Quantifying the impacts of the neonicotinoid restriction on oilseed rape pest control and productivity.

PhD Studentship

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Declaration:

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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A note should be made to the 107,122 insects sampled in creating the data for this thesis.

Contents:

0. Abstract:

Under current EU legislation the use of neonicotinoid seed treatments is banned in oilseed rape (OSR, *Brassica napus* L.) this has led to increased pest pressure and reduced cropping area of OSR in the UK. One main factor for increased pest pressure is from the cabbage stem flea beetle (*Psylliodes chrysocephala*) which also exhibits resistance to the only remaining alternative synthetic chemical control licensed in the UK. This thesis will in part examine the implications of the neonicotinoid restriction in pest control in the autumn from *P. chrysocephala* one of the primary target species for neonicotinoid seed treatments in OSR. Empirical field trials were performed to compare how alternative pest protection practices interact with pest control and crop yield. These were done alongside Neonicotinoid to gain a measure of the benefits of seed treatments. In control experiments undertaken during this thesis the interaction between *P. chrysocephala* and OSR are explored. Providing evidence that OSR can withstand higher pest pressure than current economic thresholds for insecticide application suggest. The relative effect of multiple pest protection methods were shown to be equal to crop protection and that neonicotinoid seed dressings did not show any observable benefit to OSR over un-treated seeds.

1.0 Introduction:

1.1 Oilseed rape:

Oilseed rape (OSR, *Brassica napus* L.) is a cultivated member of the Brassica family and is primarily grown on an agricultural scale as an oil crop (Redman, 2019), with the meal from oil processing being sold as animal fodder (Cotrill *et al.*, 2007). Oilseed rape contributes around 80% of EU biofuel production (Ouvrard and Jacquemart, 2019) and 16% of worldwide oil production from crops (FAOSTAT, 2021). It is the third most widely grown crop in the UK, after winter wheat and barley (DEFRA, 2015) with an average yearly value of £804M (Nicholls, 2016). The primary harvest is of the seed grain for oil extraction (Diepenbrock, 2000), but it is also an important break crop in arable rotations, reducing weed pressure (Haramoto and Gallandt, 2004; Rick and Ann, 1995) and fungal pathogens (Muehlchen, Rand and Parke, 1990). Increased rotational diversity has been shown to reduce pest and weed pressure over reduced crop rotations (Brust and King, 1994).

1.2 Oilseed rape in the UK:

In the UK OSR is either autumn sown or spring sown OSR, with the primary form being is autumn sown (winter oilseed rape: WOSR) which requires a period of vernalisation in order to form flowers (Tommey and Evans, 1992). Spring sown OSR (spring oilseed rape: SOSR) does not require vernalisation and is planted in the spring and develops much faster. The UK's mild winters allow WOSR to be effectively grown allowing for the increased yields, over SOSR, attributed to the longer growing season (Rameeh, 2011). The area grown in UK peaked in 2011/12 at just under 750,000 Ha dropping to below 550,000 Ha in 2015/16 (Scott and Bilsborrow, 2019). This has continued to drop to a current level of 337,000 Ha in 2020 (DEFRA statistics, Figure 1). Variations between years are to be expected as farms will not be on the same rotation stage, so some caution is needed when comparing yearly production/ planting of OSR. However, the fluctuation in market value is a major factor in the planting or OSR with crop values changing rapidly (Davies, 2019). The increase in OSR area from 2005 was in part due to EU policy to produce 20% of energy use from renewable sources of which ORS biodiesel is a component part (EREC, 2011). Then the downward trend of area grown has occurred since the restrictions (Figure 1) on using neonicotinoid seed dressings on crops that flower, came into force in 2013 and it has continued since the wider ban came into effect in 2019 (DEFRA, 2019).

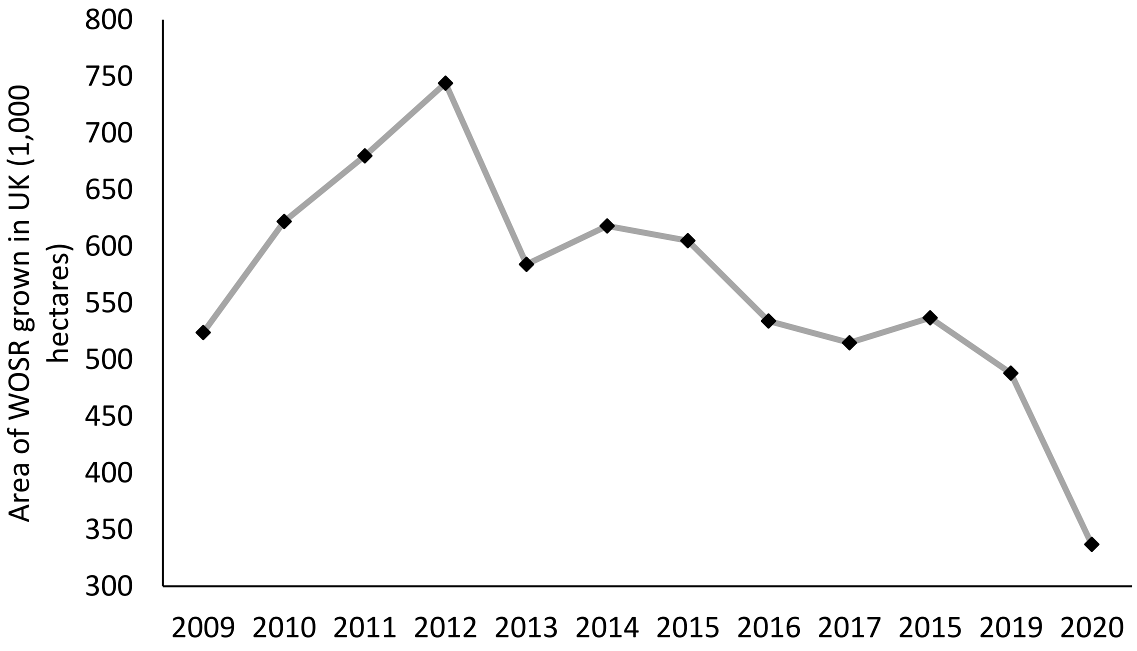

Figure 1. UK winter oilseed rape (WOSR, *Brassica napus*) cropping area from 2009 to 2020. Data from DEFRA statistics (DEFRA, 2019).

1.3 Pest issues and their control in oilseed rape:

Oilseed rape is subject to a wide array of pest species including, insects, gastropods, and birds (Williams, 2010). In the UK, the insect pests of primary concern for establishment and yield include, pollen beetle (*Brassicogethes aeneus*), cabbage stem flea beetle (*Psylliodes chrysocephala* L.), cabbage seed weevil (*Ceutorhynchus pallidactylus* M.), and aphids that vector diseases (*Myzus pursicae*). The primary methods utilised by farmers to avoid or reduce the damage caused by these pests is through the application of synthetic insecticides (Williams, 2010). These are applied as either sprays or seed dressings. Spray applications are directly onto the crop surface, are often broad spectrum and can be applied in response to pest pressure (Johnen *et al.*, 2010). Seed dressings are applied to the seed before sowing and are taken up into the plants tissues as they grow, conferring a level of protection to all parts of the plant (Simon-Delso *et al.*, 2014).

1.4 Neonicotinoids:

Neonicotinoids, including seed treatments, were first registered in the UK in 1994 and by 2008 they represented 24% of insecticides used world-wide (Jeschke *et al.*, 2011). They act by binding to the insect’s nicotinic acetylcholine receptor, causing excess synaptic firing (Tomizawa and Casida, 2005). By contrast binding to mammalian receptors is much reduced making neonicotinoids much less toxic to mammals. The seed dressings are systemically taken up by the plant as it grows, conferring protection to all parts of the plant, an advantage over repeated spraying which may lead to off target effects (Tomizawa and Casida, 2005). This systemic nature made neonicotinoids seed dressings a very attractive option to growers who could apply an insurance level of protection against pests during crop establishment. At the time of interdiction noenicitinoids also allowed the diversity of insecticide mode of action to aid in resistance management (Zimmer, 2015). All of these positive properties led to their rapid adoption (Jeschke *et al.*, 2011). However, the systemic nature, hailed by farmers as being more targeted (UK, 2020) and easier to use had the downside of resulting in their presence in nectar and pollen and therefore to potential exposure of non-target insects such as bees (Goulson, 2013; Gross, 2014).. Research has indeed shown detrimental effects on n bees (Gill and Raine, 2014; Raine and Gill, 2015; Henry *et al.*, 2012; Whitehorn *et al.*, 2012) and also on birds (Gibbons, Morrissey and Mineau, 2015; Hallmann *et al.*, 2014). The level of environmental toxicity has been brought into question with studies showing cumulative effects (Gill and Raine, 2014). These concerns led to increased political pressure and public concern over their use.

1.5 Neonicotinoid legislation:

On the 1st of December 2013, the European commission restricted the use of three neonicotinoid pesticides, Imidacloprid (registered for use in 2000, Chinook, Bayer), Clothianidin (registered for use in 2008, Modesto, Bayer) and Thiamethoxam (registered for use in 2007, Cruiser, Syngenta) regulation 485/2013 prohibited the use as seed treatments, soil treatments and foliar supplication to bee-attractive crops and seed and soil treatments in cereals sown between Jan and July (EU, 2013). This was expanded in 2018 under regulation 2018/783-785, which prohibited the outside use of neonicotinoids on all crops and only allowing use on for plant production which is 100% inside glasshouse (EU, 2018). Other countries have also banned the use of some neonicotinoids, for example Fiji banned the use of imidacloprid as of January 2020 (Government, 2020), the US, Canada, Australia and New Zeeland governments have all announced a review of neonicotinoid use (Goverment, 2019; Zealand, 2018) and a recent report covering 17 African nations has called for a review of neonicotinoid use across Africa (NASAC, 2019). Again, the main reason for restrictions was in response to concerns over the potential effects of these chemicals on non-target species, especially bees (Blacquiere *et al.*, 2012).

1.6 Post neonicotinoid restrictions:

The restriction on the use of neonicotinoids in 2013 included the loss of seed treatments in OSR and this led to widespread farmer concerns (UK, 2020; White, 2016) and ultimately threatened UK OSR production (Graham and Alford, 1981). One of the main concerns was the growing incidence of cabbage stem flea beetle (*P. chrysocephala)* in crops and this led to the NFU to seek a derogation to use neonicotinoids in 2015 to protect OSR (Case, 2015). This was granted on a small scale in Suffolk, Cambridgeshire, Bedfordshire, and Hertfordshire covering around 30,000 Ha of cropping area equivalent to 5% of the UK total (Case, 2015). More recently the French government is considering reducing the ban on sugar beet over concerns of aphid virus transmission reducing crop yields (Case, 2020). The UK government has recently issued an emergency authorisation to use a neonicotinoid (Syngenta: Cruiser SB AI: thiamethoxam to protect sugar beet, under strict regulations (DEFRA, 2021).

The reductions in cropping area of OSR in the UK since 2013 (DEFRA, 2019) have been attributed to increased pest pressure from the lack of seed the treatments. Scott and Bilsborrow (2017) identified (*P. chrysocephala* L.) in the top three most important reasons for the reduced cropping area of OSR observed since the neonicotinoid restriction in 2013

(Impey, 2020). They also estimated prevention/ mitigation of *P. chrysocephala* including costs of other insecticide, application costs, and re-drilling costs , totalling £18,369,369 in 2015/16, with a reduction of WOSR in the UK by 13% in 2016 compared with 2015 (White *et al.*, 2020).

This reduction in area of OSR, brought about at least partly by the neonicotinoid restriction in 2013, may have had adverse effects on the flower-visiting insects the ban was introduced to protect, because OSR provides an important spike of floral resources for pollinators (Holzschuh *et al.*, 2012) and has a wide diversity of floral visitors (Garratt *et al.*, 2018; Garratt *et al.*, 2014). (Stanley, Gunning and Stout, 2013; Garratt *et al.*, 2014). The reduction in OSR, with some farmers stopping it altogether, will cause a shortage in floral resources especially for early season bee species (Budge *et al.*, 2015).

1.7 *Psylliodes chrysocephala* life cycle:

With the importance of OSR to pollinators, rotations, and the UK economy it is important to explore alternatives to neonicotinoid-based insecticides. This requires knowledge of the pests to be controlled and for OSR this is *P. chrysocephala,* which is distributed across the UK and northern Europe. The life cycle of has been studied in many countries across Europe (Graham and Alford, 1981; Winfield, 1992; Oakley, 2003; Holland and Oakley, 2007; Cox, 1998) and is relatively well understood (Williams, 2010). Adults migrate into the crop during the autumn (Thioulouse, Debouzie and Ballanger, 1984; Thioulouse, 1987) where they feed on leaves, producing characteristic shot holing and necrosis (Alford, 2003) before reproducing within the crop and laying eggs in the soil and at the base of plants (Alford, 1979; Bonnemaison and Jourdheuil, 1954). The larvae then emerge, burrow into plant stems and feed throughout the winter within the plant petioles and stem before exiting the plant to pupate within the soil. The two forms of feeding make *P. chrysocephala* an economically important pest of OSR (Nicholls, 2016). The timings of each stage are dependent on environmental conditions and can vary from year to year (Williams, 2010). The natural enemy community is little understood, but it is known to be parasitized by *Tersilochus tripartitus*, *T. microgaster*, and *Aneuclis melanarius* at the larval stage and *Microctonus melanopus* at the adult stage (Williams and Ferguson, 2010). More recently the parasitoid *Microctonus brassicae* has been examined as a potential biocontrol agent for *P. chrysocephala* showing 44% of populations being infected in controlled environments (Jordan *et al.*, 2020).

1.8 *Psylliodes chrysocephala* impact on OSR establishment and yield:

Feeding by adult *P. chrysocephala* on the early growth of OSR can reduce stands and In years of high abundance, can decimate whole fields ((2015), personal observations). making establishment a lottery. There is some evidence that OSR can tolerate very high levels of leaf area loss before the plant exhibits yield reductions (Freyman, Charnetski and Crookston, 1973; McCormick, Virgona and Kirkegaard, 2013; Ulas *et al.*, 2015) and the impact of injury akin to adult feeding (shot hole injury) on early growth has been assessed for plant biomass (Nowatzki. T and Weiss. M, 1997; Ellis, 2015; DEFRA, 2014) or response in plant tissue concentrations of glucosinolate (Koritsas, Lewis and Fenwick, 1989; Koritsas, Lewis and Fenwick, 1991; Bartlet *et al.*, 1999; Döring and Ulber, 2020). These studies examined the initial response of the plant to injury and did not measure the chronic effects on later seed production. The larvae are considered to be of greater concern than the adults (Williams, 2010) and once inside the plant stem they are difficult to target with insecticide spays, but were controlled previously through the systemic nature of neonicotinoid seed dressings, as shown with the reduction in spray applications at sites under the 2015 DEFRA derogation (Case, 2015; Scott and Bilsborrow, 2019). The wounds caused by *P. chrysocephala* larvae can also leave plants at a higher risk of secondary infections such as stem canker (*Leptosphaeria maculans*. K.) which has been reported to be “associated with insect wounds” (Newman, 1984).

Since the neonicotinoid restrictions, there have been increasing reports of adult *P. chrysocephala* preventing crop establishment in OSR (Nicholls, 2016) and it has been reported that the control threshold level, 5 larvae per plant from a sample of 25 plants; has been exceeded in 46% of the planted crop (White, 2016).

1.9 Resistance to pyrethroid in *Psylliodes chrysocephala:*

The removal of neonicotinoid seed treatments has led to an increase in pyrethroid application, up to four times the levels used before the ban (White, 2016). Scott and Bilsborrow (2019) report an estimated cost of £22.2million for agrochemical (primarily pyrethroids) purchase and application in 2014/15, derived from Farm Business Survey of >200 farms. This represents an increase in the number of applications per crop from 1.4 to 2.0 (Scott and Bilsborrow, 2019) and this has increased the pressure on the insects to develop insecticide resistance (Mallet, 1989). The repeated use of a single mode of action can allow the percentage of pest population which exhibit resistance traits to proliferate and become a major problem (Helps *et al.*, 2020). Resistance to pyrethroids has been reported in *P. chrysocephala* , first in Germany in 2014 (Zimmer *et al.*, 2014) and now across continental Europe (Højland *et al.*, 2015). In September 2014 resistance was found in 73% of the adults in UK populations (Foster and Williamson, 2015).

This was shown to be associated with a target site mutation similar to the knock down resistance mutation found in other insect species (Castberg and Kristensen, 2018). This mutation was selected quickly and has ultimately spread across populations, thus reducing the susceptible proportion of the population making the insecticide less efficient and driving further resistance.

1.10 Protecting crops from *Psylliodes chrysocephala*:

Given the restriction on neonicotinoid use, coupled with growing pyrethroid resistance and projections of milder, less snow-covered winters in the UK (McCabe and Wolock, 2010), the impact of *P. chrysocephala* on OSR production in the UK is expected to increase. It has already been suggested that an extra 38,000 ha of OSR would have been planted if seed dressings were still available (White, 2016), highlighting the farmer's response to the restriction.

With growing concern on the environmental impacts of farming, the need for alternative and sustainable crop production methods is paramount.

1.11 Increased floral diversity:

One area which has shown potential as a pest protection method either without, or with reduced synthetic chemical application, is increasing the botanical diversity within the cropping system (Ratnadass *et al.*, 2012). This can be either ’trap cropping’ or ‘intercropping’. For trap cropping a plant species which is more attractive to the pest than is the cash crop, is grown alongside the cash crop to divert pest pressure towards the trap crop, reducing infestation in the main crop (Hokkanen, 1991; Shelton and Nault, 2004; Shelton and Badenes-Pérez, 2006; Cook *et al.*, 2007; White, Ellis and Kendall, 2018) Trap crops may also influence the levels of natural enemies by provision of nectar, alternative prey, shelter, and non-crop habitat (Skellern and Cook, 2018). Intercropping’ is a a system whereby the desired marketable crop is grown in conjunction with one or more other species, as a means of increasing diversity and exploiting the within-field ecology to benefit the desired crop (Costello, 1994; Hooks and Johnson, 2003; Hooks and Johnson, 2004; Vandermeer, 1992).

In OSR, turnip rape (*Brassica rapa*) used as a trap crop has been shown to reduce numbers of pollen beetle (Skellern and Cook, 2018; Cook *et al.*, 2007) and seed pod weevil (Cárcamo *et al.*, 2007) in spring OSR and *P. chrysocephala* in winter OSR (Barari *et al.*, 2005).

Barari *et al* (2005) reported higher numbers of *P. chrysocephala* larvae (and percentage parasitism) in Turnip rape grown in asscotion woth OSR

Intercropping has often shown reductions in pest damage compared to crop monoculture (Baux and Schumacher, 2019; Brandsæter, Netland and Meadow, 1998; Gombac and Trdan, 2014; Hooks and Johnson, 2004; Prasifka *et al.*, 2006) and in turn, biological control of pests has been shown to be influenced by intercropping, through the increase of natural enemies active within intercropping systems (Hooks and Johnson, 2003).

One drawback of intercropping is the competition for resources with the crop (Verret *et al.*, 2017b)., including for light, water, nutrients, and growing space (Carof *et al.*, 2007; Kloen and Altieri, 1990). In an autumn sown crop competition can be mitigated by use of frost-sensitive companion plants, that will be destroyed over winter (Verret *et al.*, 2017a; Lorin *et al.*, 2015). The use of herbicide resistant OSR cultivars (Clearfield) can also be utilized with susceptible companion species (Cadoux *et al.*, 2015). Clearfield cultivars exhibit resistance to the herbicide used alongside to control within field weeds (BASF, 2018). Thereby mitigating the competition during the reproductive stage and grain filling periods of the crop.

1.12 OSR response to *Psylliodes chrysocephala*:

The high capacity of OSR to compensate from defoliation prior to flower bud formation has been reported in several studies (Freyman, Charnetski and Crookston, 1973; McCormick, Virgona and Kirkegaard, 2013; Ulas *et al.*, 2015), but in general, there is a lack of knowledge about the extent of the compensation. Studies which have mimicked the effect of feeding damage on OSR using mechanical shot holing of early growth stages have measured plant biomass (Nowatzki. T and Weiss. M, 1997; Ellis, 2015; DEFRA, 2014) or glucosinolate production (Koritsas, Lewis and Fenwick, 1989; Koritsas, Lewis and Fenwick, 1991; Bartlet *et al.*, 1999; Döring and Ulber, 2020) and did not measure the chronic effects on seed production. Antwi et al (Antwi, Olson and DeVuyst, 2008) did simulate injury and actual feeding of *Phyllotreta cruciferae* on early growth spring canola and grew plants to pod production, showing no effect of simulated injury as opposed to significant reductions from actual beetle injury. Similarly, Susko & Superfisky (Susko and Superfisky, 2009) showed differing compensatory responses from OSR from patch defoliation (akin to slug injury) and shot hole injury (akin to *P. chrysocephala*) with patch defoliation giving full recovery but injury reducing seed grain yield.

Assessments have been made on the effect of to *P. chrysocephala* infestation on OSR growth rate (Döring and Ulber, 2020) and the response of secondary metabolites (Döring and Ulber, 2020; Koritsas, Lewis and Fenwick, 1989; Koritsas, Lewis and Fenwick, 1991; Bartlet *et al.*, 1999). These have only grown the OSR for a limited period after infection and little to no data is available on the chronic effects of larval infestation on the seed yield.

1.13 *Psylliodes chrysocephala* adult feeding preference:

With reduced pesticide options available there is a need for better targeting of those that can be used, including knowing which growth stages are preferred by the beetles and therefore in most need of protection. Diehl, (2017) fed leaf discs cut from leaves of varying ages to *P. chrysocephala* , demonstrating low feeding on cotyledons but high feeding and damage on all true leaves, with lower levels on the first leaf for the first 24hours, and then comparable levels on the first, second and third leaves after 48hours.

1.14 Insecticide application thresholds:

Thresholds to determine when insecticides should be applied are derived from the economic injury levels and are defined as “the lowest population density that will cause economic damage” (Stern, 1973). Such economic thresholds are a valuable tool to reduce the cost of application by aiming at keeping them to the lowest possible level needed (Ramsden *et al.*, 2017). They are often defined in terms of the abundance of a pest per unit area, per plant, or per part of plant, above which the economic losses are equal or greater than the cost of control (Ramsden *et al.*, 2017). However, the thresholds are only as efficient as the data they incorporate on the costs of applications, finance and labour, against the efficiency of the application to reduce pest pressure and avoid resistance, usually without detailed data on the risks the application poses to the wider environment and any confirmation of effectiveness.

Current thresholds in the UK for *P. chrysocephala* are based on economic factors of low-cost application insecticides, are > 25% of leaf area loss at BBCH Growth Stage (GS, Lancashire *et al.,* (1991)) 11-12 and 50% of leaf area at GS 13-14, or if significant plant loss is occurring during adult feeding (Oakley, 2003). However, these thresholds are based on early growth green leaf area and dry matter and do not take account of yield and compensation ability (Ellis, 2015). Currently insecticide applications to control larvae are recommended if larval numbers average 5 or more per plant (AHDB, 2019), although Green (2008) has suggested that thresholds should be as low as 2 larvae per plant. It is clear that the effect of the growth stage of the plant, the time when the damage occurs (i.e., at cotyledon stage, 1 or 2 leaf stage or later) and the interactions between leaf area loss and larval infestation have not been studied at the level of effects on subsequent seed production.

1.15 Aims of Thesis:

This thesis will examine the impacts of the neonicotinoid restriction on OSR pest control, pollination, and productivity by testing how neonicotinoid seed treatments compare to alternative synthetic chemical and ecological pest protection methods as a first step to defining the level of crop protection being lost through seed treatment restrictions. It is vital to understand what level of crop protection and yield production is achieved through the application of neonicotinoids as any quantification of impacts of their loss from the arsenal will be purely speculation.

See Chapters:

2.0 Nurse crops can reduce cabbage stem flea beetle (*Psylliodes chrysocephala* L.) damage to oilseed rape (*Brassica napus* L.) without neonicotinoids.

3.0 Potential of Brassica trap crops for protecting winter oilseed rape (*Brassica napus* L.) from *Psylliodes chrysocephala* damage.

4.0 Comparison of crop protection strategies for *Psylliodes chrysocephala* in winter oilseed rape (*Brassica rape*): past, present, and future.

The second section of this thesis collects empirical data on how OSR compensates from *P. chrysocephala* damage and how such damage impacts flower visiting insects.

See Chapter:

5.0 The impact of injury caused by *Psylliodes chrysocephala* L. adults and larvae on flower and yield production in *Brassica rapa* L.

The feeding preferences of *P. chrysocephala* are also tested in a whole plant choice experiment to define the most susceptible growth period of OSR and finally the diurnal activity of *P. chrysocephala* in the field. This second section expands on knowledge on the life history and *P. chrysocephala* interactions.

See Chapters:

6.0 Feeding preference of *Psylliodes chrysocephala* L. on early true leaves of Oilseed Rape (*Brassica napus* L.)

7.0 Assessing the circadian rhythm of *Psylliodes chrysocephala* L. in UK winter oilseed rape (*Brassica napus* L.).

The final section of this thesis was a survey of UK farmers opinions of the current neonicotinoid ban, pest pressure and how they would interact with the pest protections methods tested in the field trials of this thesis.

See Chapter:

8.0 Are farmers really concerned about the neonicotinoid ben? Comparison of UK farmers views on crop protection strategies for *Psylliodes chrysocephala* in winter oilseed rape (*Brassica rape*): past, present, and future.

