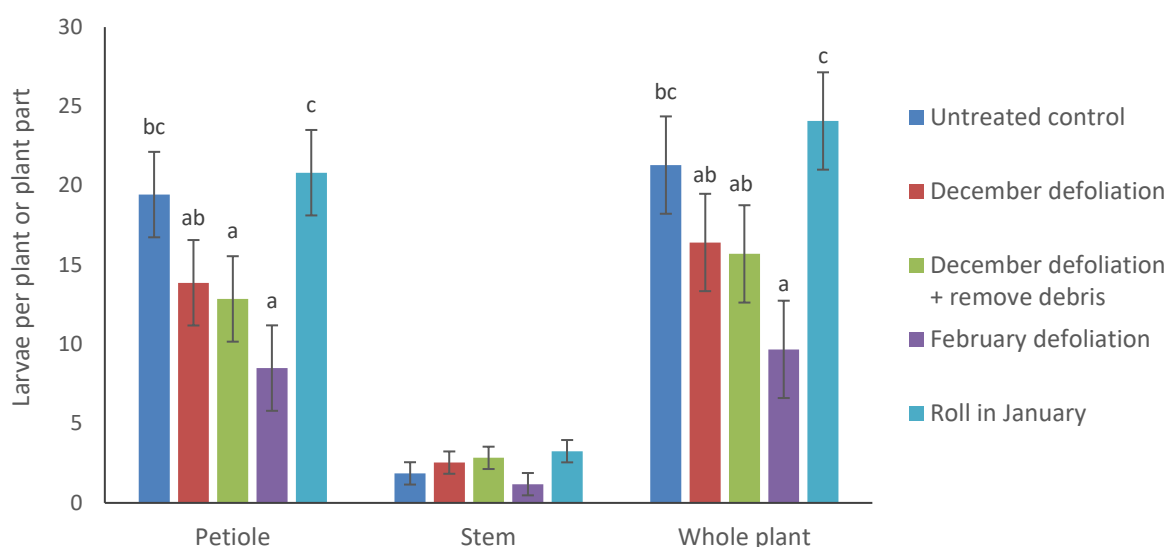
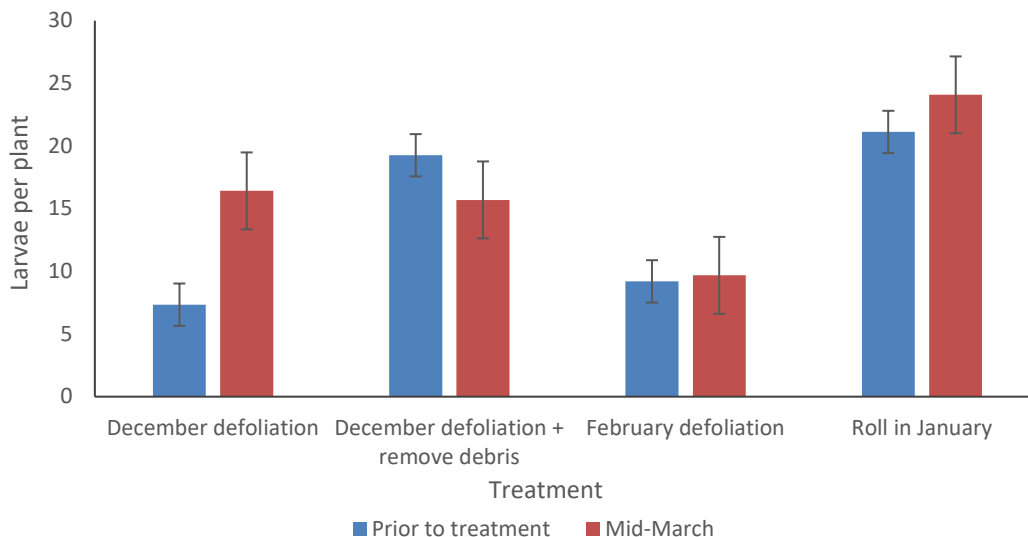


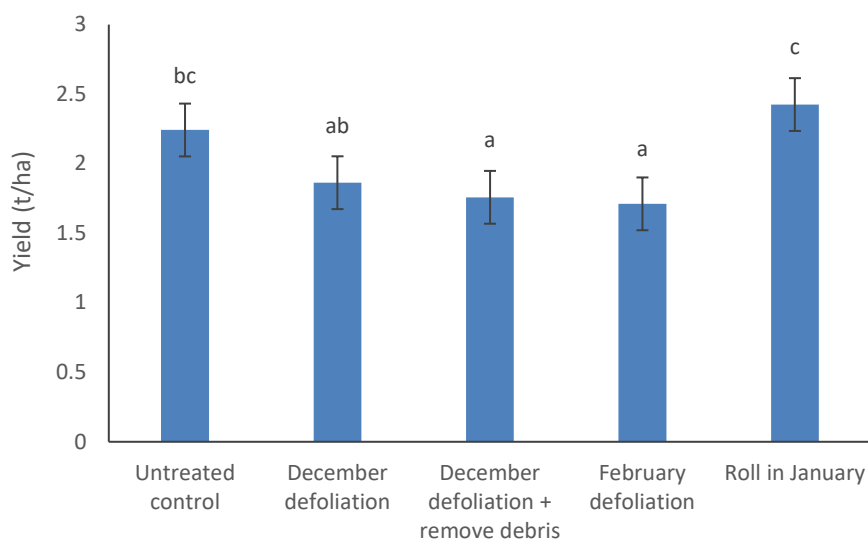
Figure 160. Mean numbers of CSFB larvae in OSR petioles, stems and whole plants in each treatment at the final assessment (8 March 2019). Letters indicate significant differences between treatments for each plant part separately. Bars followed by the same letter or by no letter are not significantly different ( $P = 0.05$ ). Bars indicate the SED.

Comparison of larval numbers before defoliation and afterwards at the final assessment show that the mean larvae per plant increased 124%, 14% and 5% between the treatments and the final assessment in the December defoliation, roll in January and February defoliation respectively, and decreased 18% in the December defoliation followed by debris removal treatment (Figure 161).



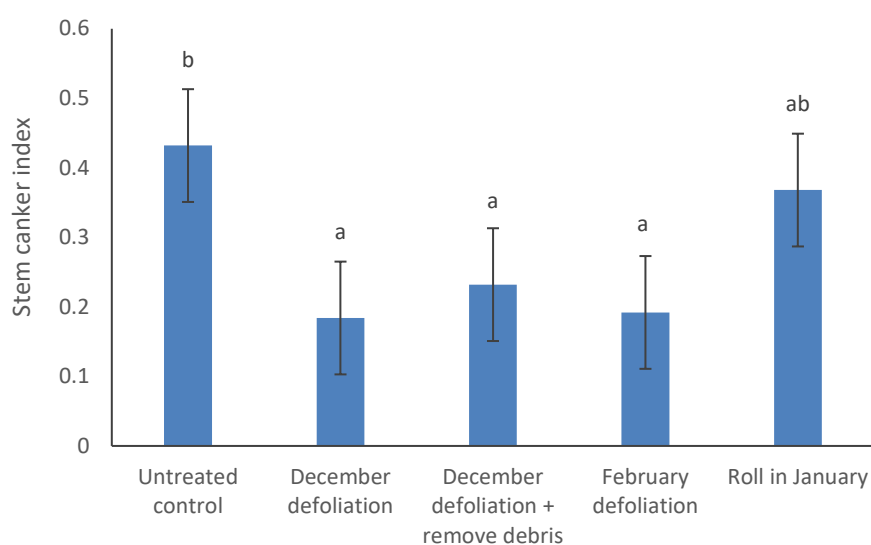
*Figure 161. Mean numbers of CSFB larvae in OSR plants immediately before each treatment and at the final assessment in mid-March. Bars indicate the SED.*