This project aims to combine data from field experiments on the relative pest protection and yield return multiple autumn pest protection practices all tested against neonicotinoid seed dressing. This data will link to haw farmers respond to the practices tested in the online survey. Bringing together data on more specific interactions between *P. chrysocephala* and OSR to better understand how pest control can adapt into a more sustainable future.

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Cox, M. J. (1998) 'The genus *Psylliodes* Latreille (Chrysomelidae: Alticinae) in the U.K.', *The Coleopterist,* 7, pp. - N2 - As in most groups of insects, scientific research on the Chrysomelidae began in Europe in 1758, with the description of a few genera and species by the Scandinavian entomologists C. von Linne, I.C. Fabricius, and others. As the 19th century dawned, many systematic entomologists took up the study of chrysomelid beetles, together with other groups of beetles, and many new species and genera were described from all parts of the world. This trend has, of course, continued down to the present time. However, researches on the Chrysomelidae did not remain restricted to systematics, and many new lines of study have been followed, especially in the present century, by workers who have benefitted from the advances made in related fields of pure and applied entomology. Much has been achieved in the study of the Chrysomelidae, as elsewhere, and it is the aim of the present book to provide a summary and guide to these achievements. It is also to be expected that this book will provide a stimulus for further studies on the Chrysomelidae, so that we can anticipate continuing progress in our knowledge and understanding of this group through the endeavours of an ever-increasing number of scientists. I offer my congratulations to all concerned in the preparation of this book and my best wishes for its success.

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2.0 Nurse crops can reduce cabbage stem flea beetle (*Psylliodes chrysocephala* L.) damage to oilseed rape (*Brassica napus* L.) without neonicotinoids:

2.1 Abstract:

The use of neonicotinoid seed dressings has been restricted in oilseed rape (OSR, *Brassica napus* L.) in the UK since 2014 with a complete ban issued in 2018 (EU, 2018). Since then, the area of oilseed rape grown in the UK has reduced, with pest pressure from the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) being a major factor. Here we test if using a 'nurse crop' inter-sown with oilseed rape can alleviate the pest pressure. Four different mixes of frost-sensitive nurse crops were tested. Due to extreme pest pressure from *P. chrysocephala*, pigeons and slugs, none of the treatments survived beyond January 2016. However, during the early development of the crop, plots sown in association with a nurse crop mix comprising brassicas did reduce the level of *P. chrysocephala* feeding damage on the crop. Data presented here suggests that nurse cropping with companion brassicas has potential to reduce pest pressure in less severe years. The potential mechanisms of action and implications for development of alternative pest protection methods and Integrated Pest Management (IPM) are discussed.

2.2 Introduction:

Winter oilseed rape (OSR: *Brassica napus* L.) is an important break crop in northern European agricultural systems (Redman, 2019) and the most produced vegetable oil crop (Commission, 2020). Many factors influence decisions to grow or not, such as market value fluctuations, crop rotation and pest /disease pressure (AHDB). In recent years pest pressure from the cabbage stem flea beetle (*Psylliodes chrysocephala,* L.) has become a greater concern to UK OSR growers (Coston et al in prep, Chapter 9 this thesis). This is largely thought to be due to the EU ban on the use of neonicotinoid seed treatments (EU, 2018) and the increase of resistance to pyrethroid insecticides, the only other registered alternative chemical control currently approved for use against *P. chrysocephala* in OSR. Target site resistance has been reported from Denmark (Castberg and Kristensen, 2018; Højland and Kristensen, 2018), France (Bothorel *et al.*, 2018), Germany (Brandes and Heimbach, 2018; Heimbach and Brandes, 2016; Zimmer *et al.*, 2014) and the UK (Foster and Williamson, 2015) and a form of metabolic resistance also reported in Denmark (Højland and Kristensen, 2018; Castberg and Kristensen, 2018). Metabolic resistance has been highlighted in the UK with increases in resistance seen (Willis *et al.*, 2020). This is thought to have contributed to a reduction in the area of OSR grown, in the UK in particular (Nicholls, 2015; Zhang *et al.*, 2017; Scott and Bilsborrow, 2019; DEFRA, 2019), and has subsequent implications for crop rotations, as OSR is an important break crop from cereals in the arable rotation (Redman, 2019).

With growing concern on the environmental impacts of farming (Chagnon *et al.*, 2015; Goulson, 2013), the need for alternative and sustainable crop production methods is paramount with the sustainable use of synthetic chemicals being just one component of an integrated pest management (IPM) system (Nilsson, 2010). One area which has shown potential as a pest protection method either without, or with reduced synthetic chemical application, is increasing botanical diversity within the field (Ratnadass *et al.*, 2012). Intercropping is an example of this; defined as a system whereby the desired marketable crop is grown in conjunction with one or more other species, as a means of increasing diversity and exploiting the within-field ecology to benefit the desired crop (Costello, 1994; Hooks and Johnson, 2004; Vandermeer, 1992). In turn, biological control of pests has been shown to be influenced by intercropping through the increase of natural enemies active within the economic crop (Hooks and Johnson, 2004). The pest reductions seen in intercropping systems can be attributed to several phenomena as outlined by Finch and Collier (2012) as i, disrupting the pests ability to locate the crop, ii, act as an alternative host to the pest or iii, act as a repellent to the pest.

Intercropping has been extensively trialled with brassicas (Cadoux *et al.*, 2015; Hooks and Johnson, 2003; Lorin *et al.*, 2015; Lorin *et al.*, 2016; Theunissen, 1994; Verret *et al.*, 2017a), often with reductions in pest damage achieved compared to crop monoculture (Prasifka *et al.*, 2006; Baux and Schumacher, 2019; Brandsæter, Netland and Meadow, 1998; Gombac and Trdan, 2014; Hooks and Johnson, 2004). For example, reductions in the numbers of cabbage aphid, *Brevicoryne brassicae*, and the green peach aphid, *Myzus persicae,* have been reported in broccoli (*Brassica oleracea* var. *botrytis* L.) intercropped with legumes (Costello, 1994; Costello and Altieri, 1995; Cadoux *et al.*, 2015). For OSR, reductions in damage from the stem weevil, *Ceutorhynchus picitarsis,* have been recorded for intercropping with: (i) faba bean (*Vicia faba*) + lentil (*Lens culinaris*), (ii) grass pea (*Lathyrus sativus*) + fenugreek (*Trigonella foenum-graecum*) + lentil, and (iii) purple vetch (*Vicia* *benghalensis*) + common vetch (*Vicia sativa*) + berseem clover (*Trifolium alexandrinum*), when compared to a monoculture of OSR (Cadoux *et al.*, 2015). Although the levels of reduction were not significant, this study highlights the potential of using intercropping to protect *Brassica* crops from stem boring pests like *P. chrysocephala.*

One major drawback of intercropping systems is the potential impact of interspecific competition between the companion crop and the main economic crop (Carof *et al.*, 2007; Verret *et al.*, 2017b). This can be mitigated by using frost-sensitive companion plants, that will be destroyed over winter by frost damage or, if necessary, by the application of a specific herbicide which only kills the companion species so that it is no longer present during the main crop's most important growing period (Cadoux *et al.*, 2015). Such companion planting of OSR with frost-sensitive legumes, cultivated through the autumn only and either killed off by frost or from spraying with a specific herbicide, has been shown to reduce the levels of damage from *C. picitarsis*, although not to significant levels (Ruck, Cadoux and Robert, 2018; Cadoux *et al.*, 2015). In a follow up report, (Ruck, Cadoux and Robert, 2018) showed that the number of *P. chrysocephala* larvae in OSR stems was reduced as the biomass of legume nurse plants increased. The process of winter die-off or by herbicide use means that the companion species cannot be considered as an intercrop as the companion species are only present during the period of high pest pressure and are then removed and not harvested. The term 'nurse' crop is used, as the hypothesis is to protect the crop specifically from autumn pest damage therefore acting as a 'nurse' to the main economic crop.

Another agronomic option to mitigate damage from *P. chrysocephala* is to increase the seed rate, whereby the extra seed act to dilute the activity of *P. chrysocephala* over a greater total number of plants (AHDB, 2019a). The planting rate of the OSR crop can have a significant impact on the crop productivity (Momoh *et al.*, 2004), with recommended seed application of 30seeds/m^2 suggested to improve yield by £29/Ha (Roques and Berry, 2016). Although reductions in yield as seed rate increases have been reported (Roques and Berry, 2016; Shah *et al.*, 2014; Momoh and Zhou, 2001), current advice is to increase the seed rate to compensate for expected losses due to *P. chrysocephala* (AHDB, 2019b; BASF, 2018), however, there is no qualitative data or published evidence to support this advice.

2.2.1 Aims of study:

I aimed to assess the impact of OSR seed rate and the efficacy of using frost-sensitive nurse plants as part of a modified companion planting system to mitigate *P. chrysocephala* damage in a replicated field experiment conducted in collaboration with NIAB, Cambridge, UK at a field site near Duxford. I hypothesised that:

(i) Levels of damage will be lower on OSR associated with a nurse crop due either to reduced host plant location, reduced acceptance (Finch and Kienegger, 1997b) or by ‘’trap crop’’ effects (Finch and Collier, 2012; Barari *et al.*, 2005).

(ii) Levels of damage will be reduced as OSR seed rate increases due to dilution effect

Three distinct types of nurse crop mixes were tested, based on commercially available cover crop mixtures and the plant species commonly found in them (see Methods, Table 1). Each was grown with the OSR, which was itself sown at four seed rates, two ‘standard’ rates (60, and 80, seeds/m^{2}and two increased rates (100 and 120 seeds/m^2). Levels of *P. chrysocephala* injury to the OSR and nurse species were assessed twice in the autumn, along with a measure of the percentage survivorship of the nurse plants and crop plants. Using these data, I build on knowledge of nurse cropping systems for OSR and highlight avenues for further research into cultural control methods for protecting OSR from *P. chrysocephala*.

2.3 Methods:

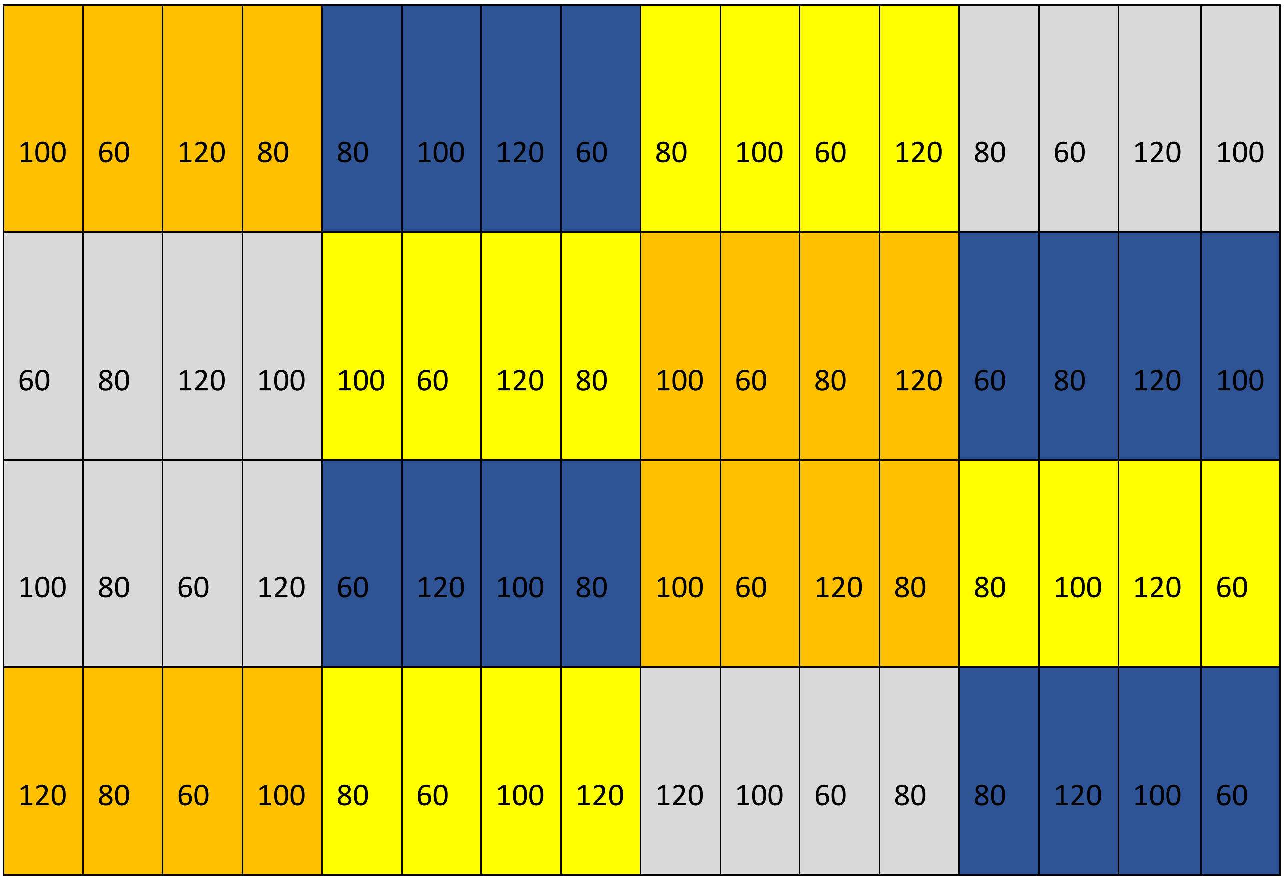
2.3.1 Treatments and experimental layout:

Data for this study was collected from an already established NIAB trial at Duxford (Lordship Farm, Hinxton, Cambridgeshire), which was on light, sandy soil. Field trials were designed and implemented by NIAB in autumn 2015. The experiment was designed in a randomized block, with four treatment mixtures (Figure 1). Each treatment was sown in a plot (nurse type) split into four sub-plots of OSR cv Charger, sown at four seed rates: 60, 80, 100 and 120seeds/m^2 representing two relatively standard seed rates and two higher rates (Figure 1), i.e., plot level is nurse crop mix and sup-plot the OSR seed application rate. Seeds were supplied by Kings seeds (https://www.kingsseeds.com/Home). Plots were 12 x 8m and subplots were 12m x 2m and were separated by a tractor wheeling lines along all sides, 1m (Figure 1).

Plots were drilled on 08th September 2015. The nurse crop mix was applied using the same drill but with coulters raised to allow seed to land on the soil surface. The plots were then rolled, to distribute and compact the seed mix; this also helps to conserve moisture. The aim was to apply the OSR and nurse mixtures using standard farm machinery to simulate a drilled crop with a broadcast sown nurse mixture. The resulting plots of OSR alone (control) and OSR with the nurse crops are shown in Figure *4*2 and Figure *3*3.

Table 1. Nurse crop mix composition. Grown in companion with oilseed rape (*Brassica napus*) at potential pest control measures for the cabbage stem flea beetle (*Psylliodes chrysocephala*). Details given of species and cultivar where available and the seed application rate in seeds/m^2.

Seed mix code	Species	Seed application rate (m^2)
A	Fenugreek (*Trigonella foenum-graecum*)	300
B	Pak Choi (*Brassica rapa var. chinensis*) cv. Joi Choi	75
	Chinese Cabbage (*Brassica rapa subsp. Pekinensis* Lour.)	75
	Salad rocket (*Eruca sativa*)	150
	Linseed (*Linum usitatissimum*) cv. Abacus	150
C	Common vetch (*Vicia sitiva*)	45
	Crimson clover (*Trifolium incarnatum*)	150
	Berseem clover (*Trigolium alexandrinum*)	150
	Persian clover (*Trifolium resupinatum*) cv. lightning	150
D	Oilseed rape (*Brassica napus*) cv. charger	60, 80, 100 or 120



100	60	120	80	80	100	120	60	80	100	60	120	80	60	120	100
60	80	120	100	100	60	120	80	100	60	80	120	60	80	120	100
100	80	60	120	60	120	100	80	100	60	120	80	80	100	120	60
120	80	60	100	80	60	100	120	120	100	60	80	80	120	100	60

Figure 1. Schematic of the field experiment used to test the effects of different nurse crop mixtures and crop seed rate on *Psylliodes chrysocephala* damage to oilseed rape. Positions of each treatment in the randomized block structure. Colour denotes plot: Blue – Mix A (Fenugreek: *Trigonella foenum-graecum*), Orange – Mix B (Pak Choi: *Brassica rapa var. chinensis* cv. Joi Choi, Chinese Cabbage: *Brassica rapa subsp. Pekinensis* Lour, Salad rocket: *Eruca sativa,* and Linseed: *Linum usitatissimum* cv. Abacus), Grey – Mix C (Common vetch: *Vicia sitiva*, Crimson clover: *Trifolium incarnatum*, Berseem clover: *Trigolium alexandrinum*, and Persian clover: *Trifolium resupinatum* cv. lightning) and Yellow – Mix D (OSR: *Brassica napus* cv. charger monoculture) and numbers show the OSR seed rate in sub-plots. Each sub-plot was 2m x 12m with a tractor wheeling line on all sides.

2.2.2 Assessment of plant density:

The establishment success of both the OSR crop and the nurse crop species was assessed once (26-27th October 2015) using 0.25 m^2 quadrats. The total number of crop plants and each of the nurse crop species was recorded from four randomly placed quadrats per plot (Figure 2). These values were combined to give a measure for plant establishment per $1m^2$.

Due to the difficulty of discriminating between the three species of clover (mix C,Table 1) at early growth stages they were recorded collectively (‘clover’).

Values for plant establishment for each species were calculated using the following formula:

$$\textit{(species total plants in the 4 quadrats/ seed rate per } m^2 \textit{ applied) X 100.}$$

2.2.3 Assessment of leaf area damage by adult *Psylliodes chrysocephala*:

Plant damage resulting from adult *P. chrysocephala* feeding was assessed twice on 26-27th October (average GS= 12-15) and 24th November 2015 (average GS= 13-19). Within each plot, 10 plants of OSR and five plants for each nurse crop species (clover scored collectively) were scored to the nearest 5% of leaf area lost (Figure 8). Injury was measured as a percentage leaf loss and estimated by eye and calibrated by consensus of field survey team (Sam. Cook, Martin. Torrence, and Trish. Wells), with 10 plants being assessed as a group to form a relative measure of injury. Adult feeding of *P. chrysocephala* can be identified as they produce circular holes in the leaf, which form characteristic ‘shot holing’ patterns (Figure 8).

However, damage levels were high and could not always be attributed unequivocally to *P. chrysocephala* because slug and pigeon damage are likely contributing factors. The growth stage of each OSR plant assessed was also recorded according to the BBCH scale (Lancashire *et al.*, 1991).

2.2.4 *Psylliodes chrysocephala* larval infestation:

Comparisons between *P. chrysocephala* larval infestation of OSR in each treatment was planned. However, poor crop survival meant that this was not possible.

2.2.5 Crop yield:

Comparisons of crop yield attributes between treatments were planned but assessments were not possible due to the total loss of the OSR crop in the experimental area. This led to the trial being terminated in January 2016.

2.2.6 Statistical analysis:

Crop density was was analysed using a generalized linear mixed model due to the treatment layout and assuming a binomial distribution from the proportion of seed survival to plant at time of assessments. A mixed model was used due to the split-plot design of the experiment.

Differences between treatments in crop growth stage and leaf area damage at the two assessment dates were analysed using analysis of variance (ANOVA) as a split plot treatment design.

2.3 Results:

2.3.1 Plant density:

Total plant density (companion and OSR plants combined) did increase in the mixtures over OSR monoculture ($F_{273.35,9}$=91.21; *P* <0.001) with higher plant density seen in treatment B > C > A and lowest in D, confirming germinations of the nurse species (Figure 2). The density of OSR crop plants was not affected by nurse crop mix type ($F_{1.94,\ 9}$ =0.65; p=0.605, Table 2 and Figure 3). However, there was an effect at sub-plot level with increasing OSR plant density as OSR seed rate increased ($F_{65.32,\ 35.9}$ =21.77; p<0.001, Table 2 and Figure 4). There was no interaction between mix type and seed rate ($F_{8.9,\ 35.9}$ F=0.99; P=0.466, Table 2).

Table 2. Oilseed rape (*Brassica napus,* OSR) mean plant density 48 days after drilling when grown with nurse crop mix at four seed rates (60,80,100 and120seeds/m^2). Treatment codes; A = Fenugreek *(Trigonella foenum-graecum);* B = Brassica *mix (Chi*nese cabbage *(Brassica rapa subsp. Pekinensis (Lour.) Rupr),* Pak Choi *(Brassica rapa var. chinensis), Salad rocket (Eruca sativa) and Linseed (Linum usitatissimum*); C = Legume mix (Common vetch *(Vicia sitiva),* Crimson clover *(Trifolium incarnatum),* Berseem clover *(Trifolium alexandrinum)* and Persian clover *(Trifolium resupinatum*); D = Oilseed rape crop monoculture control. Standard error of differences given in brackets, approximate average standard error of differences: 1.546 (calculated on variance scale). No statistically significant difference observed between nurse mix (rows), increasing OSR seed rate significantly increased OSR density (column).

	Nurse mix treatment			
OSR seed rate	A	B	C	D
60	6.062 (1.306)	5.625 (1.306)	6.375 (1.306)	4.875 (1.306)
80	8.964 (1.285)	5.750 (1.306)	6.562 (1.306)	7.312 (1.306)
100	8.375 (1.306)	8.062 (1.306)	10.437 (1.306)	9.500 (1.306)
120	10.500 (1.306)	9.687 (1.306)	11.625 (1.306)	10.875 (1.306)

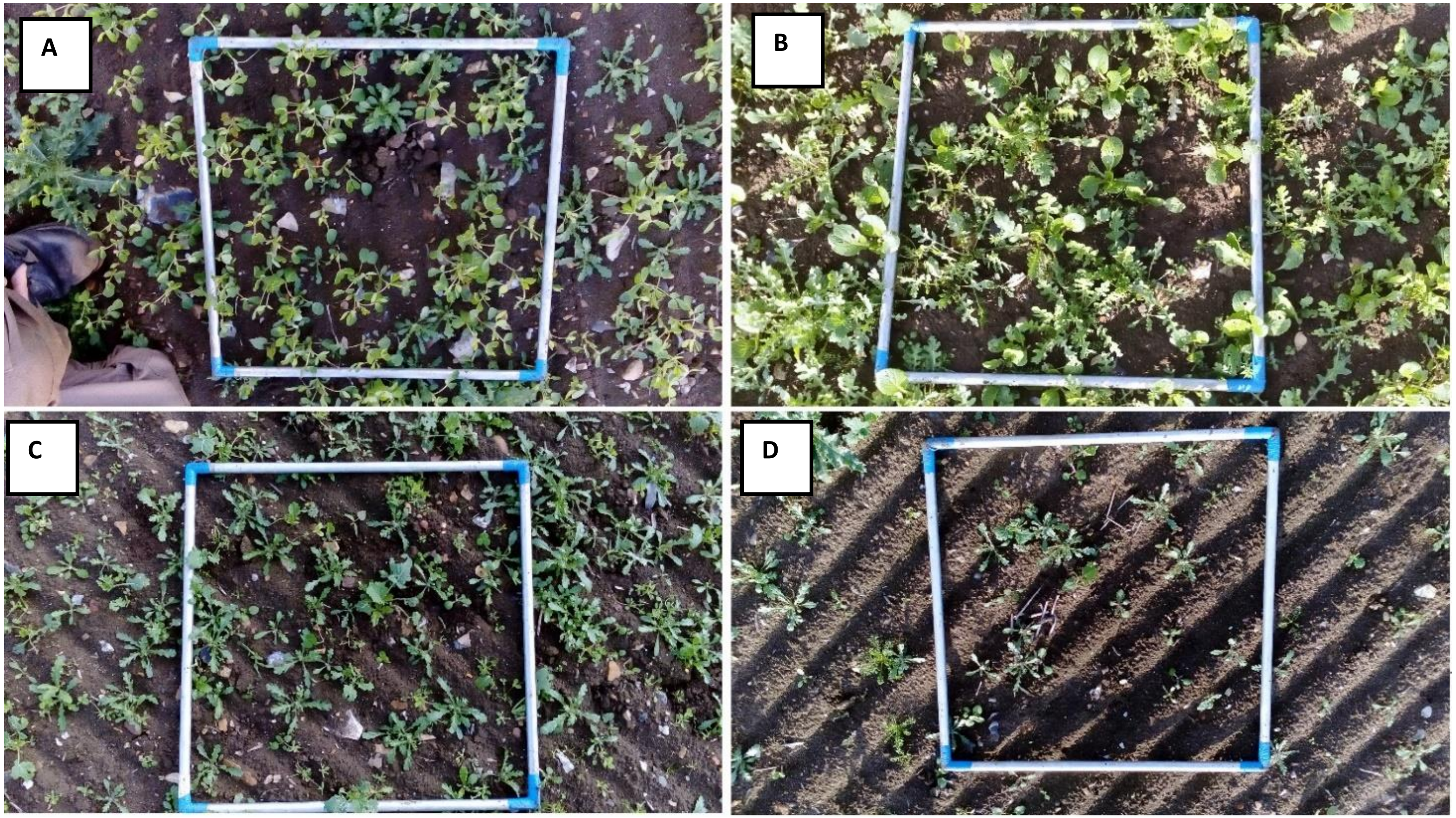


Figure 2. Representation of plant establishment in plots of oilseed rape (*Brassica napus*) with different ‘nurse’ companion crops, showing one randomly selected quadrat per treatment (plant density in four quadrats were assessed per plot). A = Fenugreek *(Trigonella foenum-graecum*); B = Brassica mix (Chinese cabbage *(Brassica rapa subsp. Pekinensis (Lour.) Rupr*), Pak Choi *(Brassica rapa var. chinensis),* Salad rocket *(Eruca sativa)* and Linseed (*Linum usitatissimum*); C = Legume mix *(*Common vetch *(Vicia sitiva),* Crimson clover *(Trifolium incarnatum),* Berseem clover (*Trifolium alexandrinum*) and Persian clover (*Trifolium resupinatum*); D = oilseed rape crop monoculture control. All images of plots with oilseed rape at 100 seeds/ m2. Photographs taken 26/10/2015.