Crop yield differed significantly between defoliation treatments ( $F = 5.5$ ,  $df = 16$ ,  $P = 0.005$ ; Figure 162). In contrast to the 2017 data, defoliation reduced yield in comparison with the untreated control. Plots defoliated in December and had the debris removed and those defoliated in February had significantly lower yield than those that were not defoliated or were rolled ( $P < 0.05$ ). Defoliating in December reduced yield in comparison with the untreated control by 17%, defoliating December and removing the debris reduced yield by 22% and defoliating in February reduced yield by 24%. Plots defoliated in December also had a lower yield than those that were rolled ( $P < 0.05$ ). The highest yields were found in the rolled plots.



**Figure 162.** Mean yield of OSR (t/ha @91% DM) in July 2019 following defoliation and rolling treatments to control CSFB larvae. Letters indicate significant differences between treatments. Bars indicate the SED.

There was no significant difference in phoma incidence between treatments at BBCH 19 on 4 December 2018 before imposing any defoliation/rolling treatments, with an average incidence of 45% plants affected and an average severity of 0.8% leaf area affected. However, canker index differed significantly between treatments on 8 July (BBCH 80-85,  $F = 3.8$ ,  $df = 16$ ,  $P = 0.023$ ). All defoliation treatments significantly decreased the canker index in comparison with the untreated control by 57%, 46% and 56% for December mowing, December mowing and removal of debris and February mowing respectively (Figure 163). Data on phoma incidence at BBCH 19 and phoma canker index at BBCH 80-85 are presented in Table 58.



**Figure 163.** Mean stem canker index in July following defoliation and rolling treatments to control CSFB larvae. Letters indicate significant differences between treatments. Bars indicate the SED.

Table 58. Mean leaf phoma incidence (% plants infested) at BBCH 19 and phoma canker index at BBCH 80-85 before and after imposing defoliation and rolling treatments at ADAS Boxworth in 2018/19. (a and b are Duncan's multiple range test indices, values followed by the same letter are not significantly different  $P>0.05$ ).

Phoma assessment	Defoliation/rolling treatment					Probability P	SED (16 DF)
	UTC	Dec mow	Dec mow + remove debris	Feb mow	Roll in Jan		
Incidence on leaves	44.8	48.0	40.8	46.4	46.4	NS, 0.548	4.37
Canker index	0.43 b	0.18 a	0.19 a	0.23 a	0.37 ab	<0.05	0.081

### 8.3.2. Investigating whether CSFB larvae can re-invade OSR plants from mown OSR debris

#### 2017/18 Pot trial

Mean numbers of larvae were significantly lower in the petioles and whole plants in the pot-grown OSR on 9 March than the OSR taken from the field and added to the pots on 30 January ( $P<0.001$  in each case, except for scarring on the stems and numbers of larvae in the stems, Table 59).

Larval numbers in the stems were so low that any differences between the field and pot-grown plants would not be apparent. This suggests that just 3.5% of larvae were able to move from plant debris to invade nearby plants.

Table 59. Total numbers of scars on the petioles and stems and number of larvae in the petioles and stems per plant in pot grown and field grown oilseed rape plants in 2017/18. (F = field, P = pot, t = t statistic, P = probability, SED = standard error of difference, DF = degrees of freedom). N/A = no larvae were found in the stems of field or pot grown plants.

	CSFB scarring						CSFB larvae					
	Petioles		Stems		Total		Petioles		Stems		Total	
	F	P	F	P	F	P	F	P	F	P	F	P
		32.1	0.8	0.2	0.1	32.3	0.9	8.5	0.2	0	0	8.5
t	8.79		0.89		8.82		6.14		N/A		6.11	
DF	19.0		23.8		19.1		19.0		N/A		19.0	
P	<0.001		0.383		<0.001		<0.001		N/A		<0.001	
SED	3.6		0.1		3.6		1.4		N/A		1.4	

### **2018/19 Pot trial**

Mean numbers of larvae per plant were significantly lower in the pot-grown OSR on 19 February than the OSR taken from the field and added to the pots on 4 December ( $F = 7.0$ ,  $df = 33$ ,  $P = 0.003$ ), with 7.7, 3.2 and 3.1 larvae per plant in the field-grown OSR, regularly watered pot-grown OSR and minimally watered pot-grown OSR respectively. Watering regime had no significant effect on the number of larvae able to reinvade nearby OSR. In this trial, 42% and 40% of larvae in the regular watering and minimal watering regime respectively were able to move from plant debris to invade nearby plants. The majority of larvae in both watering treatments were small (less than 3 mm long) (Table 60). A higher percentage of larvae were large (more than 5 mm long) in the minimal watering treatment (21%) than in the regular watering treatment (9%) (Table 60).

*Table 60. Percentage of larvae in each size category on 19 February in the 2018/19 pot trial. Small = <3 mm, medium = 3-5 mm, large = >5 mm.*

<b>Watering regime</b>	<b>Small</b>	<b>Medium</b>	<b>Large</b>
Regular watering	50	41	9
Minimal watering	42	37	21

## **8.4. Discussion**

Defoliating OSR overwinter effectively reduced larval populations in both 2017 and 2019. Reduction in the numbers of CSFB larvae ranged from 23% to 55% over the two years, with greater reductions seen in later defoliation timings. In addition to the field experiments in this project an Innovative Farmer Field Lab (funded by Innovative Farmers, AHDB and Syngenta) was also organised in 2018/19. This involved eight farmers defoliating 12 fields to investigate the impact on CSFB larvae. A topper was used to defoliate the crop or it was grazed off using sheep. Results showed that defoliation was able to significantly ( $P < 0.05$ ) reduce larval populations in all but two of the 12 fields. Across the sites, defoliation reduced larval populations by 39% on average, with grazing slightly more effective (51% reduction compared to the undefoliated areas) than topping (25% reduction compared to the undefoliated areas) (White & Kendall, in press). That growers were able to produce similar larval reductions on a field-scale to those seen in the plot trials described here is encouraging.

A review of previous trial data found that, in the absence of pyrethroid resistance, a well-timed pyrethroid spray targeting larvae could provide 79% control, but in the presence of pyrethroid resistance control drops to 9% (see Section 3.4.2 for further details). Pyrethroid resistance is now widespread in the UK (S. Foster, pers. comm.) so, whilst the larval control achieved by defoliation is lower than might be expected from a well-timed insecticide treatment in the few areas in which

pyrethroid resistance is absent, in most other areas defoliation would still be a useful component of an integrated strategy which is reliant on a range of strategies to control the pest.

Removal of crop debris following December defoliation in the 2018/19 field trial had little impact on level of control of CSFB larvae, with the number of larvae per plant where the debris was left in situ similar to that where the debris was removed (16.4 and 15.7 per plant at the final assessment respectively). However, the treatment in which debris was removed was the only one to produce reductions in larvae per plant between the assessment immediately prior to the treatment and the final assessment in the 2018/19 trial. In all other treatments in either field trial (except for the March defoliation) larval numbers increased between the assessment immediately prior to the treatment and the final assessment. This suggests that some larvae are able to recolonise the defoliated plants. This was backed up by results from the pot experiments, which showed that 3.5% and 40-42% of those larvae in defoliated material were able to reinvade the OSR in the 2017/18 and 2018/19 pot trials respectively. It is difficult to explain the discrepancy between the pot trials in 2017/18 and 2018/19. It is possible that differences in larval size and plant growth stage affected the ability of larvae to reinvade. Larvae were introduced to the pots later in 2017/18 than in 2018/19 and so plants would have been at a later growth stage. More mature plants may be more difficult to invade. While larval size was not measured in 2017/18, it is likely that these larvae were smaller at the time of reinvasion than those in 2018/19 as temperatures were cooler in November and December in 2017 than 2018 (Met Office, 2020), which would have slowed larval development, and this may have affected their ability to reinvade.

Whilst these results demonstrate that recolonisation from debris can occur, it is clear that considerable late egg hatch leading to larval invasion also occurred in the field trials. In both field trials, larval numbers in undefoliated plots were observed to increase throughout the winter (193% increase between December and February 2016/17, and 203% increase between December and March 2018/19). Indeed, the increase in larval number due to late egg hatch in undefoliated plots appears to account for much of the change in larval numbers in the defoliated plots. For example, larval numbers increased 112% and 124% between the December assessment immediately prior to defoliation and the final assessment in March in 2016/17 and 2018/19 respectively. This suggests that the majority of the increase in larval number between the defoliation date and March was due to late egg hatch rather than reinvasion from defoliated debris. The reduction of larvae between the defoliation in early March and the final assessment in late March in the 2016/17 trial provides further evidence for larval increases post-defoliation being largely due to late egg-hatch rather than reinvasion from debris, as the lack of increase in larval numbers is likely to be due to the short period in which late egg hatch could occur. Removing plant debris would potentially complicate the use of defoliation to control larvae so it is reassuring that this appears not to be necessary.



It was proposed that rolling the crop after a hard frost and before stem extension might help to reduce CSFB larval numbers. This was shown not to be the case, however this may be because only light frosts occurred during the trial meaning the desired splitting of petioles did not occur. Also, simulating rolling experimentally is difficult as there is the potential to damage large areas of crop while manipulating a heavy roller. In this experiment a water container was used to simulate rolling and it is possible that this was not sufficiently severe. It would be interesting to investigate the potential of rolling again in the future.

It has been suggested that defoliating the plants and/or leaving crop debris in situ could increase the risk of fungal pathogens infecting the surviving crop but this was shown not to be the case for phoma. Although the level of phoma incidence and severity were relatively low before defoliation in 2018/19, the 45% observed incidence would have been sufficient to trigger a fungicide treatment as the threshold is 10% plants infected (AHDB, 2015). However, the ultimate level of stem canker was significantly lower where plots were defoliated in comparison with the untreated control ( $P < 0.05$ ). The level of stem canker was reduced by 57%, 46% and 56% for December mowing, December mowing and removal of debris, and February mowing respectively. This is approximately equivalent to the effect of a good fungicide spray (P. Walker, pers. comm.). It was likely that mowing occurred during a period when phoma infection was already present on leaves, therefore mowing removed the opportunity for the disease to spread from the leaves to the main stem. ADAS plant pathologists report that in the 2018/19 season, phoma and stem cankers were at low levels but the degree of control provided by defoliation would likely have given up to a 0.1 t/ha yield response. This suggests that defoliation can not only reduce numbers of CSFB larvae but also reduce phoma stem canker risk if it coincides with leaf infection.

The difference in larval pressures between the trials and the change in larval numbers throughout the winter provide some insight into the life cycle of CSFB. The peak larval population was 75% higher in 2018/19 than 2016/17 (24 and 14 larvae per plant respectively). As mentioned above, larval numbers increased throughout the winter in both field trials, except for in March 2019 when a small decrease in larval numbers was observed. This is in line with trends in recent years but counter to the long-term trend, where overwinter increases in larval number were generally very small (see Section 3.4.4). Increases in larval pressure have been linked to mild autumn and winter temperatures (Collins, 2017), and modelling work in this project appears to confirm this (see Section 4.4). In the 2016/17 trial, both February and March 2017 temperatures were markedly different to the 1961-1990 average, being 2-3°C warmer (with an average temperature of 5.7°C and 7.9°C respectively) (Met Office, 2020). These temperature fluctuations are in line with the observed changes in larval populations, with significant increases seen between January and March but not between December and January.

In the 2018/19 field trial, conditions were unusually mild, with every month in autumn, winter and early spring warmer than the 1961-1990 average, particularly November (1-2°C warmer with average temperature 8.5°C), December (1-2°C warmer with average temperature 7.1°C), February (2-3°C warmer with average temperature 5.6°C) and March 2019 (2-3°C warmer with average temperature 8.0°C) (Met Office, 2020). The warmer conditions in 2018/19 may explain the increased larval populations observed in this trial compared to 2016/17. The warm early winter conditions in 2018/19 would also have encouraged the large increases in larval numbers recorded between December and January in the 2018/19 trial, while the warm conditions throughout (and especially in February and March) 2018/19 may have encouraged early maturity of larvae and the reduction in larval numbers seen between February and March, presumably due to larvae leaving the plant to pupate. Late hatching larvae would have been smaller than those that hatched earlier in the season and so are likely to have less of an impact on yield. Controlled environment experiments (Alford, 1979; Mathiasen *et al.*, 2015a) indicate that mild winter temperatures could shorten pre-oviposition and egg development times, increase total egg production, and extend the period over which egg-laying and development can occur (i.e. further into the winter). However, the impact of fluctuating field temperatures on egg-laying and -development is not well understood. Nor is the relationship between larval size and yield impact. These relationships are potentially important, especially if mild winters become more common.

It was interesting that in both years of field experimentation, assessments of the location of larvae within WOSR plants showed that the majority were still present in the leaf petioles in March. In 2016/17 only 8% of larvae were in the stems in March and in 2018/19 the equivalent figure was 13%. Larvae are thought to begin moving from the petioles to the stem from March (Green, 2008) so it is likely that numbers in the stem would have increased, although other work has shown that even in April stem larval numbers can be relatively low (White, 2016). Larvae may move to the stem due to overcrowding in the petioles or to benefit from the increased flow of assimilates as the plant reaches the stem extension stage. It is possible that the location of larvae within the plant is affected by temperature so it would be interesting to see if the proportion in the stems in March changed significantly after a severe winter. In 2016/17, defoliation had no effect on stem larval numbers but in 2018/19 larval numbers were recorded to increase in the stems, though non-significantly, in the December defoliations and rolling treatment compared to the untreated control. It is generally thought that larval feeding in the stems has a greater impact on yield than that in the petioles (Williams & Garden, 1961), and this may explain some of the reductions in yield seen in the 2018/19 trial. A better understanding of the relative impacts on yield of larval feeding in the stems and petioles respectively is needed (see Section 6). If stem feeding does have a greater impact on yield than petiole feeding then it may be possible to manipulate larval behaviour to minimise stem feeding, e.g. through plant growth regulator (PGR) use.