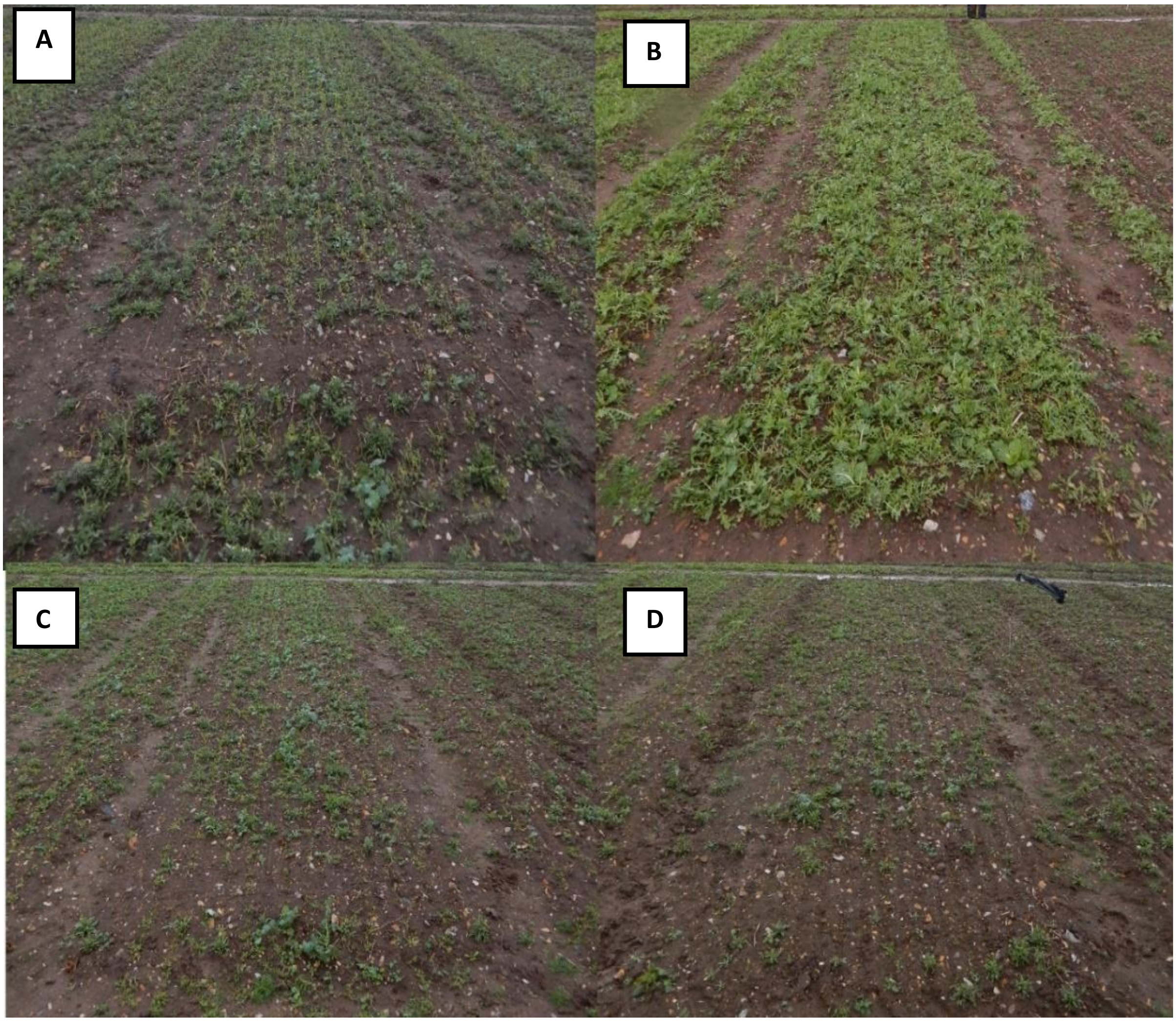


Figure 3. Full plot photographs of oilseed rape (*Brassica napus*) with different ‘nurse’ companion crops mixtures: Treatment A = Fenugreek *(Trigonella foenum-graecum*); B = Brassica mix *(Pak Choi (Brassica rapa var. chinensis),* Chinese cabbage *(Brassica rapa subsp. Pekinensis (Lour.) Rupr),* Salad rocket *(Eruca sativa*) and Linseed *(Linum usitatissimum*); C = Legume mix *(Common vetch (Vicia sitiva),* Crimson clover *(Trifolium incarnatum),* Berseem clover *(Trifolium alexandrinum)* and Persian clover *(Trifolium resupinatum);* D = Oilseed rape crop monoculture. All are plots sown at 120 oilseed rape seeds/m^2. Images taken on 24/11/2015, 7 weeks post drilling.

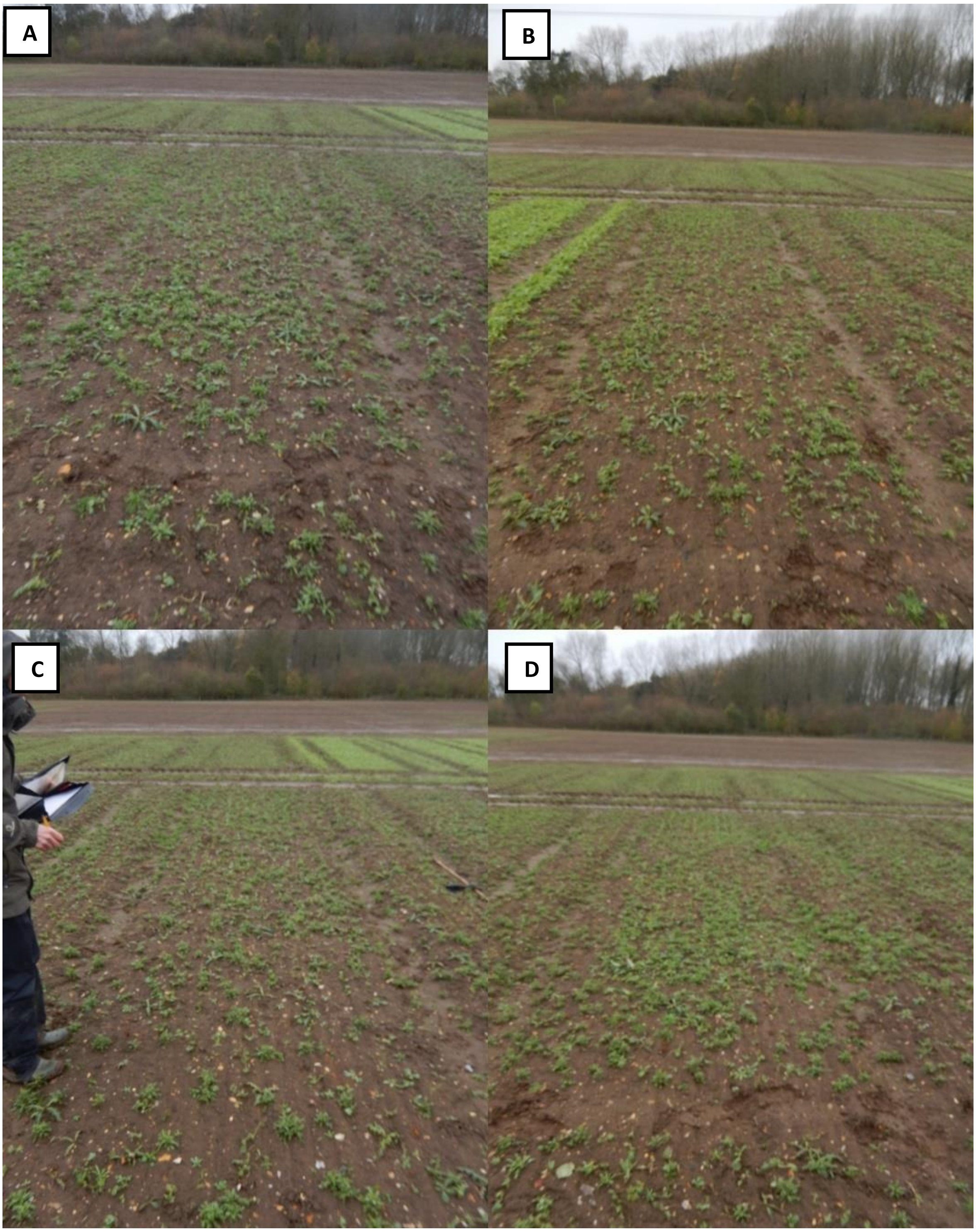


Figure 4. Oilseed rape (*Brassica napus*) monoculture plots (Mix D) showing the four seed rates applied: A. 60, B. 80, C. 100, and D. 120 seed/m^2. Images taken on 24/11/2015, 7 weeks post drilling.

2.3.2 Crop growth stage:

When crop growth was assessed in October all plants were at GS 13 to GS 14; there was no statistically significant difference between nurse crop mix type ($F_{3, 9}$ 1.06; p=0.412) or OSR seed rate ($F_{3, 9}$ 0.72; p= 0.549) and there was no interaction ($F_{9, 36}$ 1.09 P=0.394). There was more evidence of a treatment effect in the November assessment, with OSR plants in treatment B (the Brassica species nurse mix) being at less developed growth stage than those in other treatments, although this was not statistically significant ($F_{3, 9}$ =3.44, p=0.065, Table 3).

Table 3 Oilseed rape (*Brassica napus*) mean growth stage (BBCH scale (Lancashire *et al.*, 1991)) for October (26/27/10/2015) and November (24/11/2015) in plots with different OSR seed rates and nurse crop mixtures: **A =** Fenugreek (*Trigonella foenum-graecum*); **B =** Brassica mix (Chinese cabbage (*Brassica rapa subsp. Pekinensis* (Lour.) Rupr), Pak Choi (*Brassica rapa var. chinensis*), Salad rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum*); **C =** Legume mix (Common vetch (*Vicia sitiva*), Crimson clover (*Trifolium incarnatum*), Berseem clover (*Trifolium alexandrinum*) and Persian clover (*Trifolium resupinatum*); **D =** Monoculture oilseed rape crop control. No statistically significant difference observed.

	A - Fenugreek		**B** - Brassica		**C** – Legume		**D** - Monoculture	
OSR seed rate	Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov
60	13.7	16.2	13.6	15.6	13.7	16.3	13.7	16.1
80	13.7	16.2	13.4	15.7	13.6	16.3	13.6	15.9
100	13.8	16.2	13.6	15.5	13.6	16.2	13.7	15.8
120	13.5	16.0	13.5	15.5	13.8	16.5	13.6	16.2

2.3.3 Crop injury:

A high level of damage was observed on the OSR plants for all nurse crop treatments and all OSR seed rates (Figure 5). There was a significant effect of nurse crop mix B in which a reduction of the percent leaf area loss was observed for OSR plants assessed in October ($F_{3,9}$ =11.48, p=0.002) and November ($F_{3,9}$ =13.28, p=0.001). Crop damage with mixtures A and C was comparable to controls (Figure 6 and Figure 7).

There was also a significant effect of OSR seed rate on damage observed in October ($F_{3,36}$ = 3.07, p= 0.040) with reduced damage as seed rate increased (Figure 6), however, this was not apparent in the November assessment ($F_{3,36}$ = 0.42, p= 0.737; Figure 7). There was no significant interaction between nurse crop mix type and OSR seed rate for either month (October= $F_{9,36}$ = 1.59, p=0.155, November= $F_{9,36}$ = 0.76, p=0.657).

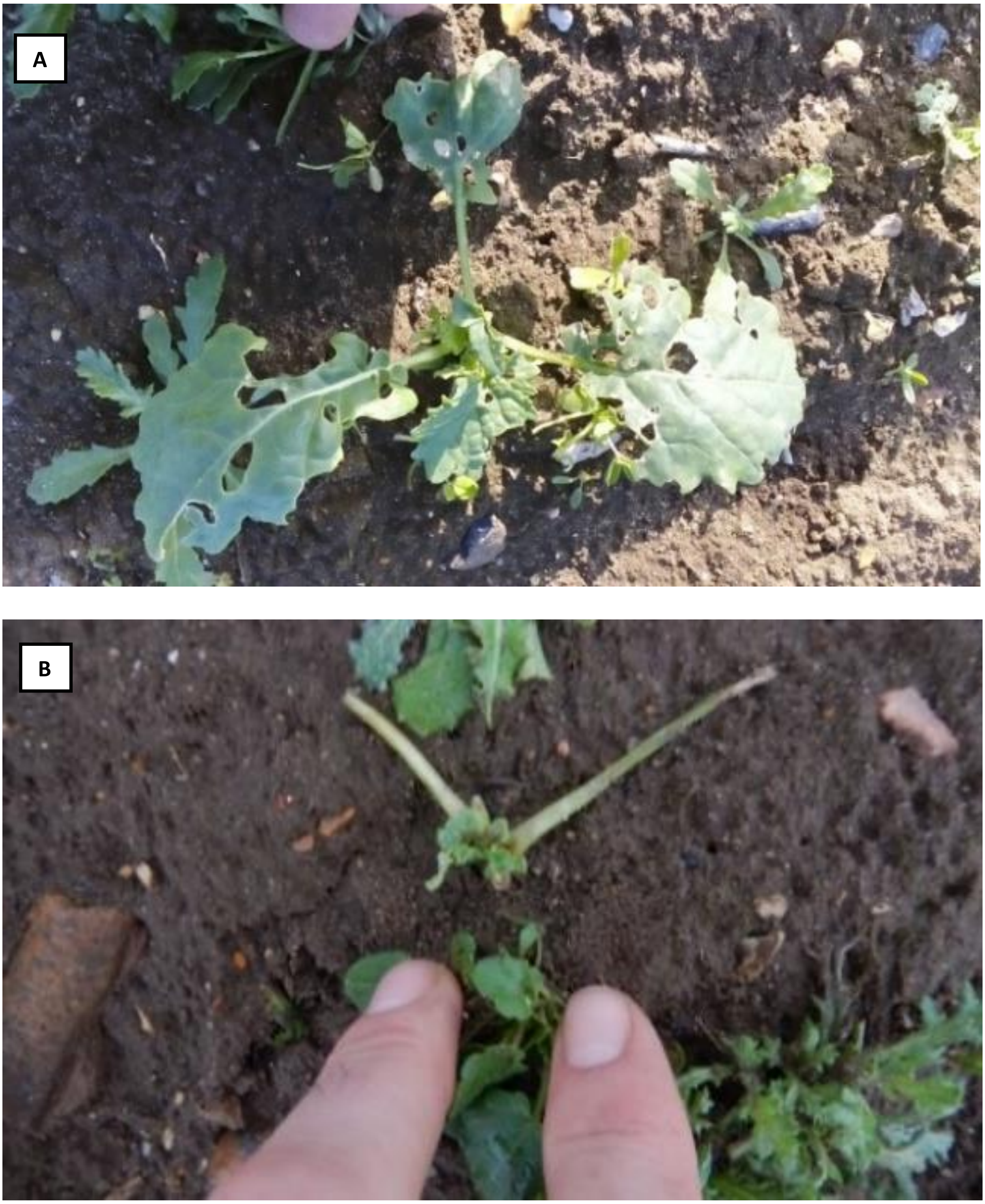


Figure 5 Representative photographs of leaf area injury seen on oilseed rape (*Brassica napus*) showing signs of shot-hole feeding injury, indicative of *Psylliodes chrysocephala* (A) and high levels of leaf area loss (B). Photographs taken: 24/11/2015.

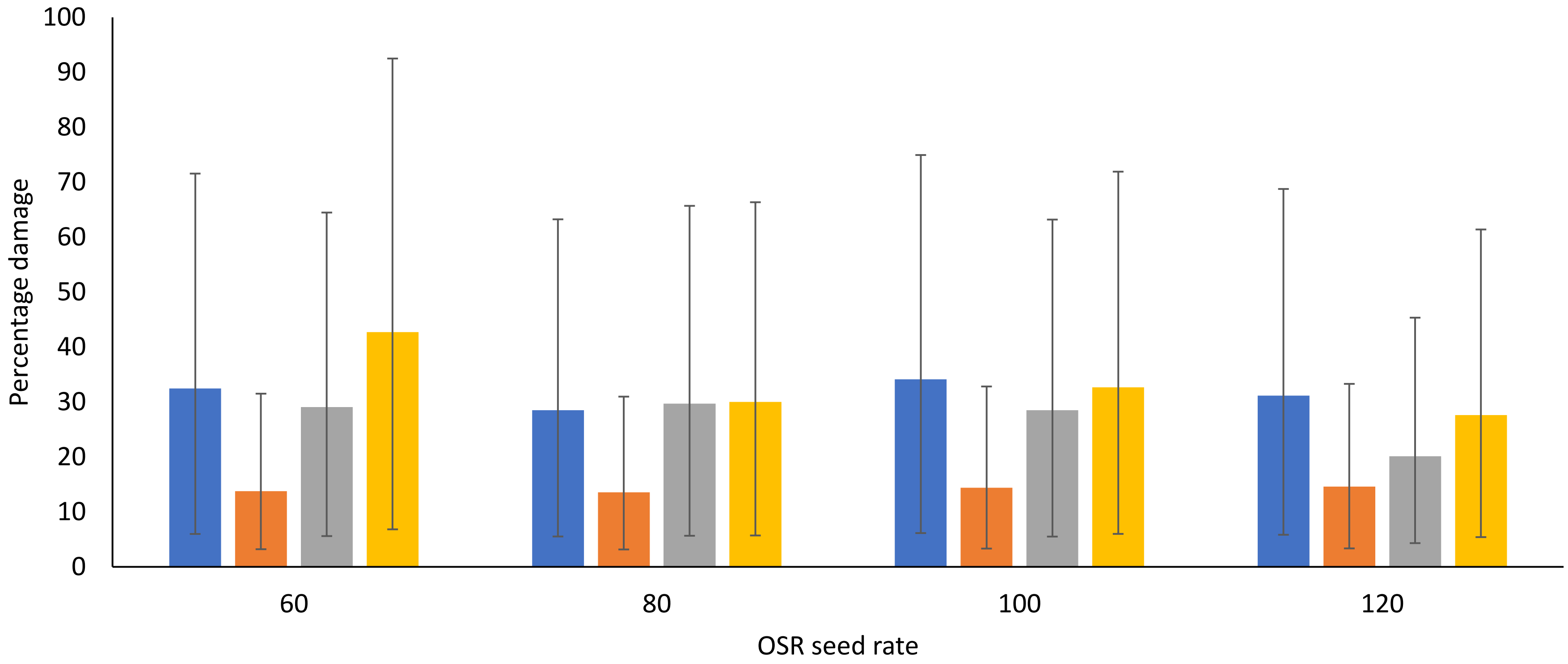


Figure 6. October leaf area injury recorded on oilseed rape (OSR, *Brassica napus*) as percentage leaf area lost. Grown with four 'nurse crop' mixtures and using four OSR seed rates. Treatment; Blue = Fenugreek *(Trigonella foenum-graecum);* Orange = Brassica *mix (Chi*nese cabbage *(Brassica rapa subsp. Pekinensis (Lour.) Rupr),* Pak Choi *(Brassica rapa var. chinensis), Salad rocket (Eruca sativa) and Linseed (Linum usitatissimum*); Grey = Legume mix (Common vetch *(Vicia sitiva),* Crimson clover *(Trifolium incarnatum),* Berseem clover *(Trifolium alexandrinum)* and Persian clover *(Trifolium resupinatum*); Yellow = Oilseed rape crop monoculture control. Bars show 95% CI.

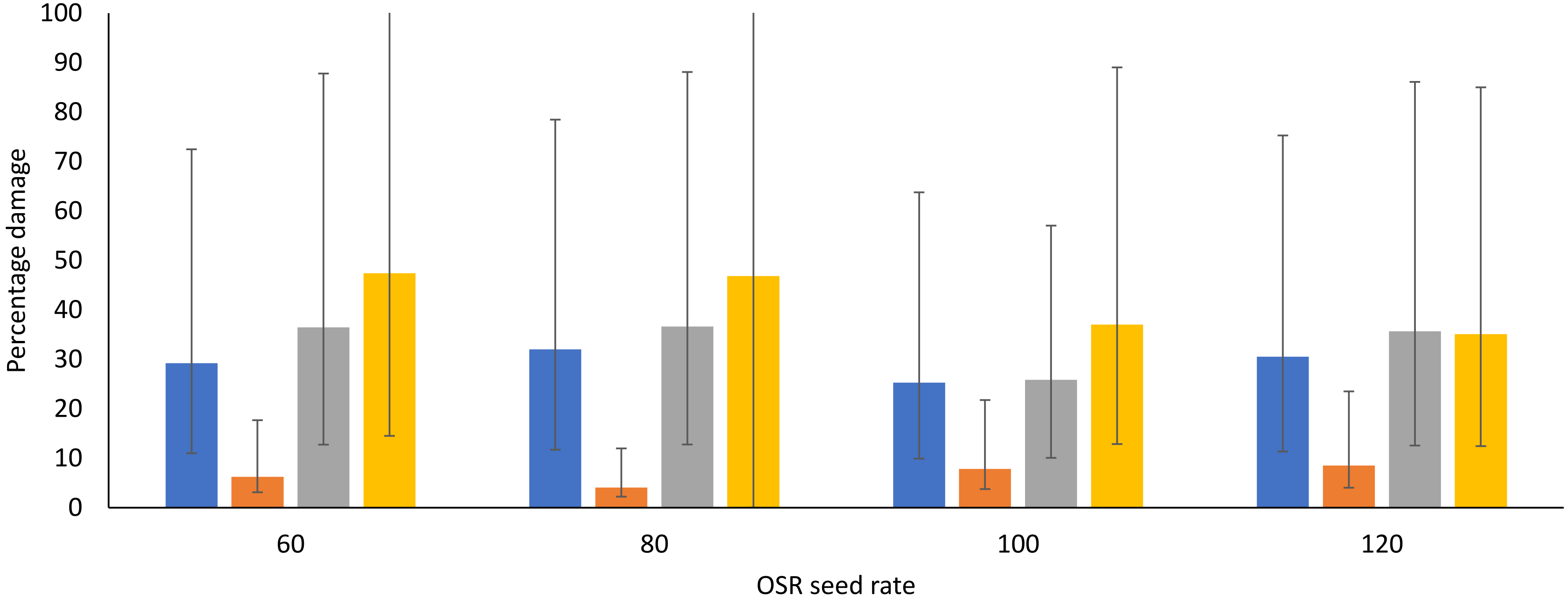


Figure 7. November leaf area injury recorded on oilseed rape (OSR, *Brassica napus*) as percentage leaf area lost. Grown with four 'nurse crop' mixtures and using four OSR seed rates. Treatment; Blue = Fenugreek *(Trigonella foenum-graecum);* Orange = Brassica *mix (Chi*nese cabbage *(Brassica rapa subsp. Pekinensis (Lour.) Rupr),* Pak Choi *(Brassica rapa var. chinensis), Salad rocket (Eruca sativa) and Linseed (Linum usitatissimum*); Grey = Legume mix (Common vetch *(Vicia sitiva),* Crimson clover *(Trifolium incarnatum),* Berseem clover *(Trifolium alexandrinum)* and Persian clover *(Trifolium resupinatum*); Yellow = Oilseed rape crop monoculture control. Bars show 95% CI.

2.3.4 Nurse crop injury:

Levels of *P. chrysocephala* feeding injury on the non-brassica nurse crop species was either not observed or was negligible. Injury was observed on just three fenugreek plants (n=160) and only at 5% area injury. Leaf injury was only observed rarely on clover (22/160 plants) and Vetch (16/160 plants). The only nurse crop plants to be injured frequently by *P. chrysocephala* were the Pac Choi and Chinese Cabbage in mix B, which had levels of injury akin to the OSR (Figure *8*8 and Figure *9*9). Both the Pac Choi and Chinese Cabbage had lower injury levels than the OSR crop plants in October ($F_{2,295}$=11.95, $P < 0.001$) but higher levels in the November sample ($F_{2,296}$=52.32, $P < 0.001$). There was no effect of seed rate in either month (October: $F_{3,295}$=0.14, $P = 0.931$, November: $F_{3,296}$=0.91, $P = 0.474$). The linseed in this mix was not observed to be injured at either month. Salad rocket was observed to be injured by *P. chrysocephala* but only to low levels with only four times the injury covered more than 15% of leaf area.