Whilst defoliation consistently reduced numbers of CSFB larvae, the impact on yield varied between 2017 and 2019. In 2017, there was a trend for defoliation to increase yield, although the differences were not statistically significant. Yield was 14% higher than in the untreated control where plots were defoliated in December and 7% higher when plots were defoliated in January. Defoliating in March reduced yield by 12% compared to the untreated control despite this treatment timing having the greatest impact on larval numbers. This is probably because defoliation occurred after stem elongation, which Kirkegaard *et al.* (2012) showed could have a significant impact on crop development. In 2019, the impact of defoliation was to decrease crop yield. Reduction in yield compared with the untreated control ranged from 17% to 24%. Removing debris resulted in a further reduction in yield, possibly because the crop was not able to utilise the debris for nutrition. The 2018/19 Innovative Farmer Field Lab also saw a trend for yield reductions in defoliated areas, with an average yield reduction of 14% (White & Kendall, in press). However, others reported no yield loss in 2019 where they defoliated to control CSFB larvae (P. Trickett, pers. comm.).

Clearly, earlier defoliations have a smaller impact on yield, presumably because these crops have a longer time to produce compensatory growth. This finding is in line with feedback from growers and agronomists, who commented that defoliations prior to January (either grazing livestock for feed or picking leaves as a substitute for saag) tended not to affect yield at harvest whereas later defoliations tended to reduce yield (White & Kendall, in press). Previous work has shown that defoliating WOSR in the winter need not adversely affect yield (Spink, 1992; Dove & Kirkegaard, 2014; Sprague *et al.*, 2015; Ellis *et al.*, 2017) and that yield reductions were associated with later defoliations, e.g. March (Ellis *et al.*, 2017). It may also be the case that yield reduction following defoliation occurs where larval numbers remain high even after defoliation, as in the 2018/19 trial where defoliated crops still had 10-16 larvae per plant by the final assessment in March.

These results also suggest that the ability of the crop to compensate for defoliation will vary between seasons. This is likely to be affected by weather conditions. Spring 2019 was generally dry in the east (Figure 164), which is likely to have reduced nutrient uptake in OSR, limiting the ability of defoliated crops to produce additional branches to compensate for the defoliation. Cool conditions (including ground frosts) in early May in some areas of the country (Figure 164) may also have affected crop development, coinciding with, and limiting, pod set, especially in backward, defoliated crops. As it is difficult to make long term predictions of the spring weather this could have an impact on the sustainability of defoliation as a means of controlling CSFB larvae.

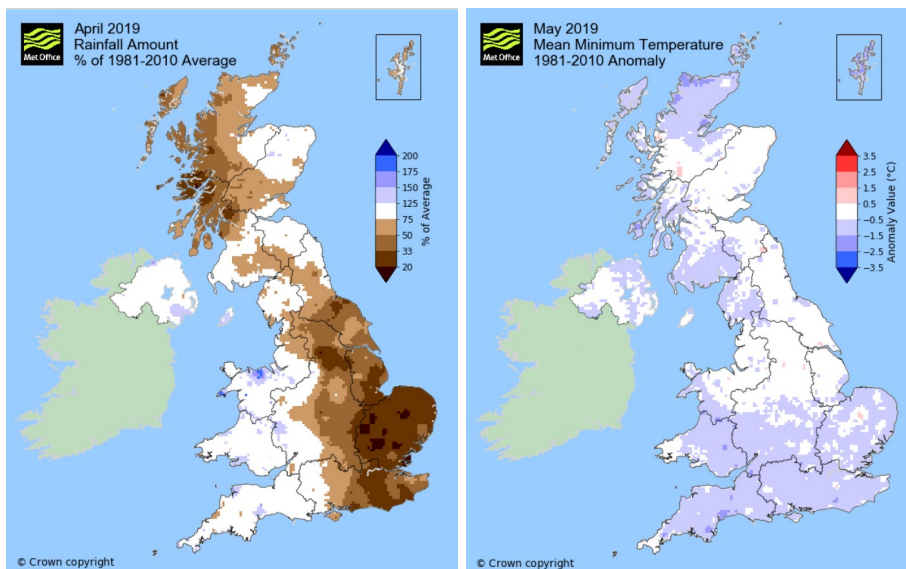


Figure 164. Rainfall 1981 - 2010 anomaly maps for April 2019 (left) and mean minimum temperature 1981-2010 anomaly map for May 2019 (right). Taken from <https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-actual-and-anomaly-maps>.

Further discussion with crop physiologists will be necessary to attempt to predict those years and situations when defoliation is likely to be beneficial to crop yield. Measurement of various growth parameters (e.g. rooting) may be helpful in predicting those crops which have the greatest compensatory ability. Several factors are likely to affect the efficacy of defoliation in terms of reducing larvae and yield loss. These include i) the trade-off between larval reduction and yield loss of defoliating at different times, ii) the effect of drill date in that earlier drilled crops are likely to both experience higher larval loads (see Section 4.4) and be better able to compensate for defoliation (Dove & Kirkegaard, 2014), iii) the nutrition and PGR requirements of defoliated crops, iv) whether some varieties are better suited to defoliation than others, and v) the importance of weather conditions for crop recovery. A further Innovative Farmer Field Lab is investigating defoliation in 2019/20, including defoliation at earlier timings, and will provide further information on the impact of defoliation on larval control and yield.

Other methods of defoliation have been suggested, e.g. contact herbicides, however this may not be effective because it is thought that larvae can detect when leaves are senescing as they are rarely found in fallen leaves (Williams & Garden, 1961). It has also been suggested that pigeon feeding may reduce larval numbers but this is also unlikely as pigeons tend to feed on the leaf blade rather than where the larvae are located in the petioles. The 2018/19 Innovative Farmer Field Lab identified some recommendations for defoliation (White & Kendall, in press), including that pigeon damage can be higher and hinder the recovery of defoliated crops so should be avoided by choosing to defoliate crops away from woodland or with careful use of deterrents.

Defoliation was shown to be effective at reducing CSFB larval numbers and also the potential risk from phoma if it coincides with leaf infection, but impacts on crop yield over the two years of study were contradictory. The 2018/19 Innovative Farmer Field Lab also observed some other impacts of defoliation, e.g. that the provision of feed for livestock presents a potential additional source of income, that sheep appear to eat charlock preferentially and that pigeons may need to be managed to minimise damage to defoliated crops (White & Kendall, in press). Grazing with sheep may also offer other benefits in terms of providing additional nitrogen and improving soil health (AHDB, 2020a). The impact of defoliation in terms of reducing CSFB pressures in following crops is also worth consideration. Reducing larval populations is likely to reduce the numbers of adult CSFB emerging from the field in the summer, in turn reducing CSFB pressures the following autumn and lowering CSFB populations in the long-term. Managing CSFB populations in the long-term and on a regional scale is clearly needed. While defoliation could contribute to this, a number of strategies, applied over a large area, would be needed to achieve this (e.g. encouraging natural enemy populations and activity). Further work is required to fine-tune this cultural control option before it can be reliably incorporated into an integrated control strategy for CSFB larvae.

## **8.5. Conclusions**

- Defoliation decreased larval numbers by between 23 and 55%. The greatest reductions were at the latest defoliation timings (March in 2016/17 and February in 2018/19). However, later defoliations tended to result in lower yields.
- An Innovative Farmer Field Lab provided additional evidence of the potential for defoliation to decrease CSFB larval numbers. Grazing by sheep reduced larval numbers on average by 51% and topping by 25%.
- Removing mown debris appeared to have little impact on the level of larval control by defoliation.
- The degree to which CSFB larvae are able to re-invade OSR plants from mown debris is unclear. Pot experiments provided contradictory results but field data suggest that any increase in larval numbers after defoliation is primarily due to invasion by late hatching larvae rather than reinvasion from mown crop debris.
- Rolling appeared not to reduce CSFB larval numbers but may be more effective after more severe winter conditions than experienced in the project.
- Defoliation decreased the severity of stem canker at levels equivalent to a fungicide spray.
- CSFB larval numbers increased over winter and this is probably related to the mild winter temperatures.
- Most CSFB larvae are still present in the leaf petioles in the spring, at least during this project, which increases their susceptibility to control using defoliation.

- The effect of defoliation on yield was inconclusive, with no significant increase in yield in the first trial and a significant decrease in the second trial. Yield was probably affected by weather conditions following defoliation, which influence the compensatory ability of the crop.
- Based on current data, defoliation is likely to of greatest benefit when used in early drilled crops with moderate to severe larval infestations. Further data from the 2019/20 Innovative Farmer Field Lab will improve our understanding of this approach.

## 9. Create an IPM strategy for CSFB

CSFB is currently the most important pest of WOSR in the UK, although the degree of damage varies markedly across the country. The loss of neonicotinoid seed treatments and the presence of resistance to pyrethroid sprays means that chemical control options are very limited. The development of an IPM strategy for this pest is urgently required particularly involving non-chemical control options which will help to rationalise chemical control and prolong the use of existing approved products in areas where they are still effective. Although new chemical control treatments have and will become available, these might only provide moderate control of CSFB (e.g. Lumiposa, a.i. cyantraniliprole, Corteva Agriscience) and will very likely be more expensive than pyrethroids. Therefore, it is vital that alternative control options for CSFB are investigated to decrease reliance on pesticides. This approach is advocated by the Sustainable Use Directive and is one that that will become increasingly important as the number of insecticides available for control of pests declines.

The current project has investigated crop agronomy, risk factors, the relationship between CSFB infestation and yield, thresholds, pest monitoring, varietal resistance, seed rate, trap cropping and defoliation as potential components of an IPM package. The ultimate aim of the project was to propose an IPM strategy for farmers and agronomists that would enable them to predict the likely risk of CSFB damage and allow rational decisions to be made on the need for control measures. It is likely that this strategy will require the combination of a number of components that individually are unlikely to achieve the level of control that was possible with a good insecticide treatment. However, in combination they would be able to contribute additively to a level of control which would allow sustainable production of WOSR in the UK. A similar approach has been taken with the control of black-grass in cereals (Moss *et al*, 2017). It was suggested that the key to increasing farmer acceptance of cultural control methods was to show that, if several are combined, they will have an additive effect.

This chapter discusses the various aspects of cultural control investigated in this project that could be included in an IPM strategy for CSFB and how successful they have been in combatting the pest (Objective 5). The intention is to increase awareness of factors that might influence CSFB pressure rather than prescribe a particular IPM strategy, particularly as few have been evaluated to any extent in the field. The chapter also discusses the natural enemies of the CSFB and, although these have not been part of this project, the important role these may play in any future IPM strategy. Whilst the project has increased our understanding of the biology of CSFB it has also highlighted how little was known about this pest and where future work is still required. A discussion of future research priorities is included as part of this chapter.

## 9.1. Biological control and biopesticides

The main natural enemy of CSFB larvae is the wasp *Tersilochus microgaster*, which parasitises larvae in the spring and therefore has an impact on pest numbers in the following season. *T. microgaster* emerges in April and peak activity coincides with flowering (Ulber *et al.*, 2010). The wasp locates the larvae while the larva is in the plant, ovipositing its egg through the stem or petiole tissues and into the larva. The new wasp emerges when the CSFB larva has left the plant to pupate in the soil (Ulber *et al.*, 2010). A study in the UK found 11% of CSFB larvae to be parasitised by the wasp but higher parasitisation rates occur in other countries (Ulber *et al.*, 2010), suggesting that populations of the wasp can be encouraged. It is worth noting that insecticides are applied to WOSR in April and May for pollen beetle and seed weevil control (Garthwaite *et al.*, 2018; Garthwaite *et al.*, 2019) and that where these are applied they are likely to coincide with and harm *T. microgaster* activity, especially broad spectrum insecticides such as pyrethroids. Intensive cultivation methods have been found to affect parasitoid populations as they damage soil-borne pupal stages. Indeed, the survival of *T. microgaster* has been found to be improved by the adoption of conservation tillage practices (Nilsson, 2010). Other parasitoids of CSFB larvae include *Aneuclis melanarius*, *Diospilus oleraceus* and *Diospilus morosus* (Holland & Oakley, 2007).

The main natural enemy of adult CSFB is the parasitic wasp, *Microctonus brassicae*. This species was first reared from CSFB in 1996 (Ortega-Ramos & Cook in prep.). Parasitism occurs in summer and into autumn. The female inserts its ovipositor into the host beetle, laying eggs into its body. The larvae of this parasitoid develop inside CSFB adults, feeding from the inside out. When larvae are fully developed, they kill the CSFB when exiting their body. Then they pupate, giving rise to a new generation (Ortega-Ramos & Cook in prep.). Experimental work in which 1-3 *M. brassicae* were introduced to boxes of 10-45 CSFB adults resulted in parasitism rates between 23-56% with adult CSFB dying after approximately 30 days (Jordan *et al.*, 2020). These parasitism rates suggest that if populations of this wasp could be encouraged then it could potentially play an important role in managing CSFB populations. *M. brassicae* is currently the subject of a PhD at Rothamsted Research (Ortega-Ramos & Cook in prep.). *Microctonus melanopus* is another wasp reported to attack adult CSFB but information on this species is sparse (Holland & Oakley, 2007; Ulber *et al.*, 2010). The spider *Centromerita bicolor* has been reported as a predator of the adult and larval stages (Holland & Oakley, 2007).

The most important natural enemy of CSFB eggs is thought to be the ground beetle, *Trechus quadristriatus*, and may be an important component in reducing larval pressures in the autumn and winter (Warner *et al.*, 2003). Means of encouraging populations of these beetles should be investigated. Pyrethroid usage against CSFB in late summer and autumn has increased dramatically in recent years (see Section 2.3) but where pyrethroid resistant CSFB populations are present (currently most of England – see Section 2.3) these are likely to provide little control.