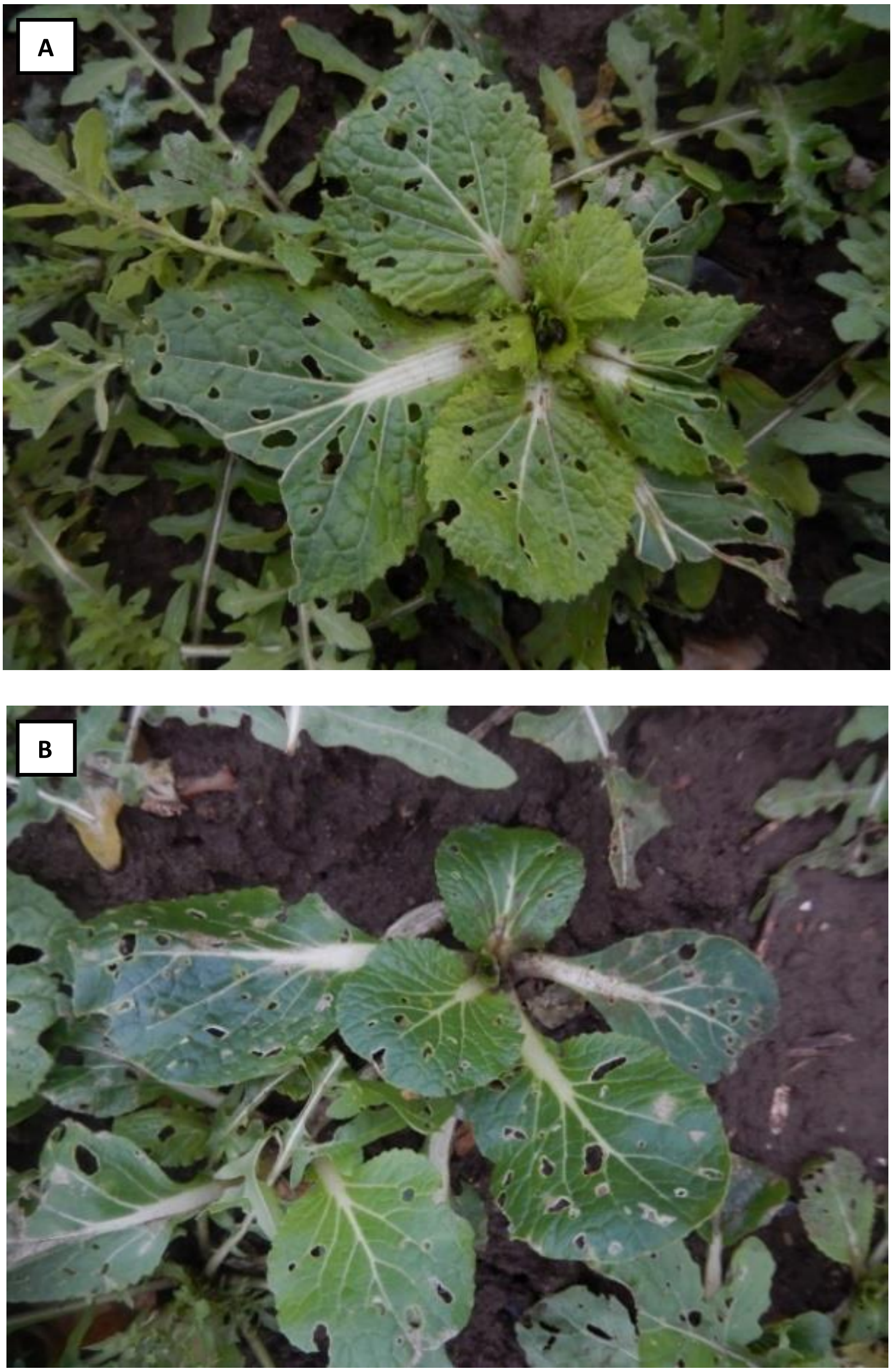


Figure 8. Representative photographs of leaf area injury from *Psylliodes chrysocephala* present on A, Chinese cabbage (*Brassica rapa subsp. Pekinensis (Lour.) Rupr*) and B, Pac Choi *(Brassica rapa var. chinensis*). Photographs taken: 24/11/2015.

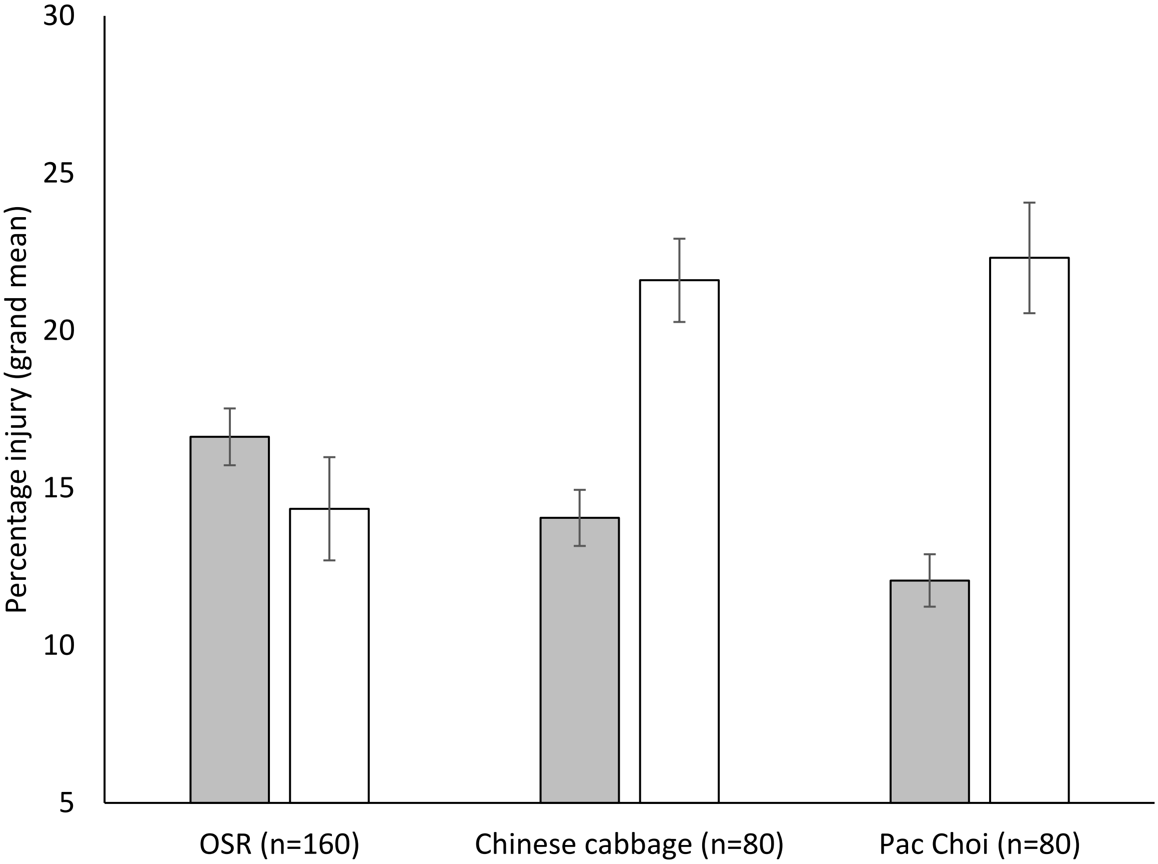


Figure 9. Percentage leaf area injury observed in on Oilseed rape (OSR, *Brassica napus*), Chinese cabbage and Pac Choi from Mix B (Brassica mix: (Chinese cabbage *(Brassica rapa subsp. Pekinensis* (Lour.) Rupr), Pak Choi *(Brassica rapa var. chinensis*), Salad rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum*) showing injury attributed to *Psylliodes chrysocephala*. Data shown is grand mean of all plants measured in October (**grey**) and November (**clear**). Sample number and standard error of difference of mean shown.

2.4 Discussion:

In the autumn/ winter of 2015-16 the abundance of *Psylliodes chrysocephala* was very high resulting in extremely high feeding injury (Figure 6 and Figure 7). This led to the abandonment of this trial in January 2016 as all the OSR within the field was destroyed. However, data collected during crop establishment and early crop growth did show that increasing in-field plant diversity reduced feeding injury from *P. chrysocephala* (Figure 6), supporting hypothesis (i) – but the composition of the nurse crop mixtures was found to be important in realising this effect. Increasing seed rate did show reductions in the levels of injury to OSR plants supporting hypothesis (ii).

Levels of injury to the OSR was only significantly reduced compared to the control in Mix B (Pack choi, Chinese cabbage, salad rocket and linseed). This effect was persistent and was observed in both the October and November assessments (Figure 6 and Figure 7). This shows that there is potential for feeding injury on the crop to be diluted in the early growth phase when grown in combination with other brassicas. The reduction of injury to OSR was apparent during the early growth stages, although the level per plant was higher in the OSR than the brassica companion plants (October assessment, Figure 9). The distribution of injury was reversed later in the season with the Chinese Cabbage and Pac Chois both receiving more feeding injury than the OSR suggesting they may be a preferred forage source at later growth stages and the potential to act as trap crop (Shelton and Nault, 2004). High levels of feeding observed on the Chinese Cabbage and Pac Choi (Figure 9). Showing potential of both Chinese Cabbage and Pac Choi to act as a suitable trap crop under the definitions of Finch and Collier (2012) as a suitable host plant.

No injury was recorded on the linseed and little on the salad rocket suggesting they are not palatable to *P. chrysocephala.* The reductions on OSR plant leaf injury observed here may partly be due to dilution of the crop amongst other suitable host plants as was seen when the OSR seed rate is increased the level of injury per plant reduces. The level of injury per plant when in combination of a diverse brassica mix is greater than the increase in seed number along. This apparent extra effect from diverse brassica mix however, may be conveying secondary benefits from increased green surface area. As the other mixtures did not show any reduction in the injury on OSR the dilution of feeding is more likely than inherent benefits from increased green surface area. White *et al,.* (2020) reports of similar reductions of *P. chrysocephala* injury to the OSR crop when grown with mustard (*Sinapis sp*) and berseem clover (*Trifolium alexandrinum*).

Fenugreek was tested as a potential nurse crop as it has a very pungent odor (Leela and Shafeekh, 2008); it was postulated that this could mask host plant location and/or repel or deter P chrysocephala infestation. There was no evidence for this, with no reductions in feeding observed in this treatment compared to the control (Figure 6 and Figure 7). The presence of host masking volatile chemicals is unlikely to influence host location in a specialist forager (Finch and Collier, 2012). *Psylliodes chrysocephala* is a crucifer specialist and has thus evolved specific chemo receptors to detect volatile chemicals from host plants (Bartlet *et al.*, 1999) and receptors for non-host volatiles would be limited (Visser, 1983). There is no reason for them to have evolved receptors for fenugreek volatiles and it would be unlikely for the fenugreek to alter the chemical composition or volume of other plant species (Finch and Collier, 2012; Bruce and Pickett, 2011). This does not preclude secondary factors from fenugreek influencing *P. chrysocephala* host location, such as increased green surface area reducing the visual cues for landing when entering a crop. The increase in the green surface index has been suggested as a mechanism behind the success of some intercrop mixtures, and not through chemical repellent or masking effects (Finch and Collier, 2012).

Legumes were present in two of the treatments in this study (Mix A and C) and showed no direct effect on the levels of *P. chrysocephala* feeding; the levels of feeding injury on the OSR in these treatments was comparable to controls (Figure 6 and Figure 7). Clover has been shown to reduce injury from *P. chrysocephala* when cover reached 200g/m2 (Ruck, Cadoux and Robert, 2018; Cadoux *et al.*, 2015). The biomass of the nurse mixtures were not recorded in this study and it is possible that it was insufficient to reduce injury as seen by (Finch and Kienegger, 1997a). Who showed reductions in multiple insect pests in OSR when clover covered >50 of the “vertical profile of the crop plant”. Postulating the lack of contrast between plants and soil affecting the insects host location ability.

Recommendations from AHDB to combat *P. chrysocephala* pressure include increasing seed rate in areas prone to high *P. chrysocephala* pressure (AHDB, 2019a). I found evidence to support this strategy; reductions in the level of *P. chrysocephala* feeding per plant was observed as seed rate increased in October (Figure 6) but the effect was transient; by the time of the November assessment the levels of damage were not influenced by plant density (Figure 7). The reductions in adult feeding observed in October is likely to be due to a dilution effect i.e., with increased host plant abundance the amount of *P. chrysocephala* feeding per plant will be diluted. Or the higher green cover at higher seed rate disrupting the host location by reducing contrast with soil (Finch and Collier, 2012). The lack of any effect by the November assessment may be due to a leveling off between seed rates of green surface cover as plants at lower density develop and cover more area. The reduced difference may also be due to high abundance of *P. chrysocephala* at the site increasing the levels of feeding observed. In a recent report by White *et al* (2020) the impact of seed rate was shown to be variable but did suggest a trend towards reductions in *P. chrysocephala* injury at higher plant density.

Increases in green cover have been shown to reduce pest landing by disrupting landing stimuli (Crawley, 1983). If this were the case for *P. chrysocephala* here, then reductions in feeding would be expected with increased green cover. Green cover was increased in the mixture plots over monoculture (Figure 3) and as OSR seed rate increased (Figure 4), but no reduction of injury was seen in any other then the Brassica mix (Figure 6 and Figure 7). This suggests that host location was not influenced by plant density alone in terms of green surface cover but by brassica density.

Cultivation of monoculture crops has become the norm for almost all arable crops grown worldwide, however, there is increasing evidence to support the hypothesis that increased botanical diversity within the crop is not only financially viable for the farmer but is also of great importance to improving within field biodiversity (Ratnadass *et al.*, 2012). In a survey of farmer opinions to increased floral diversity in Switzerland, the uptake of methods such as intercropping was higher where knowledge of their benefits was present (Baux and Schumacher, 2019). In a similar survey of UK OSR growers, the use of nurse cropping was considered to have potential, but growers lack proof of effectiveness (Coston et al 2021, Chapter 8 this thesis). Here the potential of nurse cropping in OSR with Brassicas to reduce early growth injury and promote crop establishment shows promise but is in need of further investigation to understand the mechanisms of success.

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3.0 Potential of Brassica trap crops for protecting winter oilseed rape (*Brassica napus* L.) from *Psylliodes chrysocephala* feeding:

3.1 Abstract:

In recent years pressure on oilseed rape (OSR, *Brassica napus* L.) production has increased as the result of a key pest, the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) developing resistance to pyrethroid insecticides, and the current EU ban on the use of neonicotinoid outdoor use. There is therefore an urgent need for further research into alternative pest protection methods. This study assesses the potential of two Brassica species as trap crops, and their efficacy as a pest protection method for reducing the pressure from *P. chrysocephala* on OSR. The relative effect of neonicotinoid seed dressing was also tested. In the year of the experiment *P. chrysocephala* pressure was so high it completely eradicated the trial, however, both trap crops tested survived (with and without neonicotinoid seed treatment) and data presented here suggests that both show potential as a trap crop for *P. chrysocephala*. The neonicotinoids seed treatment did not improve the survival of OSR. Implications for autumn pest control are discussed.

3.2 Introduction:

Since 2014 the use of neonicotinoid seed dressings has been restricted in OSR with a complete ban of outside use imposed in 2018 (EU, 2018). Pest management of *P. chrysocephala* is becoming particularly problematic for OSR growers, (EU 2018) and that which efficacy is under questing with increasing resistance in field populations of the beetles (i.e., the pyrethroids Brandes and Heimbach 2018, Foster and Williamson 2015, Castberg and Kristensen 2018). The reduction in control options has led to a renewed research effort into alternative non-chemically based methods of pest control.

One such method, which shows potential, is 'trap cropping', a system whereby a plant species which is more attractive to the pest than the crop, is grown alongside the crop in order to act as a trap for pests, reducing direct feeding and subsequent larval infestation in the main crop (Barari *et al.*, 2005; Hokkanen, 1991; Shelton and Badenes-Pérez, 2006; White, Ellis and Kendall, 2018). Trap crops may also influence the levels of pest species by providing valuable resources to natural enemies such as nectar, alternative prey, shelter and non-crop habitat (Skellern and Cook, 2018).

Several studies have investigated the effectiveness of trap crops in high value vegetable brassicas. For example, it has been suggested that trap cropping could be employed to protect cabbage from the cabbage flea beetle (*Phyllotreta* spp.) and therefore reduce the need for insecticide application (Bohinc and Trdan, 2012). Several studies have shown the potential of trap crops in managing diamondback moth (*Plutella xylostella*) in cabbage and cauliflower (George, Collier and Port, 2009; Shelton and Nault, 2004).

Research in oilseed rape (OSR, *Brassica napus*) has shown that trap crops can act as a pest control measure for a wide range of pests as outlined in Table 1.

Table 1 Pest insect species which show potential for control via trap cropping in brassica systems.

Pest Species	**Reference**
Cabbage stem flea beetle (*Psylliodes chrysocephala* L.)	(Barari *et al.*, 2005; Trdan *et al.*, 2005)
Flea beetles (*Phyllotreta* spp.)	(Bohinc and Trdan 2013)
Cabbage seed weevil (*Ceutorhynchus obstrictus*)	(Kovács et al. 2013)
Pollen beetle (*Brassicogethes/ Meligethes aeneus* F.)	(Cook and Denholm 2008, Cook et al. 2007, Veromann et al. 2014, Veromann et al. 2012, Kaasik et al. 2014, Čuljak et al. 2016)
Stink bug (*Eurydema* spp.)	(Bohinc and Trdan 2012)

Barari et al, (2005) examined the use of turnip rape (*Brassica rapa*) as a trap crop for *P. chrysocephala* in winter OSR showing reduced larval infestations in OSR plots grown in association with the trap crop compared with control plots and this was independent of whether or not insecticide was applied to the trap crop. Sivčev et al. (2017) also showed that the beetles were attracted to turnip rape, and more beetles emerged from soil growing turnip rape than OSR.

Winter turnip rape cv Jupiter (*Brassica rapa* var. *olifera*) has shown promise as a trap crop in winter oilseed systems against pollen beetle (Skellern and Cook, 2018). Similarly, Tyfon, a hybrid between stubble turnip (*Brassica rapa* subsp. *rapifera*) and chinese cabbage (*Brassica* *raps* subsp. *Pekinensis* (Lour.), which is used as fodder for sheep (Gottstein, 2008) has also been shown to be a possible trap crop for pollen beetle winter OSR systems (Cook *et al.*, 2013). The efficacy of these species as a trap crop for *P. chrysocephala* is unknown.

In this study, the potential of trap cropping with turnip rape or Tyfon to reduce *P. chrysocephala* adult feeding damage and larval infestation in winter OSR are tested. The interactions between the trap crop (a nature-based cultural control) and a synthetic chemical-based (neonicotinoid seed treatment: Cruiser, active ingredient thiamethoxam) crop protection strategy were tested. The aim was to test whether trap cropping is a realistic alternative to neonicotinoid seed dressings, as an example of how cultural control methods might perform compared to synthetic agrochemicals. As *P. chrysocephala* are known to feed on a wide range of brassica species (Alford, Nilsson and Ulber, 2003; Williams, 2010). It is expected that they will actively feed on both trap types and reduce the amount of damage to the OSR crop. If there is an effect from using trap crop boarders, it would be expected to reduce the level of injury on the OSR in association with a trap crop. If injury is seen to be even across treatments, there will be a lack of evidence of the effectiveness of trap cropping for *P. chrysocephala*.

3.2.1 Aims of study:

This study was designed to test the effectiveness of two trap crop species in reducing pest pressure from *P. chrysocephala* in OSR crops. The impact of treating the trap crop with a neonicotinoid seed dressing was also tested. The null hypothesis is that the OSR within plots will be unaffected by trap crop presence of seed treatment and be comparable to control plots. I hypothesized that turnip rape trap crop boarders will reduce pest pressure in the OSR plots as shown by Barari et al (2005) and a similar effect will be seen for Tyfon. I hypothesized that the neonicotinoid treatment will kill the beetles feeding on the trap crop, reducing the level of injury to the trap and the oilseed rape plot associated with it, inferring increased crop protection from *P. chrysocephala* over un-treated trap crop treatments through the toxic effect of the neonicotinoid.

3.3 Methods:

3.3.1 Treatments layout and drilling procedure:

The experiment was established on 17th September 2015 at Rothamsted Research (Figure 1) and comprised six treatments (Figure *2*2 and Table therein), laid out in plots measuring 9m x9m. Each treatment was replicated six times in a quasi-complete Latin square design, with each treatment occurring once in each row and column and every treatment is a horizontal and a vertical neighbour to every other treatment twice, to exclude directional effects and to balance, as far as possible, any effects of neighboring treatments on each other. The treatments comprised: OSR (cv. DK Exalte, *Brassica napus*) main crop with or without a 1m-wide trap crop border of either turnip rape (cv. Jupiter, a restored hybrid variety, *Brassica rapa*) or Tyfon (a hybrid between stubble rape, *Brassica rape oleifera* and Chinese cabbage, *Brassica rape* subsp. *pekinensis*). Each trap crop was sown either using neonicotinoid-treated seed (Cruiser, at 15ml/kg, active ingredient: Thiamethoxam) or untreated seed. Oilseed rape without a trap crop acted as a control and OSR sown using neonicotinoid-treated seed was used as a treated control. Plots and trap borders were drilled using a Haldrup SB-25 plot drill (Halsrup, https://www.haldrup.net/en/seeders/sb25.html). OSR was sown at 50seeds/m^2 and the trap crop borders were sown at 150 seeds/m^2, which is the recommended sowing rates based on their seed weights (LG 2016). All other agronomy was per standard farm practice.

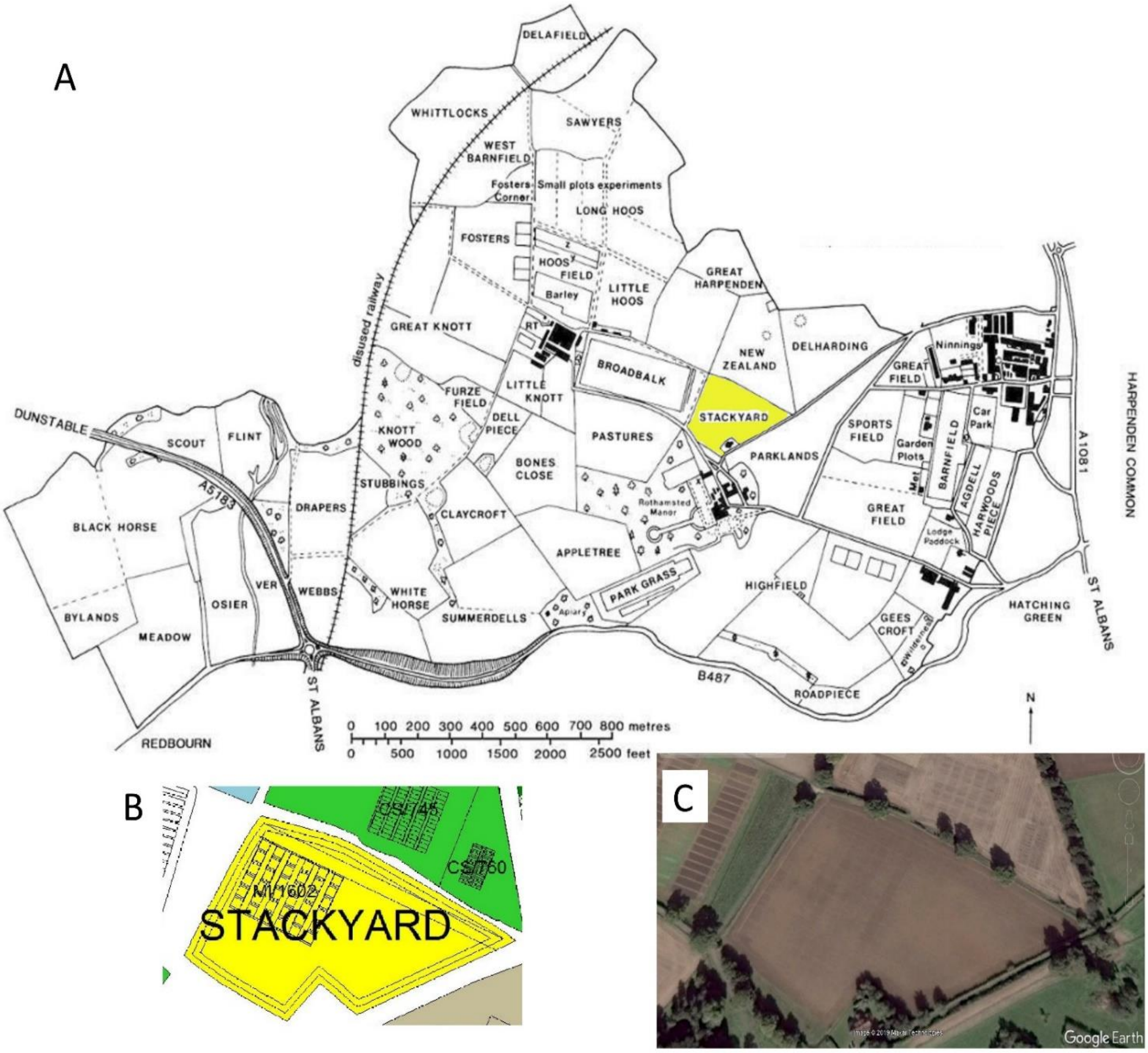
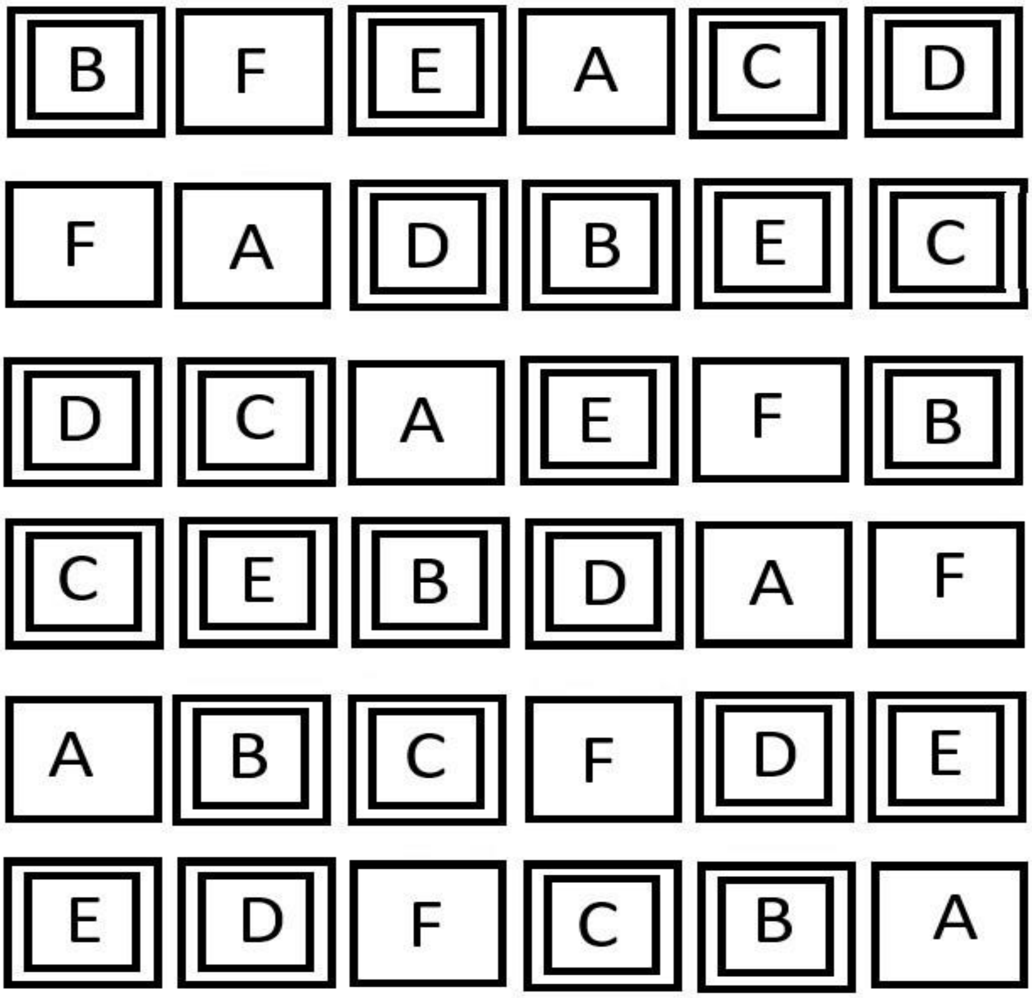


Figure 1. A. Rothamsted Farm map showing the location of the experimental field (Stackyard); B. the location of the experiment within the field; and C. Google Earth image (19th September 2015) of the field.