Moreover, they are likely to be harmful to natural enemies active at this time, including *M. brassicae* and *T. quadristriatus*.

A study investigating pest control in organic WOSR found that management techniques such as mulching, avoiding ploughing, and comb harrowing encouraged natural enemies of WOSR pests and reduced the reproductive rates of the pests (Nuss & Ulber, 2004). Clearly, crop management approaches that encourage natural enemy activity by providing suitable habitats, using appropriate cultivation methods and minimising or better targeting insecticides will help reduce CSFB pressures.

A number of potential biopesticides for the control CSFB have been identified (Butt *et al.*, 1992; Butt *et al.*, 1994; Hokkanen *et al.*, 2006). These are currently the subject of an AHDB-funded PhD studentship at Harper Adams University (AHDB Cereals & Oilseeds project code 21510042).

## **9.2. Recommended IPM strategy**

A summary of those factors that influence CSFB risk is provided in Table 61. In total 31 factors that could influence CSFB pressure were identified of which 20 decreased CSFB risk, seven increased risk and four were neutral. The reliability indices ascribed to each factor are estimates and are clearly very variable. Improving the precision with which we can predict the likely CSFB risk under different scenarios will be reliant on further research and this is discussed in more detail in the next section.

Components of an IPM strategy for CSFB are outlined in Figure 165. This indicates the reliability of control and the need for further research for each component. Some components can be utilised immediately to improve management of CSFB (e.g. trap crops and sow date) while others require further research to determine their reliability and importance (e.g. varietal breeding and winter defoliation). It is likely that more than one IPM component would be needed to reduce CSFB pressure sufficiently. Consideration should also be given to the wider effects any strategy has on the crop. For example, sowing in the second half of September is likely to reduce numbers of CSFB larvae and may reduce levels of adult CSFB feeding but would need to be balanced against the potential agronomic disadvantages of this approach. Late sown crops are at an increased risk of poor establishment and suboptimal yield, although the WOSR Yield Enhancement Network (YEN) winning crop in 2017 was drilled on 15 September and achieved a yield of 6.5 t/ha (P. Berry, pers. comm.). Conversely, early sowing might help the crop to establish if plants emerged before the main period of beetle migration but is likely to increase the risk of high levels of CSFB larvae, over-large canopies in the spring and lodging risk. Early sowing also increases the risk of cabbage root fly attack (*Delia radicum*), although cyantraniliprole seed treatments are effective against this pest (van Nieuwenhoven, 2017). Losing a crop to larval damage is more harmful than losing a crop

to adult damage because more crop inputs have been invested in the WOSR and fewer options are available regarding resowing the field with another crop. Testing various IPM strategies in the field would increase the confidence with which they could be recommended to farmers and agronomists.

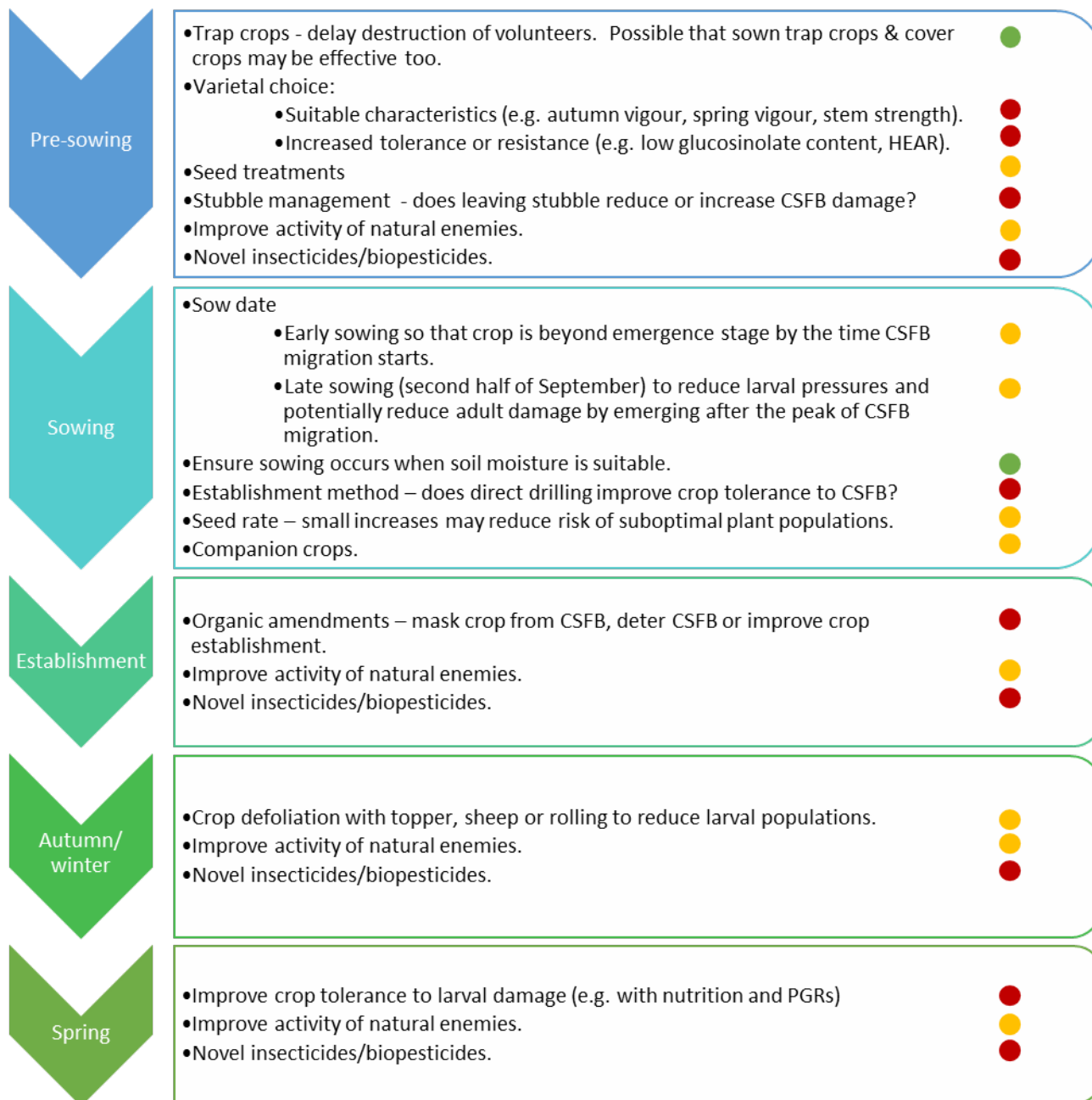


Figure 165. Potential components of an IPM strategy for CSFB. Components are given for specific stages of the crop. A traffic light system indicates the reliability of CSFB control and the need for further research for each component; green = reliable control, possibly with some further research needed, yellow = moderate control with further research required, red = control not proven and significant further research required.

Table 61. A summary of those factors investigated in the current project that might have an influence on CSFB pest pressure. An index (1-10, where 1 is poor and 10 good) is given as an estimate of the reliability of individual effects. + indicates where CSFB pressure increases due to the specific effect and - indicates where CSFB pressure decreases.