Code	Treatment description
A	OSR untreated
B	OSR with Turnip rape trap crop border
C	OSR with Turnip rape trap crop border sown using neonicotinoid-treated seed
D	OSR with Tyfon trap crop border
E	OSR with Tyfon trap crop border sown using neonicotinoid-treated seed
F	OSR with Neonicotinoid treatment

Figure 2. Treatment structure of the field experiment testing two different types of Brassica trap crop (turnip rape and Tyfon) borders on crop protection of an oilseed rape (OSR, *Brassica napus*) main crop from damage by the pest *Psylliodes chrysocephala*, and the effect of treating the trap crop with neonicotinoid seed treatment (Cruiser, at 15ml/kg).

3.3.2 Plant density:

Plant density for both the OSR and the trap crops was used as a measure of the success of establishment and was recorded on 3rd to 5th November 2015, using 0.25m quadrats. As plant density of the whole experiment could not be assessed in one day, assessment was carried out in two experimental blocks (12 plots) per day to avoid temporal bias in sampling. Four quadrats were randomly placed in the central 7m^2 area of all plots (OSR) and one quadrat was surveyed on each of the trap crop borders where present (see Figure1 and Figure 2). No assessments were done on the borders of the plots without a trap crop as plant density of OSR in the borders was assumed to be the same as the rest of the plot.

3.3.3 Leaf area injury by adult *Psylliodes chrysocephala:*

To measure the levels of feeding injury by adult *P. chrysocephala* to the OSR and the trap crop, plants were visually inspected for characteristic shot hole damage (Figure). Each plant was scored to the nearest 5% of leaf area removed, by estimated visual assessments. This rapid assessment allowed for data to be collected by one individual and ensured consistency between measurements. Within the central 7m^2 of each plot, a total of 12 OSR plants were examined. Where a trap crop was present, three trap crop plants from each border side were examined for damage (totaling 12 plants per plot). Two rounds of assessments were carried out one on all plots on the 28th of October and the second split between one block on the 12th and the other two between the 16-17th November 2015, again assessing 1 or two blocks of the experiment each time to ensure consistency between treatments. The growth stage of the plants assessed for injury was also recorded according to the BBCH scale

(Lancashire et al. 1991); at this stage of crop development this involved recording the number of true leaves present.

3.3.4 *Psylliodes chrysocephala* larval infestation:

Psylliodes chrysocephala larval infestation of crop and trap crop plants was assessed using destructive sampling of whole plants. Where present, five OSR plants were collected from the central 7m^2 area of each plot. For treatments with a trap crop border (Treatments B, C, D and E) one plant was taken from each of the four borders. Plants were sampled carefully to ensure good representation from the whole area of the plot. This mitigated possible pest congregation as *P. chrysocephala* within a field has been shown to be patchy (Ferguson *et al.*, 2002). Plants were removed gently from the ground by pulling the stem and were placed in labelled plastic bags and transferred to cold storage (5^{o}C) prior to processing. The number of larvae per plant was assessed by dissecting the stems and petioles using a scalpel under a light microscope and recording the number of larvae and the larval instar. *Psylliodes chrysocephala* larvae develop within Brassica stems passing through three distinct larval instars as described by Ebbe-Nyman (1952). Plants were collected for sampling on 10th February 2016 and dissections were completed by 2nd March 2016. Dissections were carried out to ensure that on each day equal number of plants were dissected from treatment plots within a block to limit any temporal effects of plant storage.

3.3.5 Statistical analysis:

Plant density data were transformed to $\log_{10}$ plant number recorded per quadrat to account for zero counts. An analysis of variance (ANOVA) with blocking to account for the rows and columns of the Latin square design was performed to analyze differences in plant density within the central areas of each plot. A mixed model (REML) was used to analyze differences in plant density in the trap crop borders due to unbalanced distribution of trap crop plots.

Growth stages were analyzed separately for each of the two sample rounds (1st round: 28th of October and 2nd round: the 12th, 16-17th of November). A mixed model (REML) analysis was used to incorporate the treatment structure and allow for missing values as some plants were damaged too much to accurately determine growth stage.

Leaf area damaged was logit transformed and adjusted to allow for 0% and 100% values in the data set. An analysis of variance (ANOVA) was performed on the data from plot centers. A mixed model was used to analyze differences between trap crop type in order to account for blocking structure.

When plants were collected for assessment of the numbers of larvae present in February 2016, a third of the plots (12/36) had no OSR plants and no treatment had the target number of 30 plants necessary for a robust analysis. A mixed model (REML) was used due to the data being unbalanced. Total number of larvae per plant were $\log_{10}$ transformed with an adjustment for zero observations (n+1).

All statistical analyses were performed using GenStat V18, for windows (VSN International, Hemel Hempstead).

3.4 Results:

3.4.1 Plant density:

The trap crop borders successfully established (Figure 3 and Figure) with no difference in plant density between the turnip rape and Tyfon trap crop types ($F_{0.01}$,10.6=0.01, *P* =0.943) nor was there an effect of the neonicotinoid seed treatment ($F_{1.03,10.6}$=1.03, *P* =0.332, Figure).

The presence of a trap crop increased the OSR density ($F_{1, 20}$ =10.10, *P*=0.005). The mean plant density of the OSR without a trap crop boarder were below the 20-25 plants/m^2 (Roques and Berry 2016), with or without neonicotinoid seed treatment, where a trap crop was present OSR density was above the 20-25 plants/m^2 (Figure 3 and Figure 4). There was no significant effect of the trap crop type ($F_{1,20}$ =0.21, *P*=0.648) or from the use of the neonicotinoid seed treatments to the trap crop seed ($F_{1,20}$=0.87, *P*=0.362) on OSR density.

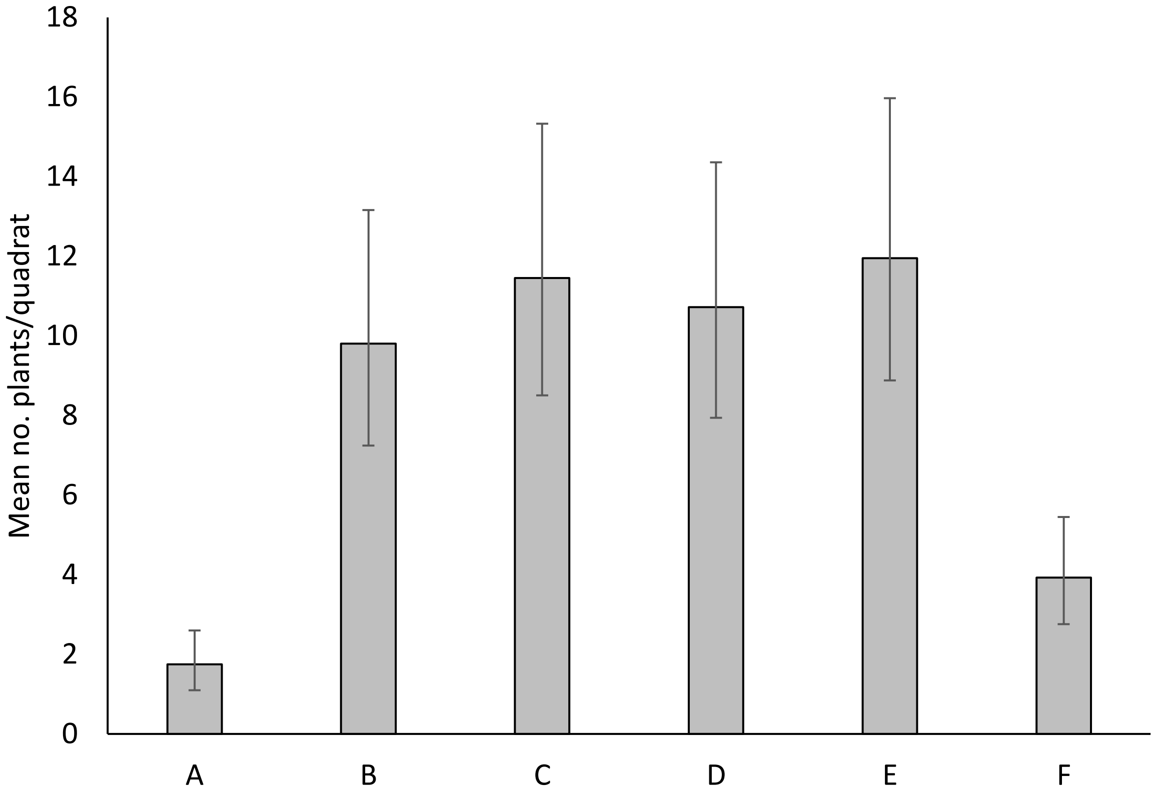


Figure 3. Grand mean number of oilseed rape (OSR) plants per quadrat for each treatment. A = Untreated OSR; B = Untreated OSR with a turnip rape trap crop border; C = OSR with a turnip rape trap crop border with neonicotinoid-treated seed; D = Untreated OSR with a Tyfon trap crop border; E = OSR with a Tyfon trap crop border using neonicotinoid-treated seed; F = OSR using neonicotinoid-treated seed. Seed treatment Cruiser, at 15ml/kg. Standard error of means shown.

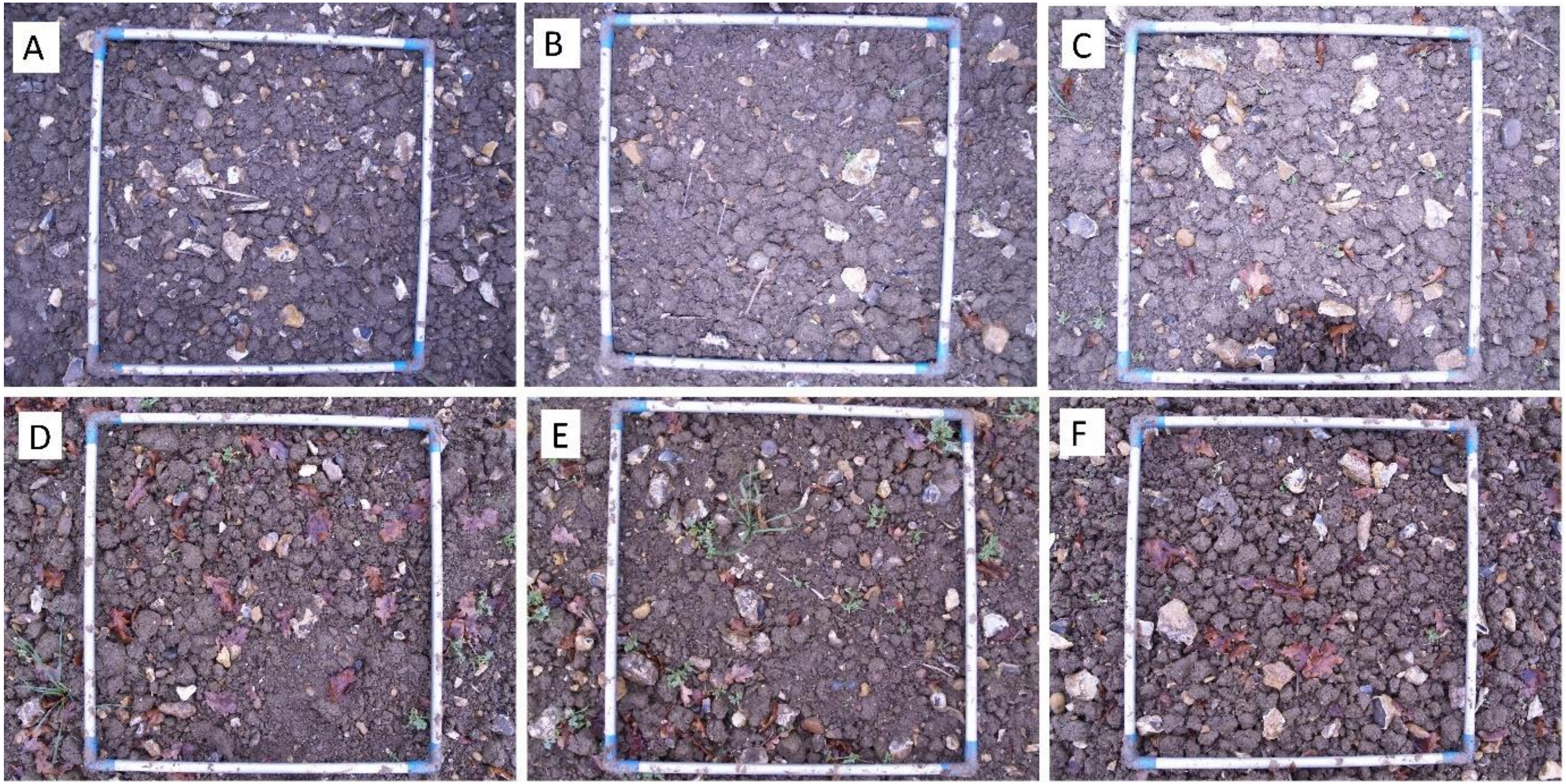


Figure 4. Quadrats from the central oilseed rape (OSR) crop area of each treatment type. (A) OSR; (B) OSR with turnip rape border; (C) OSR with neonicotinoid seed-treated turnip rape border; (D) OSR with Tyfon border; (E) OSR with neonicotinoid-treated Tyfon border; and (F) OSR with neonicotinoid seed treatment. Seed treatment Cruiser, at 15ml/kg. Photos taken 3/11/2015, 47 days after drilling.

Figure 5. Representative photograph of quadrat samples within each trap crop type. (A) Turnip rape (*Brassica rapa*) sown using untreated seed; (B) Turnip rape sown with neonicotinoid treated seed; (C) Tyfon sown with untreated seed; (D) Tyfon sown with neonicotinoid-treated seed. Seed treatment Cruiser, at 15ml/kg. Photos taken 3/11/2015, 47 days after drilling.

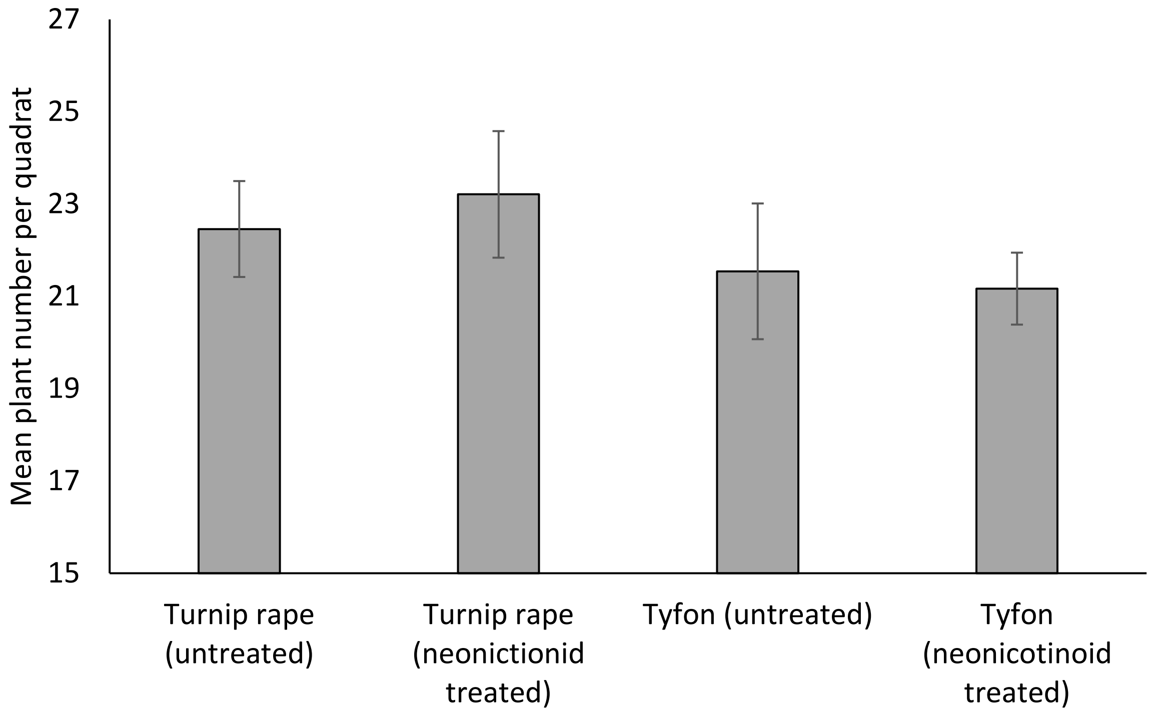


Figure 6. Mean ±SE plant density of turnip rape and Tyfon trap crop borders sown using untreated or neonicotinoid-treated seed. Seed treatment Cruiser, at 15ml/kg.

3.4.2 Growth Stage:

At the October assessment the levels of adult *P. chrysocephala* feeding damage were already high and one full plot from treatment A (Control) could not be accurately assessed for growth stage due to a complete lack of surviving plants. Overall, when grown in association with a trap crop border the OSR was at a more advanced growth stage than in the absence of a trap crop (GS 14.23 and GS 13.98, respectively; Figure ; $F_{1,19.8}$=8.43, *P*=0.009); there was no significant effect of the trap crop type on growth stage of OSR ($F_{1,18.9}$=0.02, *P* =0.883) and no significant effect of the neonicotinoid seed treatment ($F_{1,18.9}$=0.2, *P* =0.659). By November, the growth stage of the OSR in the crop centres had advanced in the more backward treatments and there were no differences between treatments (in association with trap crop GS 15.70, monoculture GS 15.55; Figure ; $F_{1,20.5}$=1.7, *P* =0.206). Due to high levels of injury, a total of 60/432 OSR plants could not be measured for growth stage in the November assessment due to a complete lack of leaf material from heavy damage.

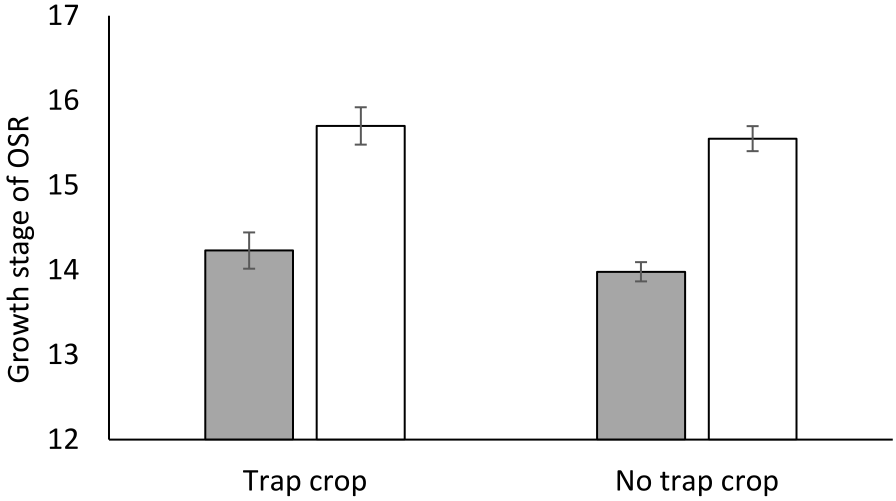


Figure 7. Oilseed rape (OSR, *Brassica napus*) growth stage in October (grey) and November (clear) when grown in association with a Brassica trap crop or not. Growth stage based on BBCH scale (Lancashire *et al.*, 1991); mean ±SE shown.

3.4.3 Leaf area injury from *Psylliodes chrysocephala*:

Levels of damage observed on the OSR crop plants were very high in all treatments (Figure and Figure). Some plots (N=12/36) did not have enough plants to assess the target number of ten plants. For example, in the first round of observations (October assessment), plot 36 (treatment A, untreated OSR control) had a total of just 8 plants within the OSR main crop central area ($7m^2$). At the October assessment there was a significant reduction in the amount of damage observed on crop plants grown in association with a trap crop border compared with treatments without a trap crop ($F_{1,20}=24.2$, $P<0.001$; Figure). There was no significant effect of the neonicotinoid seed treatments ($F_{1, 20}=0.52$, $P=0.48$) nor between the trap crop type ($F_{1, 20}=0.0$, $P=0.995$). The same pattern was seen for the November assessment with the only significant effect being the presence of a trap crop ($F_{1, 20}=17.76$, $P<0.001$); it did not make a difference if the trap crop was sown using neonicotinoid-treated seeds or not ($F_{1,20}=1.28$, $P=0.271$), nor was there any effect of the trap crop type ($F_{1,20}=0.19$, $P=0.665$).

Both turnip rape and Tyfon trap crops were attacked by *P. chrysocephala* (Figure9), with no difference in the injury levels observed between the trap crop types ($F_{0.37,9.8}=0.37$, $P=0.557$) or neonicotinoid seed treatment ($F_{2.70,9.8}=2.70$ $P=0.132$).

Due to such high levels of injury, it was not clear if only *P. chrysocephala* damage had occurred or whether there were other sources of defoliation such as slug and pigeon damage.

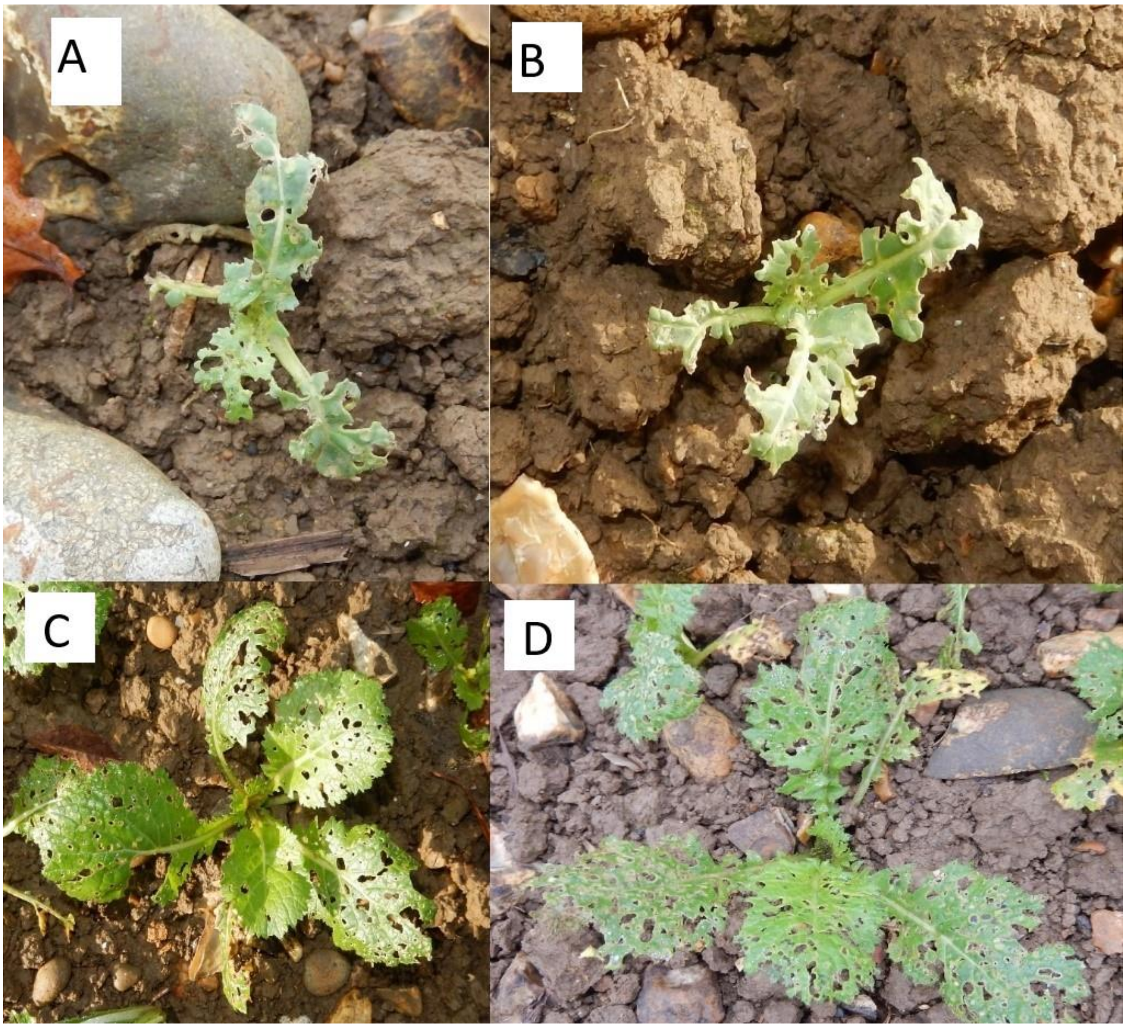


Figure 8. Damage assessment photographs, **A**=untreated oilseed rape (OSR) plant showing evidence of shot holing damage indicative of *Psylliodes chrysocephala* feeding activity (plant from plot 8). **B**= Neonicotinoid treated OSR plant exhibiting large amounts of feeding damage (plant from plot 7) **C**= Untreated turnip rape (*Brassica rapa*) plant exhibiting high levels of characteristic shot hole feeding damage (plant from trap crop border of plot 1). **D**= Untreated Tyfon plant exhibiting high levels of shot hole damage (plant from trap crop border of plot 6). Seed treatment Cruiser, at 15ml/kg. Pictures for both trap crop plants are from un-treated seed as there was no significant effect on adult feeding from the seed treatment so only one set is represented here. Photographs taken on 24/11/2015, 56 days after drilling.