Potential component of IPM strategy	Specific effect	Impact on CSFB risk (+/-)	Reliability index (1-10)	Recommendation for use (Full, provisional, further work required, none)	Comments
Novel modelling techniques					
<i>Adult CSFB damage</i>					
Timing crop emergence	Crop at or before emergence when adult CSFB arrive	+	8	Full	Explains why crops fail to emerge/establish.
Stubble management	Leaving stubble or removing stubble	+/-	5	Further work required	Analysis suggests lack of stubble decreases pest pressure. Anecdotal field evidence suggests the opposite.
Moisture at sowing	Sowing when rain is forecast	-	7	Full	Likely to also apply to establishment methods that retain soil moisture.
<i>Autumn CSFB larval numbers</i>					
Sow date	September sowing	-	8	Full	September sowing decreases autumn CSFB larval pressure. Note that early September sowing is likely to coincide with peak adult CSFB feeding.
Varietal choice	HEAR varieties	-	3	Further work required	Lower autumn larval numbers with HEAR varieties.

Table 61. Continued.

Potential component of IPM strategy	Specific effect	Impact on CSFB risk (+/-)	Reliability index (1-10)	Recommendation for use (Full, provisional, further work required, none)	Comments
<i>Spring CSFB larval numbers</i>					
	September sowing	-	7	Full	See comment above for autumn CSFB larval numbers.
Varietal choice	Low glucosinolate content	-	3	Further work required	Varieties with low glucosinolate content (Mentor, Elgar, Windozz) had less feeding damage than those with high glucosinolate content (Amalie).
	Tolerance to adult CSFB feeding	-	5	Further work required	Amalie & Django yielded well despite high levels of leaf loss.
	Tolerance to CSFB larvae	-	5	Further work required	Wembley yielded well despite high levels of CSFB larvae.
Seed rate	High seed rate on adult damage	-	3	Provisional	Inconsistent benefit of increasing seed rate on leaf area lost due to CSFB.
	High v low seed rate on plant number	+/-	5	Provisional	Increasing seed rate had little benefit on % plants lost to CSFB. A small increase in seed rate could be beneficial to ensure suboptimal plant populations are not established under higher CSFB pressure or where moderate CSFB pressure coincides with poor establishment conditions.
	High v low seed rate on yield	+/-	5	Further work required	Inconsistent effect of increasing seed rate on yield.

Table 61. Continued.

Potential component of IPM strategy	Specific effect	Impact on CSFB risk (+/-)	Reliability index (1-10)	Recommendation for use (Full, provisional, further work required, none)	Comments
Seed rate	High seed rate	+	8	None	Increasing seed rate increased CSFB larval numbers per m <sup>2</sup> .
Tolerance to CSFB attack	Increasing area of leaf loss	+	6	Provisional	50% leaf area loss had limited impact on yield.
	Increasing area of leaf loss	+	6	Full	In only 30% of experiments did yield decrease with increasing leaf area loss.
	Increasing area of leaf loss	+	7	Full	Timing of CSFB migration is critical, tolerance less important if beetles arrive as crop emerging.
	Increasing number of CSFB larvae	+	6	Further work required	In only 11% of experiments did yield loss decrease with increasing larval numbers.
	Increasing number of CSFB larvae	+	6	Further work required	Yield loss increased by 0.05-0.07t/ha for every extra CSFB larva per plant but only in 11% of experiments.
	Stem v petiole larvae	-	6	Further work required	Up to six CSFB larvae in the stems had little impact on yield.
Trap cropping (vOSR)	Impact on adult CSFB numbers	-	8	Full	Up to 88% lower numbers of CSFB adults where vOSR used as trap crop.
	Impact on adult CSFB damage	-	8	Full	Up to 76% less leaf loss where vOSR used as a trap crop.
	Impact on OSR plant populations	-	8	Full	Up to 56% increase in plant numbers where vOSR used as trap crop.

Table 61. Continued.

Potential component of IPM strategy	Specific effect	Impact on CSFB risk (+/-)	Reliability index (1-10)	Recommendation for use (Full, provisional, further work required, none)	Comments
Trap cropping (vOSR)	Impact on CSFB larval numbers	-	8	Full	Up to 69% lower larval numbers where vOSR used as a trap crop.
Defoliation	Impact on CSFB larval numbers	-	8	Full	Larval numbers decreased by between 23-55%.
	Impact on stem canker incidence	N/A	6	Provisional	Severity of stem canker reduced at levels equivalent to good fungicide spray.
	Impact on yield	+/-	5	Further work required	Results inconclusive and likely dependent on environmental conditions.
<i>Other strategies not investigated in depth</i>					
Companion crops	Various crops that reduce CSFB damage or encourage rapid establishment	-	7	Provisional	Encouraging data but further work needed to maximise benefit and improve management of the companion crop.
Establishment method	Minimum cultivation e.g. direct drilling	-	4	Further work required	Some encouraging data but further work needed to confirm benefit.

Table 61. Continued.

<b>Potential component of IPM strategy</b>	<b>Specific effect</b>	<b>Impact on CSFB risk (+/-)</b>	<b>Reliability index (1-10)</b>	<b>Recommendation for use (Full, provisional, further work required, none)</b>	<b>Comments</b>
Organic amendments	Interfere with host location or deter CSFB or encourage rapid establishment	-	3	Further work required	Further work needed to confirm benefit.
Biological control	Encourage natural enemy activity	-	6	Provisional	Research needed to identify effective methods of encouraging populations and activity.
Biopesticides	Develop effective biopesticides	-	3	Further work required	Research at early stages but immobile larval stage and aestivating adult stage may be a useful target.

### **9.3. Potential future research**

Potential future areas of research have been grouped under the various components of a CSFB IPM strategy studied in this project. Further work is likely to be required to determine which components of an IPM strategy best complement each other.

#### **Cultural control of CSFB**

Novel statistical modelling approaches were used successfully to analyse a large database of information on the impact of various agronomic factors on CSFB pressure. Adult CSFB damage and autumn and spring larval numbers were affected by a range of meteorological parameters but there were few factors that were under direct control of farmers/agronomists. Future research could include:

- Sow date:
  - Trial work to confirm importance of drill date.
  - Varietal choice in relation to this discussed below.
  - Determine the economic viability of late drilling in reducing larval numbers.
  - Early sowing remains popular and is likely to be beneficial if emergence does not coincide with arrival of CSFB. Identifying suitable crop management for very early sown crops (e.g. spring canopy management) is a priority.
- Confirm benefits of methods that minimise damage at establishment, e.g. establishment method, organic amendments and straw/stubble management.
- Optimise use of companion crops and investigate interactions with other control strategies, e.g. delayed drilling.

#### **Impact of variety and seed rate on CSFB pressure**

There was no clear evidence that any particular variety is any more or less susceptible to CSFB than any other in both work with RL trials and ADAS drilled variety-seed rate trials. There was also no clear evidence that large increases in seed rate decreases CSFB risk.