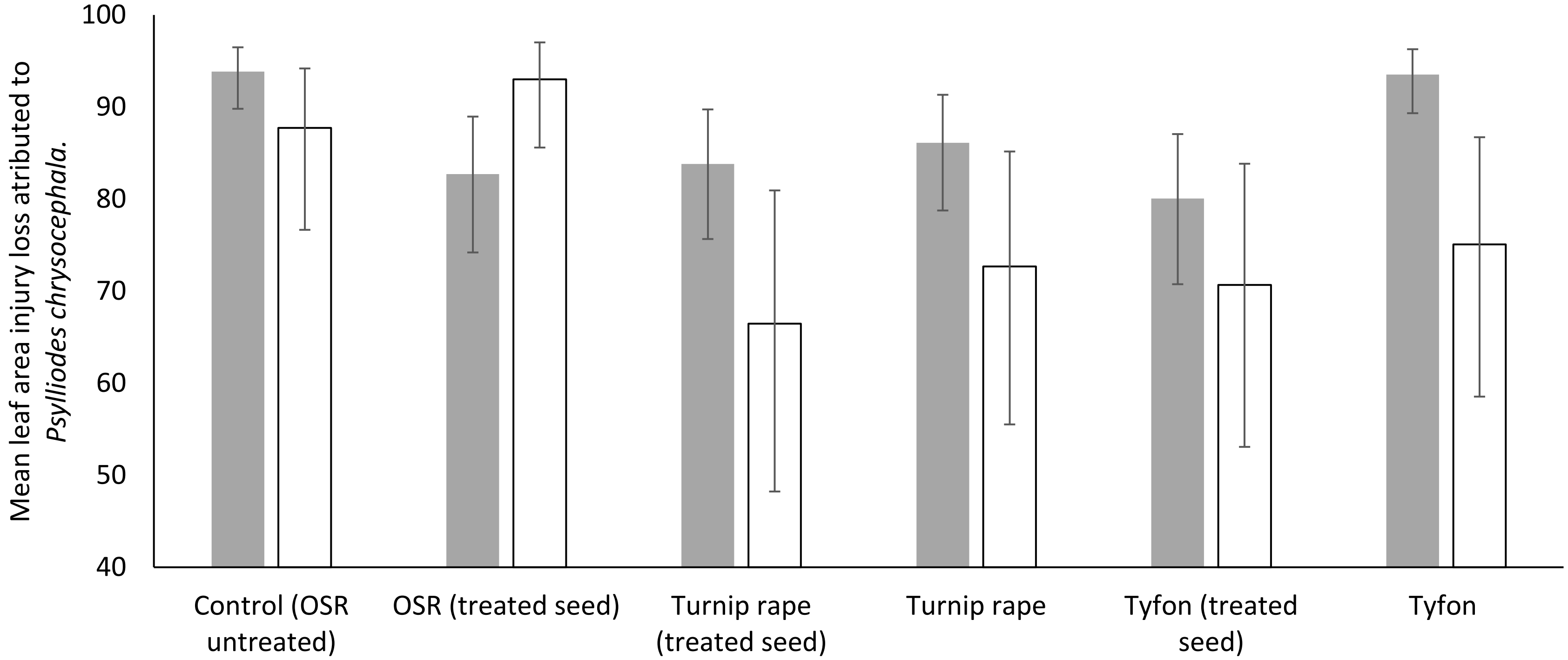


Figure 9. Backtransformed mean percentage leaf area damage to oilseed rape plants (OSR, *Brassica napus*) by *Psylliodes chrysocephala*. Grey bars = October assessment; Clear bars = November assessment. Treatment codes: A = Untreated OSR; B = Untreated OSR with a turnip rape trap crop border; C = OSR with a turnip rape trap crop border sown using neonicotinoid-treated seed; D = Untreated OSR with a Tyfon trap crop border; E = OSR with a Tyfon border sown using neonicotinoid treated seed; F = OSR sown using neonicotinoid treated seed. Seed treatment Cruiser, at 15ml/kg. Error bars are 95% confidence intervals.

3.4.4 *Psylliodes chrysocephala* larval infestation:

Not all treatments could be sampled for larvae due to poor plant survival. Only where a trap crop boarder was present did any OSR survive to allow destructive sampling for *P. chrysocephala* larvae (Table 2). Collected plants exhibited high levels of injury to both the OSR and trap crops (Figure9). The number of larvae per plant in the OSR plot centres was lower in plots with Tyfon trap crop border than Turnip rape; the difference bordered on significance ($F_{1,2.2}$=15.61, *P*=0.052). Neonicotinoid seed treatment did reduce the numbers of larvae, but not significantly ($F_{1,2.4}$ = 2.4, *P*=0.242, Figure 10).

Trap crop plants were present and available in sufficient numbers for destructive sampling in all plots (starting seed rate was higher than OSR). Larval infestation of the trap crop plants was very high; in two plants infestation exceeded 200 larvae in a single plant (one turnip rape and one Tyfon). Larvae were extracted from all but 8 of the plants dissected (n=167) clearly showing that the larvae will select and use both turnip rape and Tyfon as host plants. Although more larvae were found per plant in turnip rape than in the Tyfon plants (Figure), the difference was not significant ($F_{1,9.8}$=0.4, *P*=0.543) and there was no significant effect of the neonicotinoid treatment on larval numbers ($F_{1,9.8}$=0.93, *P*=0.357, Figure 9).

Table 2. The number of oilseed rape (OSR) plants available for destructive sampling (aim to achieve 25 per treatment) to determine the effect of treatments on *Psylliodes chrysocephala* larval infestation. (A) OSR untreated; (B) OSR with turnip rape trap crop border; (C) OSR with neonicotinoid seed-treated turnip rape trap crop border; (D) OSR with Tyfon trap crop border; (E) OSR with neonicotinoid-treated Tyfon trap crop border; and (F) OSR with neonicotinoid seed treatment. Seed treatment Cruiser, at 15ml/kg.

Treatment	No of oilseed rape plants available for sampling
A	0
B	15
C	20
D	15
E	20
F	0

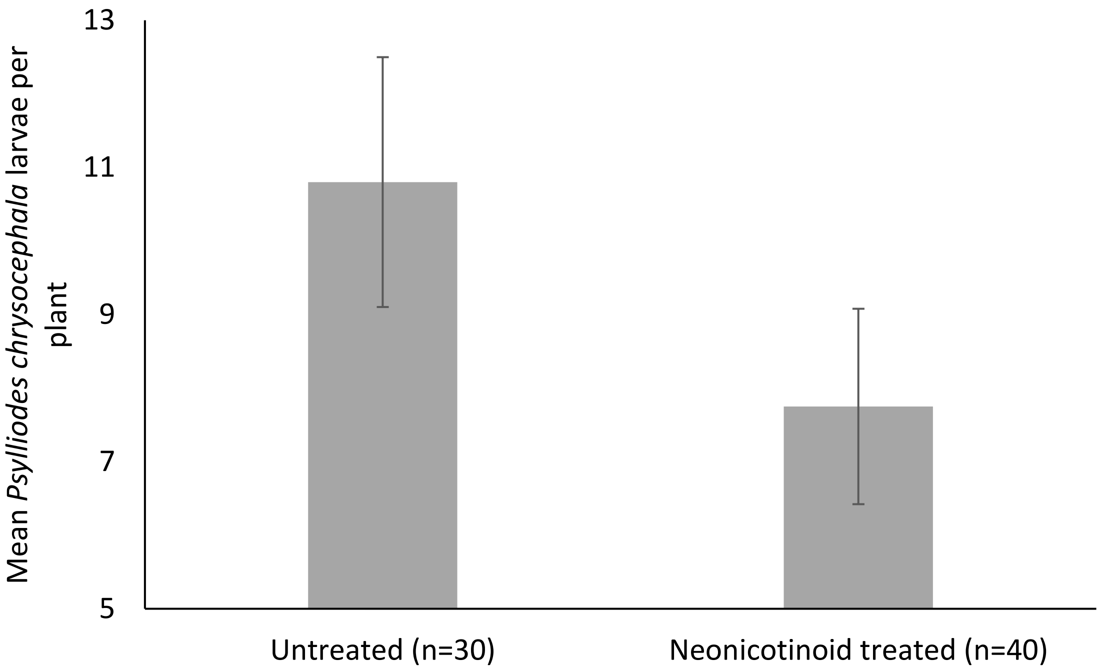


Figure 10. Mean ±SE number of *Psylliodes chrysocephala* larvae per plant in oilseed rape (*Brassica napus*) with seed either untreated or treated with neonicotinoid seed dressing Seed treatment Cruiser, at 15ml/kg.

Figure 11. Oilseed rape (OSR, *Brassica napus*) plants collected for sampling of *Psylliodes chrysocephala* larvae. Plants shown here were taken from the central crop area of OSR sown grown in association with a Turnip rape trap crop border sown using neonicotinoid-treated seed (Treatment C). Seed treatment Cruiser, at 15ml/kg. Plants collected 10/2/2016, 146 days after drilling.

Figure 12. Brassica trap crop plants collected for destructive sampling of *Psylliodes chrysocephala* larvae. (A) Turnip rape (*Brassica rapa,* from treatment D), (B), Tyfon, from treatment B); both treatments sown with untreated seeds. Plants collected 10/2/2016, 146 days after drilling.

Figure 13. High numbers of *Psylliodes chrysocephala* larvae and larval damage from test section of a turnip rape plant (untreated seed). Photograph taken 10/02/2016, 146 days after drilling.

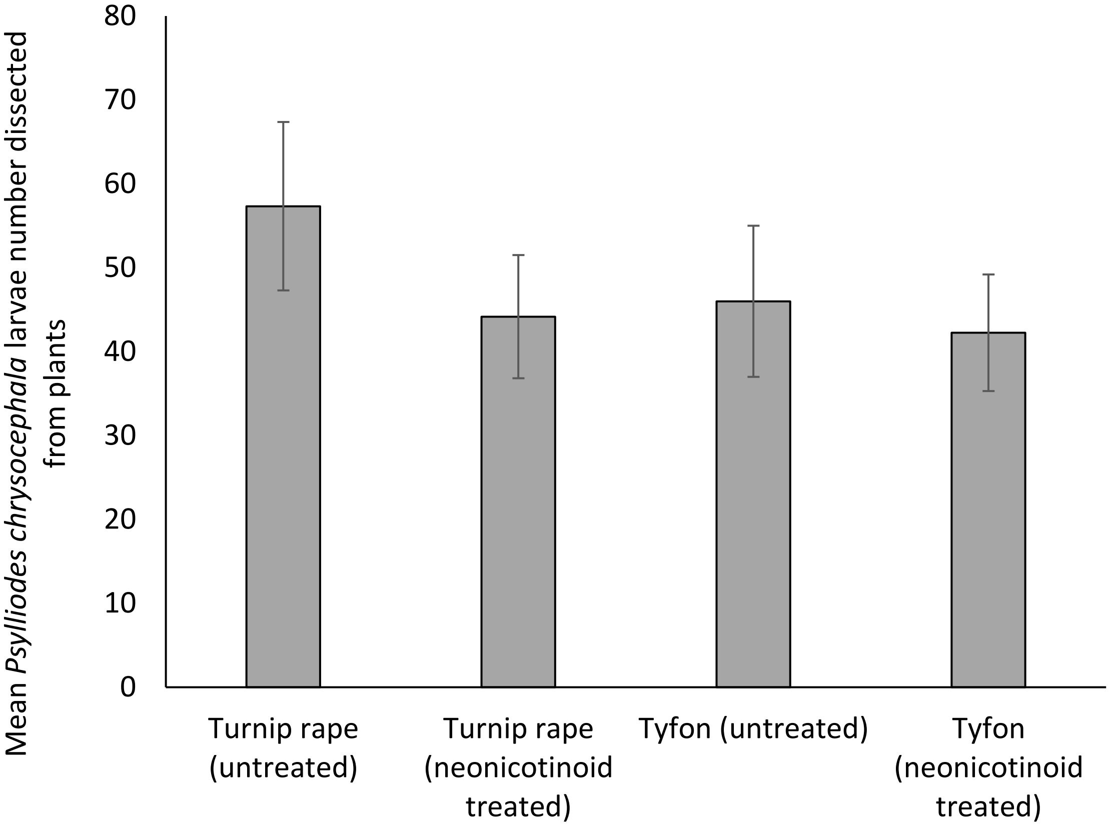


Figure 14. Mean ±SE number of *Psylliodes chrysocephala* larvae dissected from Brassica trap crop plants (Turnip rape and Tyfon) sown using untreated seed or seed treated with Cruiser, at 15ml/kg or untreated.

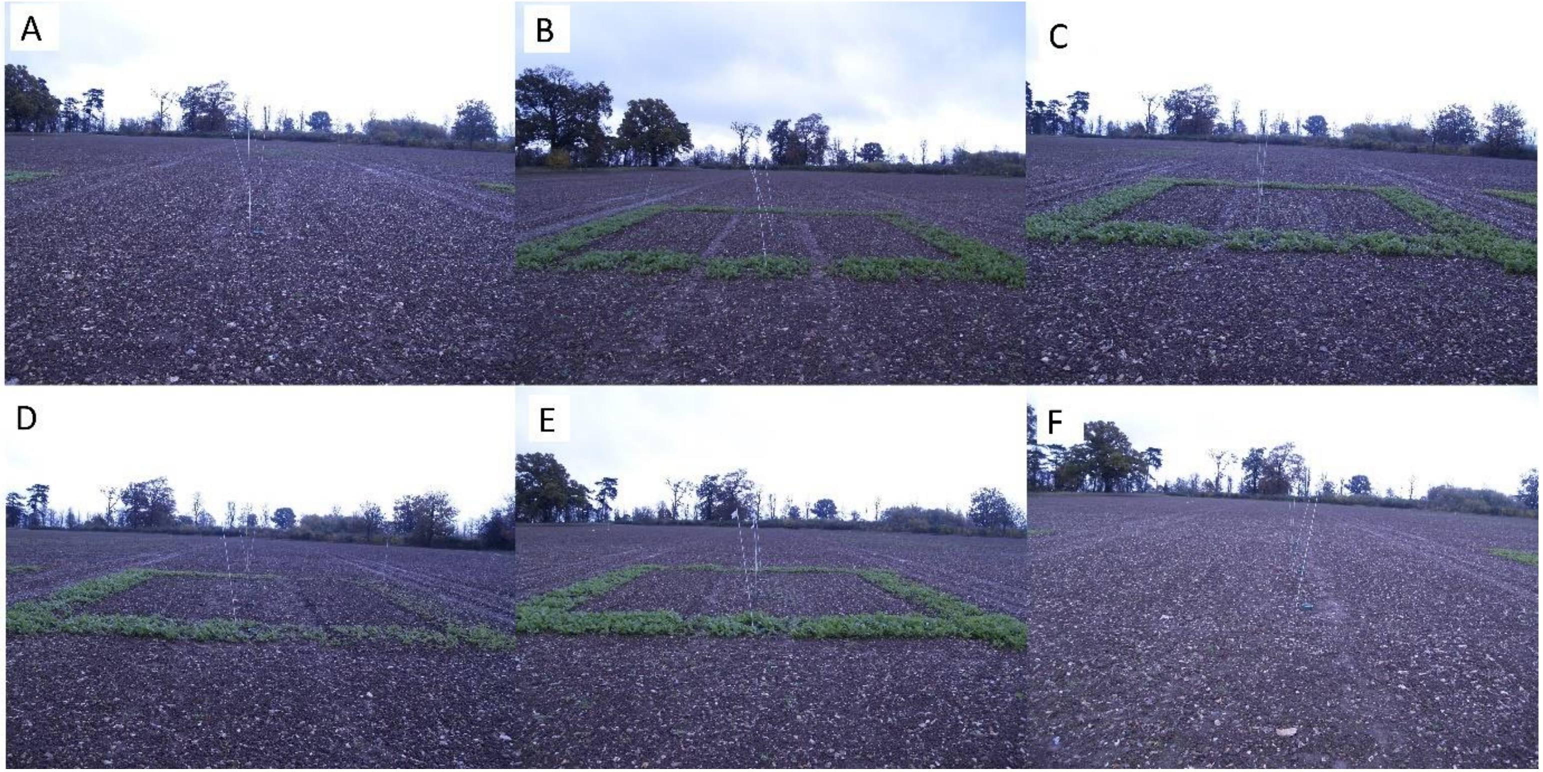


Figure 15. Representative photographs of each treatment tested to protect oilseed rape (OSR) from *Psylliodes chrysocephala* damage. (A) OSR; (B) OSR with turnip rape trap crop border; (C) OSR with neonicotinoid seed-treated turnip rape trap crop border; (D) OSR with Tyfon trap crop border; (E) OSR with neonicotinoid seed-treated Tyfon trap crop border; and (F) OSR with neonicotinoid seed treatment. Seed treatment Cruiser, at 15ml/kg. All photographs taken 22nd January 2016. Note some OSR plants can be seen in treatments C and E but mostly the only green leaf material visible is the trap crop borders and the rest of the field is barren.

3.5 Discussion:

All methods of crop protection tested, including neonicotinoid seed treatment, were insufficient to protect the crop beyond winter from high levels of adult feeding and larval infestation of *Psylliodes chrysocephala* which led to the trial being abandoned. This also occurred at NIAB Duxford site in the same season (Chapter 2 this thesis) and highlights the severe damage potential from *P. chrysocephala* when at high abundance, such as observed in 2015/2016.

Both species of trap crop, turnip rape and Tyfon, did survive the feeding damage by *P. chrysocephala* and although they had very high larval infestation (Figure 10). Both turnip rape and Tyfon therefore have potential as trap crops for *P. chrysocephala* control confirming the findings of Barari et al (2005). There is evidence that *P. chrysocephala* exhibit preference to different Brassica (White *et al.*, 2020).Turnip rape has been suggested as a preferred host to OSR (Sivčev *et al.*, 2016) with higher adult emergence from turnip rape than the OSR crop in Serbia. In the present study the OSR was eaten more readily (Figure) than either the trap crops and could not tolerate the levels of damage. Bothe the trap crops show higher tolerance to *P. chrysocephala* feeding than the OSR. This is borne out by the survival of the trap crops alone while the crop was lost.

Crop establishment (plant density) and lower levels of feeding injury was recorded on OSR when grown in association with a trap crop; the only OSR plants to survive beyond October were in the trap crop protected treatments. Further work is required to understand the underlying mechanism behind these observations. One potential explanation is the trap crops, which are taller than the OSR crops, are acting as a physical barrier to the beetle’s movement into the central area (Tillman *et al.*, 2015). Another is the higher seed rate in the trap crop boarders and subsequent higher plant density are diluting the level of feeding away from the OSR in the central area. In the experiment here the area of OSR inside the trap crop boarder was 9X9m with a 1m border and may disrupt the beetle's movement at this scale. However, a trap crop planted as a border around the edge of a field may not work when scaled-up. Unlike pollen beetles, which enters the crop from the field edges (Frearson *et al.*, 2005; Mauchline *et al.*, 2017). *P. chrysocephala* is known to migrate into the central area of a field and distribute in patches across the crop (Ferguson *et al.*, 2006; Thioulouse, Debouzie and Ballanger, 1984). Understanding on the migration pattern of *P. chrysocephala* would allow better understanding of how and where to locate trap crops. Another option to be explored would be directional trap crop areas located on in one larger area at the edge of the crop which see highest migration abundance. This directional migration of *P. chrysocephala* into OSR crop is measured using yellow water traps later in thesis (Chapter 4 and 7).

The number of larvae in both turnip rape and Tyfon were significantly higher than current thresholds for OSR (Figure10 and Figure 14) i.e., 5/plant; (AHDB, 2019). The extremely high levels of larvae recorded in the trap crop plants may be an artefact of the lack of alternative OSR host plants in the field, resulting in larval distributions being concentrated in the small areas of available plants in the trap crop (Figure 14). The survival of both trap crop types when infested with high larval numbers suggests they have a high tolerance for larval infestation. The presence of feeding injury with larval infestation coupled with tolerance to high abundance of adults and larvae supports the hypothesis that both turnip rape and Tyfon show traits for a dead-end trap crop, if they were subsequently destroyed mechanically of through sheep grazing (Shelton and Nault, 2004).

In this study neonicotinoid treatments did not reduce levels of adult *P. chrysocephala* feeding or significantly alter crop establishment over untreated OSR and they did not save the crop from total loss. The number of *P. chrysocephala* larvae in the OSR were lower in treated plots compared to plots sown with untreated seed, but not significantly, nor did seed treatment significantly reduce the levels of larval infestation in either trap crop species (Figure). The data presented here suggests that neonicotinoid seed treatments are not effective in ensuring crop establishment or reductions in *P. chrysocephala* larvae in years of high pest abundance.

The abandonment of this trial highlights the importance of multi-year studies as the treatments tested here may be effective in years of lower *P. chrysocephala* numbers. Data from Sweden (Nilsson, 2002) and Germany (Nilsson, 2002) on the cycles of *P. chrysocephala* activity suggests they exhibit a cycle of high abundances every seven years. This has not been examined in the UK and should be considered of high importance when estimating the effects of *P. chrysocephala* on an experimental trial conducted in only one year. The methods tested in this trail may be more effective in years of lower pest pressure.

3.6 Acknowledgements:

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4.0 Comparison of crop protection strategies for *Psylliodes chrysocephala* in winter oilseed rape (*Brassica rape*): past, present, and future:

4.1 Abstract:

Oilseed rape (OSR, *Brassica napus* L.) is one of the most widely grown crops in UK agriculture. The use of neonicotinoid seed treatments, used to protect early crop growth and promote establishment, has been banned in winter OSR since 2013. This restriction has led to increased pressure from autumn pests such as the cabbage stem flea beetle (*Psylliodes chrysocephala*). Using two plot-based field experiments the relative effectiveness of past control options i.e. neonicotinoid seed treatment (Active Ingredient, AI: thiamethoxam) is tested against current control options: topical sprayed applications of pyrethroid insecticides (AI: lambda cyhalothrin) and increased seed rates, and potential future options including cultural controls: nurse cropping and trap cropping, and a future chemical options: an alternative seed treatment, Lumiposa™ (AI: cyantraniliprole), and neonicotinoid applied as a spray (AI: thiacloprid). Data presented here suggests that in years of moderate *P. chrysocephala* pressure seed treatments do not significantly reduce pest pressure or benefit the end yield and that alternatives not based on synthetic insecticides are equally efficient. The implications for Integrated Pest Management are discussed.

4.2 Introduction:

A blanket ban on the use of neonicotinoid insecticide seed treatments (EU, 2018) and the development of resistance to pyrethroid insecticides in pests species (Brandes and Heimbach, 2018) has driven an increase in pressure on crop production of oilseed rape (OSR, *Brassica napus* L., Zhang *et al,.* (2017)). This has led to reductions of the cropping area in the UK since the restriction came into effect in 2014 (Garthwaite *et al.*, 2019). With pest pressure particularly from slugs and the cabbage stem flea beetle (*Psylliodes chrysocephala*) being commonly referenced as the cause of such reductions amid farmer concerns (Collins, 2017; CEH, 2017; Gillbard and Allison, 2019). When giving reasoning for insecticide application 60% of UK farmers defined pressure from *P. chrysocephala* as a main factor in applications of insecticides in 2018 (Garthwaite *et al.*, 2019).

With the area of OSR now at its lowest level in over a decade, and the need for imports of rapeseed, often from countries still allowing the use of neonicotinoid seed treatments (Harris, 2019) it is important to quantify the contributions that the lost crop protection methods made to crop protection and yield and to test the relative efficacy of alternative pest protection measures available to growers now and those that might be available in the future; research has shown several promising alternative pest protection methods available to farmers in the post-neonicotinoid era. Here I compare the efficacy of past, present, and future methods of crop protection against *Psylliodes chrysocephala* in the IK.