- Varietal choice:
  - Medium-term: Varieties with traits that complement cultural control techniques e.g. rapid establishment for early sown crops, varieties suited for late sowing, spring vigour to improve tolerance to larval feeding, varieties that respond well to defoliation (either through good rooting in the autumn or good vigour in the spring).
  - Long-term: Breeding for tolerance/resistance. Breeding for varieties with reduced glucosinolate content or higher levels of erucic acid may be beneficial. Determining their benefit when sown on a field-scale.
  - Use AHDB sponsored recommended list trials to screen varieties for tolerance/resistance/ attractiveness/ palatability to CSFB.



- Seed rate: Confirm effect of seed rate on field-scale. Investigate importance of factors such as thousand seed weight.

### **Re-evaluating thresholds for adult and larval CSFB attack**

As well as evaluating alternative methods to combat CSFB it is also vital to improve our understanding of the pest levels that justify control. Data suggests that established WOSR can withstand considerable loss of leaf area. Also that some crops are better able to tolerate to larval feeding as anecdotal evidence suggests that crops can yield well even in presence of significant larval pressure. Further research would reduce reliance on insecticides, which in turn would reduce the risk of resistance developing to any new modes of action developed for the pest. Further research could include:

- Factors that govern tolerance to larval feeding.
- Understanding the impact of spring hatching larvae on crop yield.
- Crop management methods to mitigate, and potentially manipulate, larval feeding.
- Is there a less time-consuming method of assessing larval numbers?
- Understanding larval behaviour to better target novel insecticides and biopesticides.

### **Using vOSR as a trap crop for CSFB adults**

Results showed significant reductions in adult CSFB infestation (up to 88%), CSFB adult damage (up to 76%), significant increases in plant population (up to 56%), and significant reductions in CSFB larvae (up to 69%). Further work is required to refine this technique to include:

- What area of vOSR is required to lure adults away from emerging OSR?
- How long does the vOSR need to be left?
- How important are the relative growth stages of the VOSR and the emerging crop?
- Where on the farm should the vOSR be situated?

### **Using Defoliation as a means of reducing CSFB larval pressure**

Defoliation in plot trials reduced larval numbers by 23-55% and similar results were achieved in an Innovative Farmer field lab. The incidence of stem canker was also significantly reduced. However, yield responses were very variable. Further research could include:

- Investigation of the potential of rolling to reduce larval numbers
- Improving the ability to predict those crops likely to benefit from rolling by taking into account defoliation timing, effect of drill date, autumn rooting and weather
- Identify those varieties particularly suited to defoliation
- Identify crop nutritional requirements post-defoliation.

### **Decision support systems (DSS)**

- Improving the precision of CSFB predictive models. Models exist to predict egg hatch and larval invasion. Key missing component is predicting adult CSFB migration. Understanding this would allow better targeting of treatments (be it chemical insecticides, products that mask the crop or deterrents) and prediction of larval invasion timing and magnitude.
- DSS tool that provides guidance for optimising establishment, e.g. soil moisture, cultivation methods, varietal choice.
- Modelling to identify means of reducing CSFB populations on a regional scale, e.g. through restrictions on WOSR cultivation.

### **Biocontrol and biopesticides**

- Understanding impact of natural enemies (e.g. ground beetles and parasitoids).
- Understanding means of increasing populations and activity of natural enemies.
- Develop and field-test biopesticides.
- Better understanding of CSFB lifecycle (e.g. where to adult CSFB go to aestivate? When do larvae move in and out of petioles?) to improve targeting of biopesticides.

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# 11. Appendix 1: Questionnaire for the 2017 and 2018 adult CSFB damage survey (Section 3.3)

## Survey questionnaire

### **Monitored crop**

- What is the field name?
  
- What is the area of the field (ha)?
- What was the previous crop in:
  - o 2015/16 .....
  - o 2014/15 .....
  - o 2013/14 .....
  - o 2012/13 .....
- What is the OSR variety?
- What is the soil type?
- How stony is the soil?
  - No stones                       Few stones                       Stony

### **Establishment of the monitored crop**

- Was the straw of the previous crop removed? Please tick one box.
    - Yes, baled and removed
    - Chopped and left
    - Chopped and incorporated
  - Which establishment techniques were used? Please tick one box.
    - Broadcast into standing crop/stubble ('autocast')
    - Direct Drill and roll
    - Sub-cast (e.g. drill with subsoiler)
    - Non-inversion tillage (e.g. disc, combi-drill, shallow <10cm)
    - Non-inversion tillage (e.g. disc, combi-drill, deep >10cm)
    - Ploughing systems (e.g. plough, combi-drill)
    - Other
- If 'Other' please describe:
- .....
- .....
- .....

.....  
.....  
If the stubble was left how tall was it?

- Short (< 15cm)       Tall (>15 cm)

- Was the field rolled after drilling?

- Yes       No

- Was the seed home saved?

- Yes       No

- What is the row width?

- When was the crop drilled?

- What is the drilling depth?

- What was the seed rate? Please complete as best you can.

- ..... kg/ha
- ..... seeds per m<sup>2</sup>
- ..... thousand seed weight

- What was the seedbed quality at drilling? Tick more than one box if required

- Ideal       Clean  
 Cloddy       Dry  
 Trashy       Moist

- How much rain was there during the two weeks post drilling?

- Dry       Some rain       Lots of rain

- When did plants start to emerge?

### **Fertiliser application**

- Were fertilisers applied to the crop before drilling?

- Yes       No

- If yes what was the product and rate of fertiliser application?

.....  
.....  
.....  
.....  
.....

- Were fertilisers applied to the crop at drilling?

- Yes       No

- If yes was the fertiliser broadcast or placed with the seed?

- Broadcast       Placed with seed

- What was the product and rate of fertiliser application?

.....  
 .....  
 .....  
 .....  
 .....

- Were fertilisers applied to the crop after drilling in the autumn?  
 Yes                       No

- If yes what was the product and rate of fertiliser application?

.....  
 .....  
 .....  
 .....

**Other pests & control strategies**

- What level of slug pressure was there during establishment? Please tick one box.  
 Low (no damage)  
 Medium (some damage)  
 High (significant damage)  
 Don't know

- Have you or are you using any novel strategies to control cabbage stem flea beetle?  
 Yes                       No

If you ticked yes please describe briefly:

.....  
 .....  
 .....  
 .....

- Which insecticides/molluscicides have been applied to the crop in the autumn? Please give details on the product, rate and date of application:

Product	Rate	Date


**Proximity of monitored field to previous oilseed rape**

- In 2015/16 how far from the monitored field was the nearest OSR field (in metres)?
- In 2015/16 in what direction was the nearest OSR field relative to the monitored field?
- In 2015/16, if known, what was the adult CSFB pressure in the nearest OSR field to the monitored field? Please tick one box.
  - Low (<25% leaf area lost at the cotyledon to two true leaf stage)
  - Medium (26-50% leaf area lost at the cotyledon to two true leaf stage)
  - High (>51% leaf area lost at the cotyledon to two true leaf stage)
  - Don't know
- If known, what was the larval CSFB pressure in the nearest OSR field in 2015/16? Please tick one box.
  - Low (no larvae present)
  - Medium (<5 larvae per plant)
  - High (>5 larvae per plant)
  - Don't know