4.2.1 Past control:

Between 2000 and 2014 the primary method of protecting OSR from *P. chrysocephala* in the UK was use of neonicotinoid seed treatments to protect the emerging crop from adult feeding followed by pyrethroid spray applications to control larvae of *P. chrysocephala* (Redman, 2019; Willis *et al.*, 2020). The neonicotinoid seed treatment was thought to be effective for the first 6-8 weeks after drilling and provide significant reductions in shot hole damage (Dewar *et al.*, 2016). To properly inform any amendment to the ban on neonicotinoid use requires empirical data on the current level of effect as a pest protection practice.

At the time of this trial (year) the foliar spray Biscaya® (active ingredient: thiacloprid) is registered for use as an aphicide and for use against pollen beetle in spring so was used as a model new foliar treatment in the autumn. Since the experiment, the use in spring will also be banned from 2021 (S. Cook, Personal comment).

4.2.2 Current:

Since the ban of neonicotinoid seed dressing in the UK, insecticide applications to OSR have been predominated by the pyrethroid group, with four of the top five insecticides used being a pyrethroid (lambda-cyhalothrin, tau-fluvalinate, zeta-cypermethrin, cypermethrin); the 5th being thiacloprid a neonicotinoid; applied as a foliar spray (Dewar *et al.*, 2016). With only pyrethroids registered for use in the autumn. This has led to an over reliance in their use to control *P. chrysocephala* with farmers increasing the frequency of applications (Hughes, Reay and Watson, 2014). However, growing evidence of widespread (and on some sites total) resistance of populations of *P. chrysocephala* to pyrethroids, has come to light (Willis *et al.*, 2020). With target site resistance reported from Denmark (Castberg and Kristensen, 2018; Højland and Kristensen, 2018), France (Bothorel *et al.*, 2018), Germany (Brandes and Heimbach, 2018; Heimbach and Brandes, 2016; Zimmer *et al.*, 2014) and the UK (Foster and Williamson, 2015) and a form of metabolic resistance also reported in Denmark (Højland and Kristensen, 2018; Castberg and Kristensen, 2018). The evidence for the UK shows there are areas with 100% resistance but that there are still areas where 0% of the population exhibits resistance (Syngenta, 2019; Willis *et al.*, 2020). These resistance levels are probably due to increased pressure from insecticides with one mode of action, producing ideal conditions for selection for resistance (Mallet, 1989). The level of efficiency from any insecticide is not 100% a proportion of the population are capable of tolerating the effect without dying (ffrench-Constant and Bass, 2017). With the use of only a single mode of action the proportion of the population expressing methods of tolerating the insecticide effect will increase until there is a higher proportion of resistant individuals than susceptible ones (Bass *et al.*, 2014). With the ban on use of neonicotinoid seed treatments, pest resistance to permitted foliar treatments (pyrethroids) and resulting difficulty in preventing *P. chrysocephala* damage and potential yield loss, the development of alternative pest protection methods is paramount. The current practice of UK farmers to apply pyrethroids more frequently to control *P. chrysocephala* has not be clarified to show any appreciable effect and will be part of this study to determine if this practice is achieving any benefit to the farmer.

Under current advice farmers are informed to increase seed rate as a measure to reduce pest pressure from *P. chrysocephala* (AHDB, 2019). In Chapter 2 of this thesis the impact of OSR seed rate was tested alongside the use of nurse crop mixes. This showed that seed rate did influence the level of leaf area damage, with lower leaf area loss at higher seed rate application observed in October (but no effect was seen by November). This suggests that early *P. chrysocephala* feeding can be diluted amongst plants when at a greater density. In the present study the influence of seed application rate on levels of *P. chrysocephala* feeding and larval infestation will be tested. It is not clear whether the dilution of injury will outweigh the interspecific competition between plants at higher densities (Berry and Spink, 2006).

4.2.3 Future - cultural:

Trap cropping is a system whereby the crop species at a more attractive growth stage or a plant species which is more attractive to the pest than is the crop, is grown alongside the crop in order to act as a trap for pests, reducing infestation in the main crop (Cook *et al.*, 2007; Hokkanen, 1991; Shelton and Badenes-Pérez, 2006; White, 2016; White, Ellis and Kendall, 2018). Using trap crops can decrease the need for synthetic chemical application by reducing the harmful impact of the pest to the desired crop. For example, Bohinc and Trdan (2013) suggest that trap cropping could be used to protect cabbage from the *Phyllotreta* flea beetles and therefore reduce the need for insecticide application. Turnip rape (*Brassica rapa*) strips grown at the edge of OSR plots has been shown to reduce numbers of *P. chrysocephala* larvae in the surrounded OSR (Barari *et al.*, 2005). Turnip rape, along with Tyfon (a stubble rape and Chinese cabbage hybrid) borders have also been shown to lower levels of adult *P. chrysocephala* feeding on OSR in small plot-based field trials (This Thesis Chapter 3).

Nurse crops are a class of companion plants, grown among the crop which, unlike intercrops are not harvested; being either naturally destroyed by frost or by herbicides prior to stem elongation of the OSR crop. This limited growth time of the companion crop reduces any competition from the nurse species during the bud-formation and flowering periods of the crop while being present during the vulnerable early growth stages. Altieri and Gliessman (1983) showed lower numbers of *Phyllotreta* flea beetles in Californian collard crops with retained weeds than in weed-free monocultures. Kareiva (1985) demonstrated that *Phyllotreta* beetles move further when they encounter non-host flora, suggesting greater movement of the beetle in more diverse cropping systems. Ruck et al (2018) suggests that using volunteer OSR as an adapted trap crop can reduce *P. chrysocephala* damage in OSR. This has been shown to have some success in reducing pest injury levels (White *et al.*, 2020). Reductions in *P. chrysocephala* feeding has been observed when OSR is grown with a nurse crop mix comprising Brassicas (Pak Choi (*Brassica rapa var. chinensis),* Chinese cabbage (*Brassica rapa subsp. Pekinensis (Lour.) Rupr),* Salad rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum*)s see Chapter 2 this thesis.

The defoliation of OSR by mowing or sheep grazing shows potential as a crop husbandry technique to reduce numbers of *P. chrysocephala* larvae infesting crop plants (Ruck, Cadoux and Robert, 2018). The later the mowing the greater the reduction in larval numbers (White, Ellis and Kendall, 2018). Spink (1992) cut OSR with a reciprocating blade mower at 3-6cms above the ground in January; this did not significantly affect the yield (oil content, seed weight, pods/branch, thousand grain weight) but defoliation had a definite growth regulatory effect. Studies in spring OSR show the later timing of defoliation and particularly after stem elongation the greater the yield deficit (Kirkegaard *et al.*, 2008). Flail cutting is currently being investigated for *P. chrysocephala* control along with using sheep to graze the crop as defoliation methods to reduce *P. chrysocephala* larval infestation within plants (Syngenta, 2019). Here the use of mowing the OSR in March is tested.

4.2.4 Future - synthetic chemistry:

At the time of the experiment, the seed treatment Lumiposa™ (active ingredient: cyantraniliprole) was registered for use in Poland, Hungary and Romania to protect OSR from *P. chrysocephala* attack, but not in the UK (NFU, 2018). Lumiposa™ is recommended to protect early growth OSR until BBCH 13-14 from *P. chrysocephala* and is marketed to "protect seedlings producing uniform and heathy stands" (DuPont, 2017). However, little data is available on the effectiveness of Lumiposa™ as a protective measure in OSR from *P. chrysocephala* feeding or larval infestation in the UK. Before any registration in the UK, it is crucial to test how effective a product is in un-biased field trials.

4.2.5 Aims of this study:

The relative effects of multiple pest protection options (past, present, and future) available to farmers for control of *P. chrysocephala* are tested in parallel to help quantify the effects of the neonicotinoid ban on crop protection and yield in OSR, and the effects on natural enemies and biodiversity.

It is hypothesised that neonicotinoid seed treatment will give the best control of *P. chrysocephala* and the untreated monoculture OSR will be the most severely effected by pest pressure. The ranking of other treatments in terms of how the compare in terms of pest control and in crop yield are the major interest.

4.3 Methods:

4.3.1 Experimental Design & Treatment establishment:

The experiment tested a variety of control options for *Psylliodes chrysocephala* that were available in the past (i.e. neonicotinoid seed treatment and pyrethroid sprays), that are available at the present time (pyrethroid sprays and altering the seed rate) and those that might be available in the future (new synthetic seed treatments and foliar sprays, and cultural methods such as trap cropping and nurse cropping, Table 1 and table 2).

The experiment was performed as a replicated plot trial on two fields (Great Field and West Barnfield) on Rothamsted farm, Harpenden, Herts., UK (Figure 1). Plots were 9m x 9m with a 2m tram line on all plot edges and between treatment areas and the surrounding crop (DK Imperial CL).

4.3.2 West Barnfield:

In West Barnfield 13 different treatments were tested grouped into past, present and future control groups as follows - **Past:** neonicotinoids seed dressing, foliar spray, **Current:** pyrethroid sprayed once or three times, **future cultural:** trap cropping and nurse cropping, and **future synthetic chemical**: Lumiposa seed treatment (Table 2). The number of pyrethroid applications was varied to simulate the decline in effectiveness over time (1 application to simulate a single effective treatment of the past and 3 application to represent the current efficacy levels). A control of untreated OSR (**current**) was replicated three times to produce three randomized blocks (Figure 1).

4.3.3 Great Field:

In Great Field the **Past:** neonicotinoid seed dressing, **current**: increased seed application rate, and **new cultural** trap cropping and crop defoliation methods were tested; the experiment comprised 10 treatments in three randomised blocks (Table 3 and Figure 3).

4.3.4 Link between fields:

The two experiments were linked by three common treatments (Table 1 and Table 3): **Past**: OSR with neonicotinoid seed dressing (Treatment E in West Barnfield and treatment J in Great Field). **Current**: OSR untreated (treatment A both fields), and **new cultural**: OSR with a trap cop border (Treatment D in West Barnfield and Treatment H in Great Field).

4.3.5 Seed bed preparation and plot establishment:

The same plant cultivars were used on both fields: an OSR Clearfield ® hybrid cultivar DK Imperial was selected to allow application of specific Clearfield herbicides to kill-off the nurse crop plants. Clearfield ® cultivars of OSR exhibit resistance to specific herbicides and allow applications of herbicides to control brassica weeds with little impact on the crop (BASF 2018). All cultivars and species used throughout are readily available from multiple seed suppliers (Table *22*). The OSR seed rate was standardised at 100/m^2 in West Barnfield and at varying rates of 60, 100 or 120 seeds/m^2 in Great Field where the impact of seed rate was tested (Table 3).

Both fields were power harrowed prior to OSR drilling. Great Field plots were drilled on 27th August 2016 and West Barnfield plots were drilled on 31st August 2016. Both fields were irrigated to 10mm on 1st September 2016. Other than insecticide applications, the experiment was managed by Rothamsted Farm according to standard practice.

4.3.6 Trap crops:

Trap crops were drilled in a 1m wide strip as a border to plots of the relevant treatments (Treatments C and D in West Barnfield: 1, and G, H, and I in Great Field; Table 3). The whole treatment plot remained at 9x9m with the central OSR crop comprising the central 7x7m. Turnip rape (*Brassica rapa*) cv Jupiter was selected as this species performed well in previous experiments (Chapter 2) and has the benefit that it can be cropped and can contribute to seed yield unlike other potential Brassica trap crops such as Tyfon. Seed rate of 150 seeds/m^2 as per Chapter 3. Trap crop borders were removed before flowering to avoid any seed drop and contamination of the field site.

4.3.7 Nurse crop:

In **West Barnfield** OSR sown with a nurse crop mix was tested. The mix comprised Pak Choi (*Brassica rapa var. chinensis),* Chinese cabbage (*Brassica rapa subsp. Pekinensis (Lour.) Rupr),* Salad rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum,* Table *2*2). This mix was used in this study as it showed the most promise as a nurse mix following a previous field experiment (Chapter 2 this thesis). Seed rate was maintained as in the previous experiment (Table *2*2) and was broadcast sown (by myself).

4.3.8 Defoliation:

Defoliation of OSR plants to reduce larval infestation of the stems via removal of the leaves was carried out by mechanical cutting. Cutting was achieved using a manual mower (5 cm above ground). All cut material was left on the plots, to reduce labour time. Cutting was performed on 14th March 2017.

4.3.9 Synthetic chemical treatments:

In **West Barnfield** Treatments F and H received one pyrethroid spray and Treatments G and I received three applications: Table 1). Applications occurred on 30th September 2016, 8th October 2016, and 17th October 2016; Hallmark Zeon, (75 ml/ha ai lambda cyhalothrin). The efficacy of applying a neonicotinoid spray, Biscaya® (Active Ingredient: thiacloprid 240g/l (Bayer 2019)), as a potential pest protection measure for autumn *P. chrysocephala* was tested here on treatments K, L and M (Table); Biscaya® was applied on 30th September 2016. Four treatments (E, F, G and L) were sown using seed with neonicotinoid seed dressing (Cruiser® - active Ingredient: thiamethoxam, Syngenta, Table). Also, in West Barnfield, two treatments were sown using seed treated with Lumiposa® seed dressing (Active Ingredient: cyantraniliprole, DuPont). The Clearfield herbicide Cleravo® (AI: imazamox) was applied to both fields (1Ltr/ Ha) on 8th November 2016 to control weeds and specifically, the nurse crop.

In **Great Field** application of a single pyrethroid insecticide spray (Hallmark® Active Ingredient: lambda cyhalothrin 100g/l, Syngenta) was made to all plots as the experiment. This was not part of the treatment design but was needed as the experiment was in danger of being wiped out by *P. chrysocephala*. A neonicotinoid seed dressing Cruiser® was applied to treatment J (AI: thiamethoxam, Syngenta, 600g/L at 100ml per 100,000 seeds). No other insecticide applications were used on treatment plots in Great field.

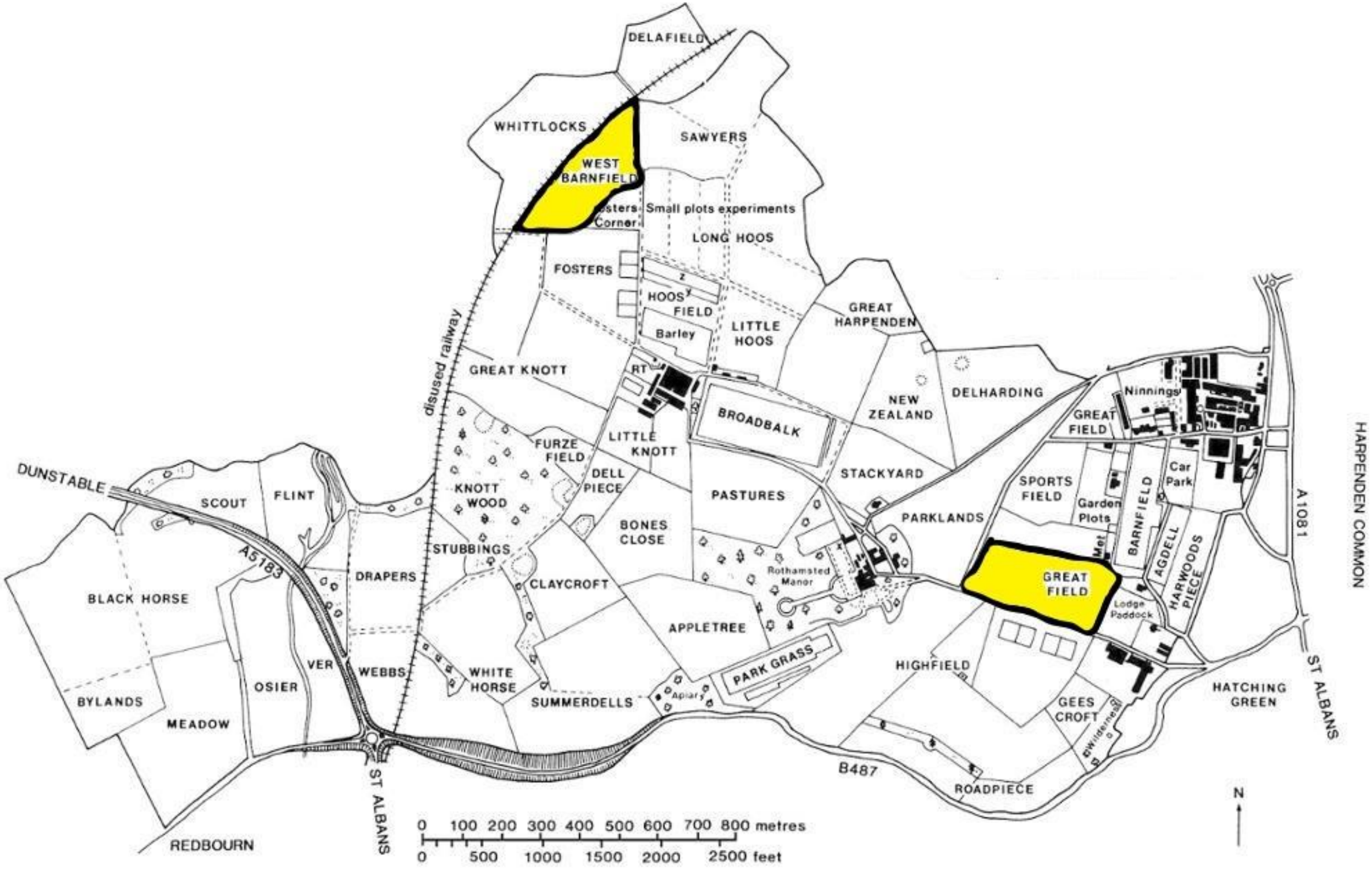


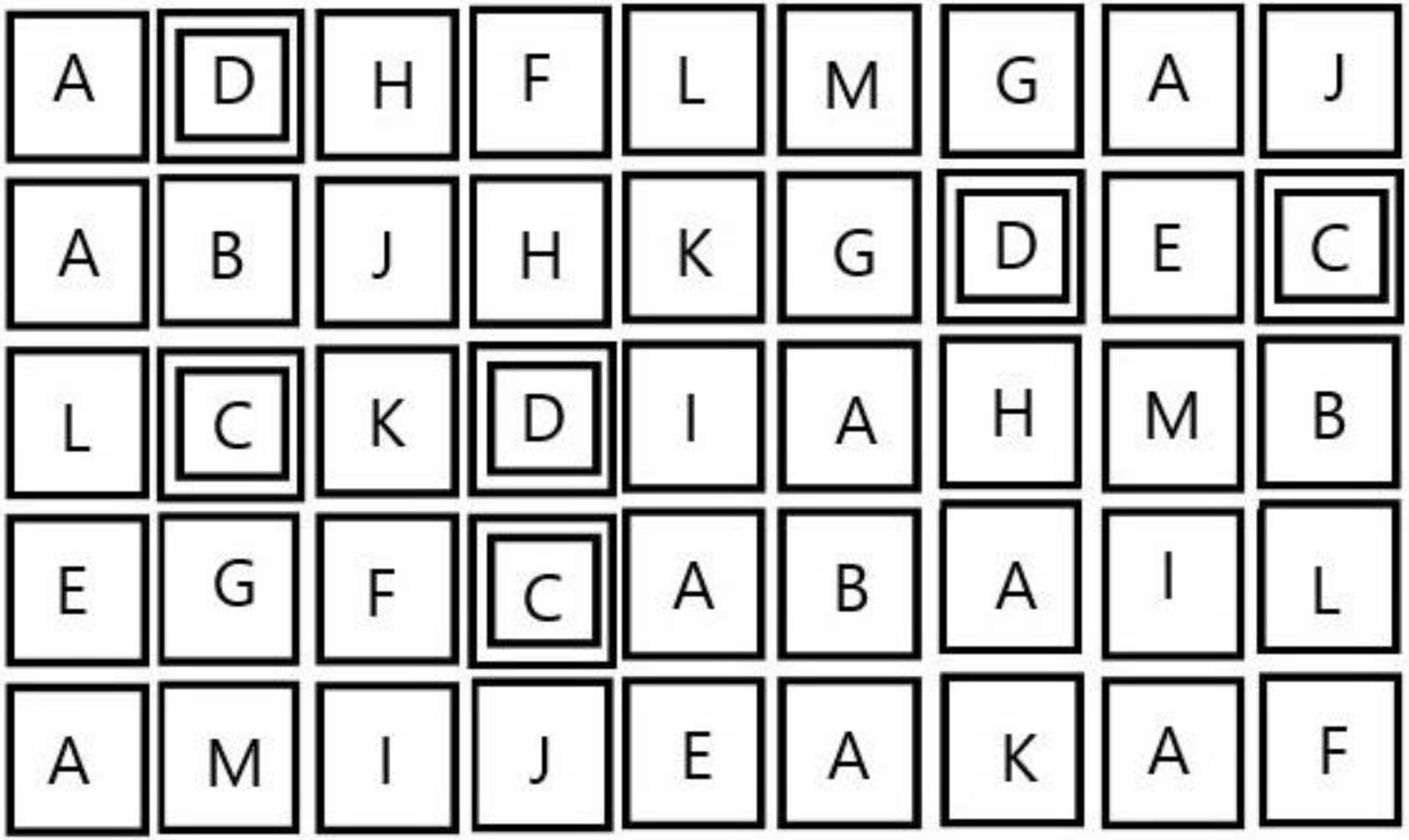
Figure 1. Field location on Rothamsted farm site at Harpenden UK. Location of experimental fields highlighted in yellow.

Table 1 West Barnfield treatment details testing control options for *Psylliodes chrysocephala* in oilseed rape (OSR, *Brassica napus*) on Rothamsted Farm (UK). OSR seed rate was always 100 seeds/m^2, trap crop border was Turnip rape (*Brassica rapa* cv. Jupiter) at 150 seeds/m^2. The neonicotinoid seed treatment was Cruiser® active ingredient: thiamethoxam; the neonicotinoid spray was Biscaya® active ingredient: thiacloprid. The pyrethroid spray used was Hallmark Zeon® active ingredient: lambda cyhalothrin at 75ml/ha; The seed treatment Lumiposa™® active ingredient: cyantraniliprole.

Treatment	**West Barnfield**
A	OSR - monoculture
B	OSR – with Brassica nurse crop mix
C	OSR – with Brassica nurse crop and Turnip rape border
D	OSR – with Turnip rape border
E	OSR – with neonicotinoid seed treatment
F	OSR - with neonicotinoid seed treatment + low pyrethroid spray (x1 application)
G	OSR with neonicotinoid seed treatment + high pyrethroid spray (x3 applications)
H	OSR untreated (no seed treatment) + low pyrethroid spray (x1 application)
I	OSR untreated (no seed treatment) + high pyrethroid spray (x3 application)
J	OSR with Lumiposa™ seed treatment
K	OSR with Lumiposa™ seed treatment + Neonicotinoid spray
L	OSR + Neonicotinoid spray
M	OSR with Neonicotinoid seed treatment + Neonicotinoid spray

Table 2 Nurse crop species composition and associated seed rate (number of seeds sown per m2). This was used in Treatments B (nurse crop) and C (trap crop) in the West Barnfield field experiment (see Table 1).

Species	**Seed rate (m^2)**
Pak Choi (*Brassica rapa var. chinensis* cv. Joi choi*)*	75
Chinese cabbage (*Brassica rapa subsp. Pekinensis (Lour.)*	75
Salad rocket (*Eruca sativa*)	150
Linseed (*Linum usitatissimum*) cv. Abacus	150

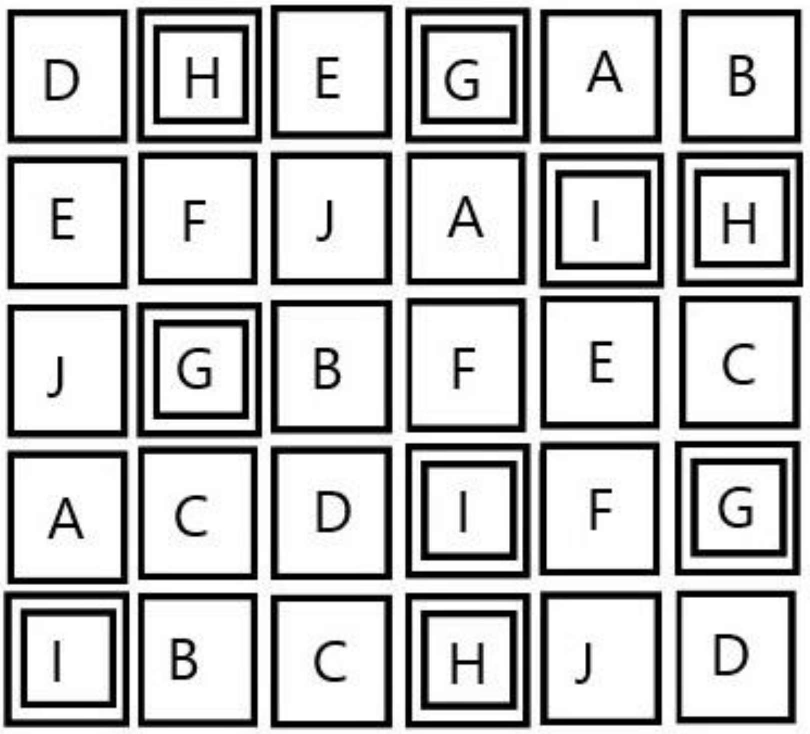


A	D	H	F	L	M	G	A	J
A	B	J	H	K	G	D	E	C
L	C	K	D	I	A	H	M	B
E	G	F	C	A	B	A	I	L
A	M	I	J	E	A	K	A	F

Figure 2. West Barnfield treatment layout as established in the field.

Table 3 Great field treatment details testing control options for *Psylliodes chrysocephala* in oilseed rape (OSR, *Brassica napus*) on Rothamsted Farm (UK). Assessing the effect of low medium and high OSR seed rate (60, 100 and 120seeds/m^2) in combination with defoliation (cut) to 5mm plant height. The effect of a 1m trap crop border (Turnip rape *Brassica rapa* at 150 seeds/m^2) around plots was also applied.

Treatment	**Great field**
A	OSR Low seed rate (60 seeds/m^2) - no cut
B	OSR Medium seed rate (100 seeds/m^2) - no cut
C	OSR High seed rate (120 seeds/m^2) - no cut
D	OSR Low seed rate (60 seeds/m^2) - cut
E	OSR Medium seed rate (100 seeds/m^2) - cut
F	OSR High seed rate (120 seeds/m^2) + cut
G	OSR Low seed rate (60 seeds/m^2) - with Turnip rape border
H	OSR Medium seed rate (100 seeds/m^2) - with Turnip rape border
I	OSR High seed rate (120 seeds/m^2) - with Turnip rape border
J	OSR Medium seed rate (100 seeds/m^2) - Neonicotinoid seed treatment



D	H	E	G	A	B
E	F	J	A	I	H
J	G	B	F	E	C
A	C	D	I	F	G
I	B	C	H	J	D

Figure 3. Great Field treatment layout.

4.3.10 *Psylliodes chrysocephala* migration:

Psylliodes chrysocephala migration into the field was recorded using yellow water traps (Ringot flora; Nickerson Brothers Ltd, Market Rasen, Lincolnshire, UK) positioned on each edge of both fields. Four traps were positioned in a square with two traps 3-meters from the crop edge in the headland and two traps 20-meters into the crop. All traps were positioned at ground level and 2/3 filled with a water with a drop of detergent (Teepol). Trapping was carried out between 26th August until 31st October 2016. Traps were emptied twice/week (every Monday and Thursday); *P. chrysocephala* numbers were recorded, and the traps were re-set with fresh water and detergent.

4.3.11 *Psylliodes chrysocephala* resistance to pyrethroids:

A measure of the percentage of *P. chrysocephala* resistance to the active ingredient of the pyrethroid Hallmark, lambda-cyhalothrin, was made for populations of beetles collected from both fields. Adult beetles (c. 50 per field) were collected using an electric aspirator from 2 meters surrounding experimental plots in each field. Collected beetles were assessed for resistance using the coated vial bioassay (IRAC 2014). Beetles were randomly assigned to treatment or control groups and introduced in groups of c.10 to glass vials coated with either a lambda cyhalothrin (full field rate 7.5g ai/ha) or acetone. Beetles were observed for movement after 24 and 48 hours and scored as unaffected, affected, or dead. The percentage population exhibiting resistance (unaffected) was calculated by the survivors in the treatment vial. This test was performed by S. Foster (Rothamsted Research) on 30th October 2016.

4.3.12 Leaf area injury:

Psylliodes chrysocephala feeding damage was assessed on plants positioned both in the border (1m from the plot edge, on each of the four sides, i.e., North, East, South, and West) and in the central 7m^2 area of the plot. Assessments were performed twice in all plots on 22nd September 2016 and 20th October 2016 in West Barnfield and 19th September 2016 and 21st October 2016 in Great Field). Five OSR plants were assessed along each border of each plot and 20 were assessed in a W-shaped sampling pattern from the crop centre, with 5 plants sampled per leg. Damage was determined by eye, estimated to the nearest 5% leaf area lost (also see chapters 2 and 3 this thesis). The growth stage of each OSR plant assessed for damage was also recorded (Lancashire et al. 1991). The leaf area loss of nurse crop plants (n=10 plants per species) and trap crop plants (five plants per plot edge, total n=20 plants per plot) were also assessed in the relevant treatments.

4.3.13 *Psylliodes chrysocephala* larval infestation:

Plants were destructively sampled to assess larval infestation. A sample of five plants for each species represented within each plot were collected, placed individually into labelled plastic bags, and transferred to the laboratory. Samples were stored at 4°C and processed as soon as possible. The stems and petioles of the plants were dissected under a binocular microscope, using a scalpel and tweezers to cut apart the plant in thin sections (Figure4). Any larvae found were identified and larval instars determined using the key in Ebbe-Hyman (1952). Plant samples were taken twice for each plot in both West Barnfield and Great Field on 3rd November 2016 and 24th February 2017; a third sample was collected on 27th March in Great Field after cutting the defoliation treatments (D-F).

Figure 4. Photograph of *Psylliodes chrysocephala* 3rd instar larvae found in the stem of an oilseed rape plant during plant dissections.

4.3.14 Pitfall trapping for ground-active arthropod diversity:

To assess the impact of the treatments on the diversity and abundance of ground-active arthropods, three pitfall traps were set in the centre of each plot approximately 1m apart in a triangular orientation. Traps catches were combined post-collection; individual trap catches within plots were not considered to be independent and were used to maximise the probability of catching representative samples of the active population. Traps in different plots where at least 10 meters apart are were considered independent. Each trap consisted of a plastic pot (7cm aperture) buried in the ground, so the pot rim was flush with ground level, with an inverted plant pot saucer supported with metal wire to restrict excess rain filling the trap pot and rendering it ineffective. Traps were 1/3 filled with a mixture of anti-freeze (ethylene glycol) and water to act as a euthaniser and to preserve catches. Traps were changed weekly from the 1st September 2016 to 3rd November 2016, then fortnightly until 1st December 2016 and subsequently monthly until May 2017. Samples from each plot were examined under a light microscope and invertebrates counted and identified to family (Chinery, 1993; Kirk, 1992), Thrips and Collembola were not counted. All samples were stored in ethanol post identification.

Figure 5. Photograph of pitfall trap sample processing and identification: arthropods are sorted into groups and counted.

4.3.15 Crop density:

A measure of crop density was carried out shortly before harvest (West Barnfield 16th June 2017, Great Field 15th June 2017). Within each plot, three 0.25m^2 quadrats were randomly placed in the central area of each plot and the number of OSR plants present was counted. From each quadrat, one whole plant was collected. Each plant was cut at ground level and individually bagged in large paper bags. Samples were returned to the laboratory and kept at 5^{0}C, until they were processed. Plants were collected when seed pods were still green (BBCH GS- 79; nearly all pods reached final size (Lancashire et al. 1991)) to avoid pod shattering. Each plant was measured for height, number of branches, set pods and blind stalks. Further measures on seed quality were not done as the seeds were not fully ripe. Collections were carried out on 15th June 2017 and 16th June 2017 from Great Field and West Barnfield, respectively.

4.3.16 Crop Harvest and yield measurements:

All plots were harvested by Rothamsted Research Farm using a plot harvester (Great Field on 18th June 2017 and West Barnfield on 19th June 2017).

From each plot a subsample of harvested grain was measured for thousand grain weight (TGW), oil and percentage moisture. This was done using standard procedures at Rothamsted Research Farm by taking fresh weight then drying the sample to remove moisture before re-weighing the sample. Seeds were counted for assessment of TGW using an Elmer C1 grain counter and weighed to calculate the weight of 1000 grains. Oil was measured using a Bruker, NMR calibrated for Rothamsted Oil and Moisture.

4.3.17 Statistical analysis:

In **West Barnfield** data were analysed according to the randomized block design. The experiment in **Great Field** was analysed using a factorial plus control (neonicotinoid seed treatment) treatment structure in randomized blocks where the factorial consisted of seed rate and control management type (trap crop/no trap crop, defoliation cut/ no cut).

Immigration Analysis of variance (ANOVA) was used to analyse differences in the numbers of *P. chrysocephala* caught in yellow water traps on each individual sample date where trap catches exceeded 10 individuals. Total numbers of beetles were also analysed. For both, each field edge was defined as a separate block and the two fields were analysed separately.

Adult feeding damage Due to incidences of injury levels scores of 0 and 100%, the data on percentage leaf area removed by feeding injury were Logit transformed and adjusted, and analysis of variance (ANOVA) with randomised blocks and nested treatment structure used to analyse differences between treatments for each sample date (September and October) separately on each field site.

Larval infestation: West Barnfield: - Total numbers of larvae per plant in the November sample were transformed ($\log_{10}$ (total larvae +1)) to account for zero counts and analysed by ANOVA. The February analysis was performed using un-transformed data due to absence of zero counts.

Great Field: - The total numbers of larvae (all three instars) per plant were analysed using ANOVA.

Pitfalls: Analysis of pitfall trap catches was restricted to Carabidae, Staphylinidae, Linyphiidae and *P. chrysocephala*. Each group was analysed separately by analysis of variance (ANOVA) on the grand total of catches over all sampling dates.

Crop Density: Plant density differences between treatments was analysed using an analysis of variance (ANOVA) on the number of plants per quadrat.

Plant productivity measures: Plant height, the number of branches, number of pods on the primary raceme, number of pods on the secondary raceme and total number of pods per plant was compared using analysis of variance (ANOVA).

Yield Thousand Grain Weight (TGW) was compared using analysis of variance (ANOVA). Oil as percentage yield was analysed using REML.

All statistical analyses were performed using GenStat V18, for windows (VSN International, Hemel Hempstead).

4.4 Results:

4.4.1 Plot establishment:

All treatments were successfully established with aerial photographs of each experiment shown in Figure 6 and Figure 7. The only issue in establishment was the salad rocket in the nurse mix in West Barnfield which did not successfully germinate. Ultimately, the nurse crop treatment (and the OSR crop plants among it) had to be manually removed, as the nurse crop plants were allowed to grow beyond the susceptible growth stage when the Clearfield herbicide was applied and was only partially effective. Due to fears from Rothamsted farm staff that the nurse and trap crop plants would become a problem in future years should they be allowed to flower and set seed; all nurse crop treatments were destroyed on 4th May 2017. The trap crop treatments had the border removed on the same day before seed set but crop plants within this treatment were not affected by this operation.

Figure 6. West Barnfield aerial photograph. Image from Google Earth (image date: 25/03/2017).

Figure 7 Great field aerial photograph. Image from Google Earth (image date: 25/03/2017).

4.4.2 *Psylliodes chrysocephala* migration:

Catches in yellow water traps showed that *P. chrysocephala* migrated into Great Field before West Barnfield with catches on the initial day of sampling (Figure 8 and Figure 9). Beetles were shown to have entered both fields and numbers were increasing at the time of the first assessment of adult feeding damage (19th September 2016). There was a directional bias in both fields with a majority of *Psylliodes chrysocephala* being caught on one field edge. Both fields had high populations to the South side of the crop and West Barnfield was also high on the East (Figure 8 and Figure 9).

In **West Barnfield**, more beetles were caught in traps placed 20m into the crop compared with traps placed 3m from the crop edge. The difference was statistically significant on 26th August ($F_{1,9}$=6.76, P=0.029) and 20th October 2016 ($F_{1,9}$=11.50, P=0.008). Again, there was some evidence of an effect on the grand total catches ($F_{1,9}$=4.62, P=0.060, Table 4).

In **Great Field** on two dates (10th and 13th October 2016) an effect of trap distance into the crop was observed with greater beetle numbers caught in traps placed 20m into the field than in traps 3m from the crop edge ($F_{1,9}$=8.87 P=0.016; $F_{1,9}$=7.21, P=0.025, respectively). There was some evidence of the total numbers of beetles caught in traps (accumulated value of all catches) being affected by distance into field, with those placed 20m onto the field catching more beetles than those place 3m from the crop edge ($F_{1,9}$=4.65, P=0.059, Table 4).

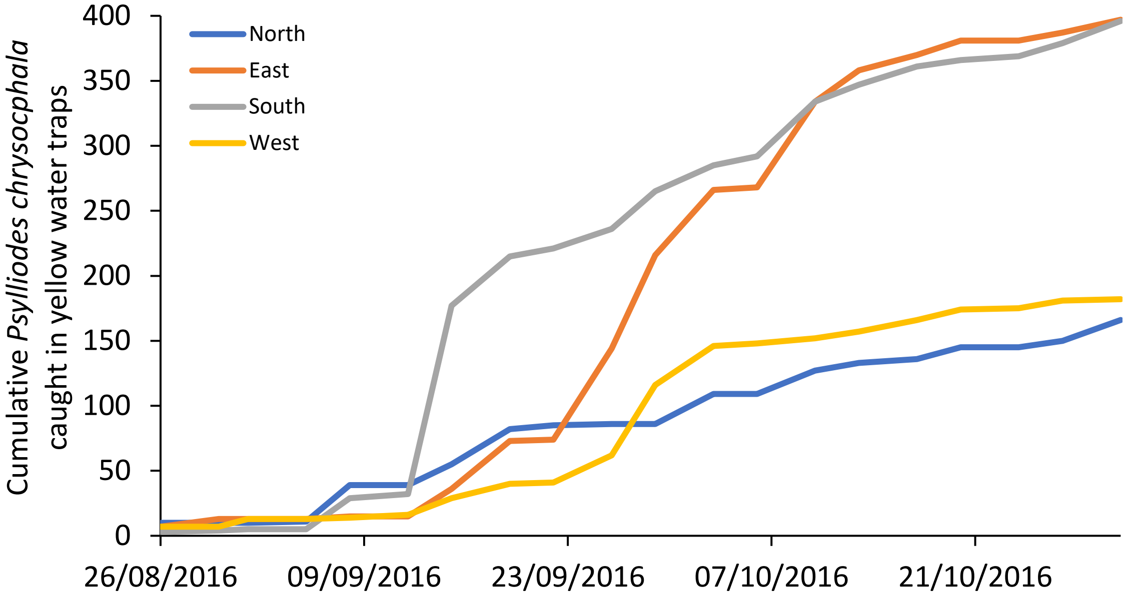


Figure 8. West Barnfield: cumulative yellow water trap counts of *Psylliodes chrysocephala* on each field edge.

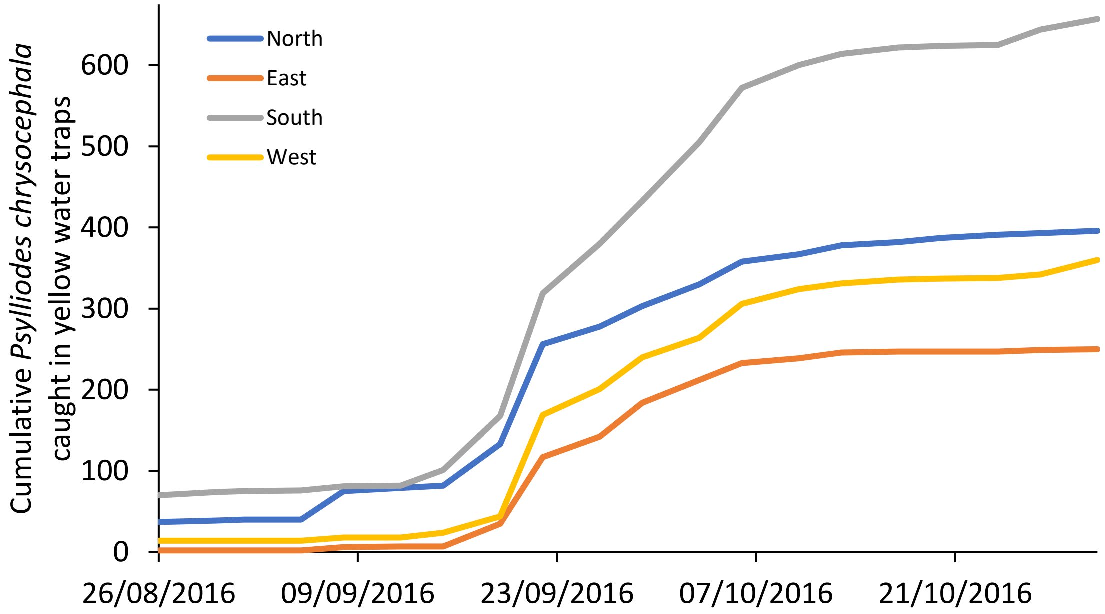


Figure 9. Great field: cumulative yellow water trap counts of *Psylliodes chrysocephala* set at each field edge.

Table 4 Grand mean and standard error of all *Psylliodes chrysocephala* adults caught in yellow water traps set 3m and 20m into two oilseed rape crops on Rothamsted Farm (UK).

	3m into crop	20m into crop
West Barnfield	52.4 (± 17.62)	90.2 (± 17.62)
Great Field	84.9 (±17.68)	123.0 (±17.68)

4.4.7 *Psylliodes chrysocephala* resistance to pyrethroids:

There was no control mortality in either sample (Table 5). There was a considerable difference in the levels of resistance between the two fields with 48.8% beetles in Great Field mobile after 48h exposure to the full field rate (7.5 g ai/ha) and 84.9% resistant in West Barnfield.

Table 5 Resistance testing of adult *Psylliodes chrysocephala* to the pyrethroid lambda-cyhalothrin at the full field rate (7.5 g ai/ha). Number of beetles active (unaffected) after 48 hours exposure and calculated percentage population exhibiting resistance.

Field	Acetone control	Lambda-cyhalothrin (7.5g ai/ha)	Percentage exhibiting resistance
Great Field	16/16	21/43	48.8%
West Barnfield	19/19	28/33	84.9%

4.4.3 *Psylliodes chrysocephala* adult feeding:

In **West Barnfield**, within the nurse crop treatments, no injury was observed on Linseed at any point during assessments. Salad rocket seed did not germinate and was never recorded in the treatments. Pak Choi and Chinese Cabbage were injured to lower levels than the OSR with a maximum percentage damage of 60% for Pak Choi and 50% for Chinese Cabbage

(Table 6). Trap crop borders were heavily damaged by adult beetles, with feeding injury observed on all assessed plants, with a maximum leaf area loss recorded of 65%. Injury levels on OSR exceeded 60% in all treatments. One incidence of 100% leaf area loss was observed in Treatment J (Lumiposa™ treated OSR).

Figure 10 and Figure 11 show the leaf area injury levels of the different treatments in September and October, respectively. Nurse crops reduced injury on the OSR (September: $F_{1,8}$=26.44, *P* <0.001, October: $F_{1,8}$=11.11, *P* =0.002) as did trap crop borders in September (September: $F_{1,8}$=25.6, *P* =0.002) but not in October ($F_{1,8}$=0.74, *P* =0.396). The level of injury was reduced by neonicotinoid seed treatment in September ($F_{1,6}$=9.52, *P* =0.005) but no difference between controls by the October assessment ($F_{1,6}$=26.9, *P* 0.948). The Lumiposa treatment also reduced leaf injury in September ($F_{2,6}$=6.27, 0.006) but not October ($F_{2,6}$=26.6, *P* =0.606). The spray applications did not reduce injury in September, although it was close to significant ($F_{2,6}$=2.97, *P* =0.069), and there was a reduction in injury in the October assessment ($F_{2,6}$=12.59, *P* <0.001). There was no effect of treatment on the growth stage of OSR (September: $F_{12,29}$=1, *P*=0.473; October: $F_{12,30}$=0.50, *P*=0.895, Table 7).

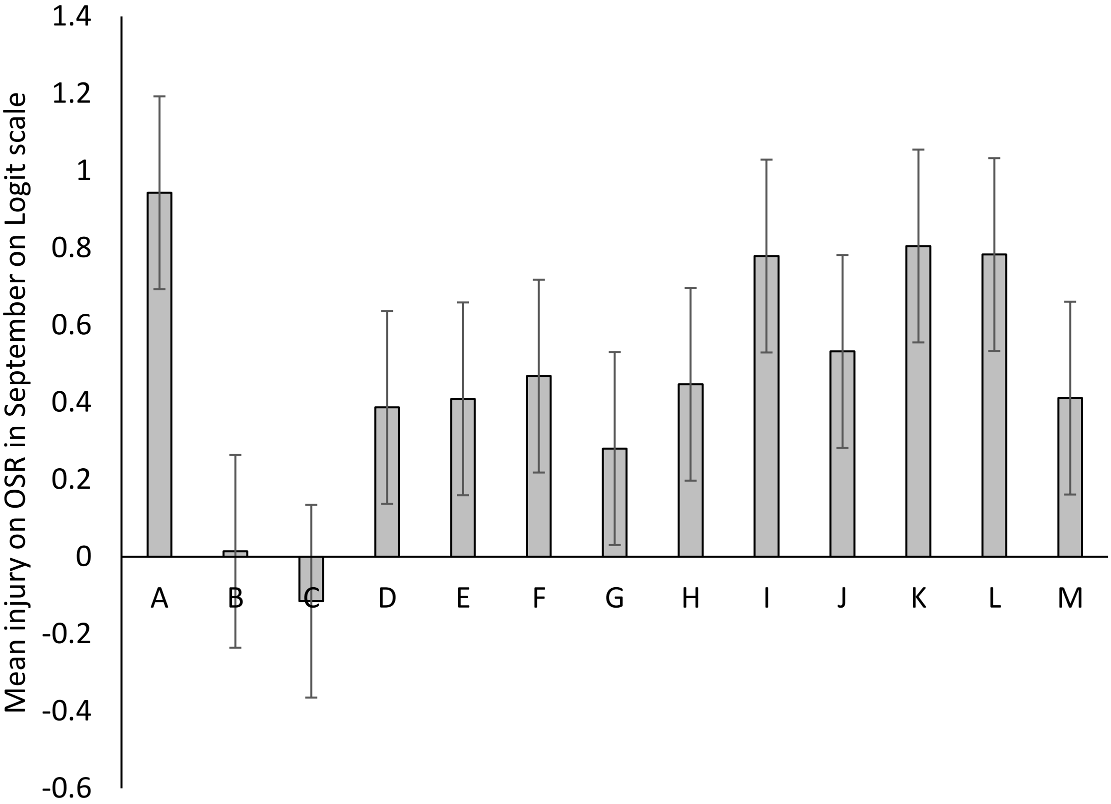


Figure 10. West Barnfield: Mean injury in September to oilseed rape (*Brassica napus*) from *Psylliodes chrysocephala* under multiple pest protection methods Treatment code: (A) OSR – untreated control, (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™ seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE shown.

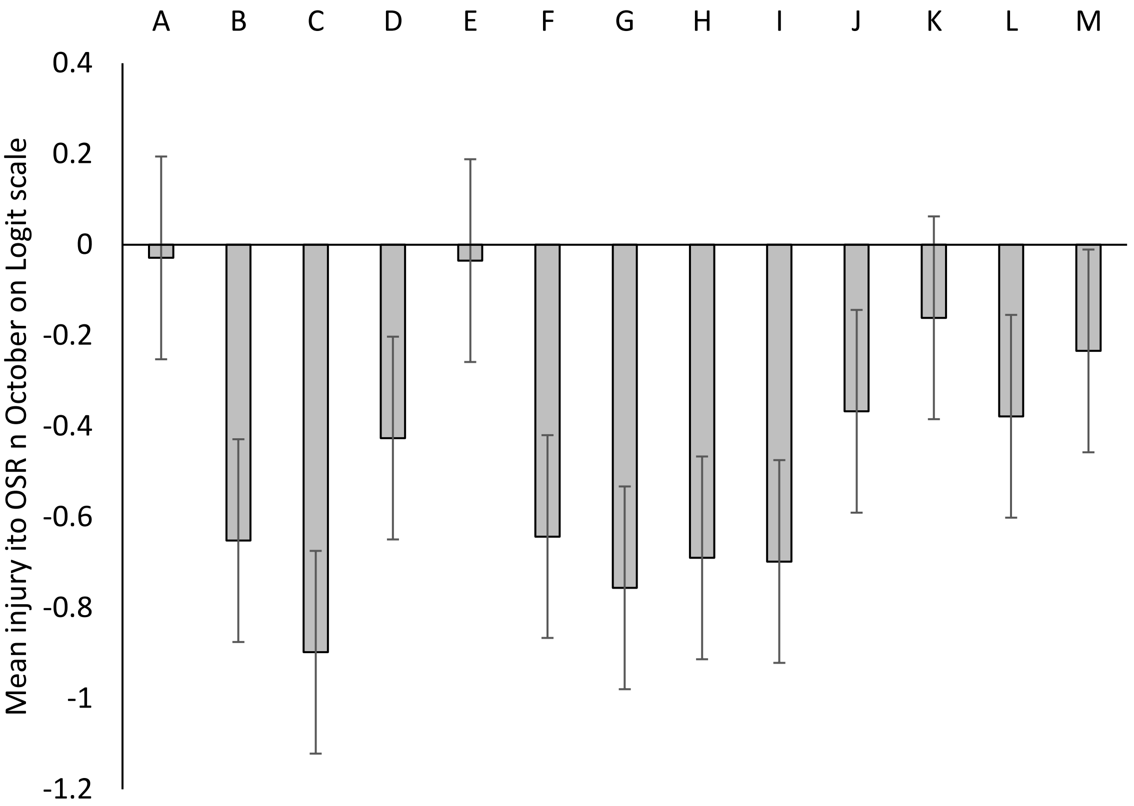


Figure 11. West Barnfield: Mean injury in October to oilseed rape (*Brassica napus*, OSR) from *Psylliodes chrysocephala* under multiple pest protection methods Treatment code: (A) OSR – untreated control, (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™ seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE shown.

Table 6. West Barnfield: percentage of leaf area lost with standard error on nurse crop species because of adult *Psylliodes chrysocephala* injury in September (20/09/2016) and October (20/10/2016) assessments. No Linseed shown as no leaf area loss was observed and no salad rocket because none germinated in the field.

	Pak Choi	Chinese Cabbage
September	45.6 (±2.91)	54.74 (±2.89)
October	21.77 (±1.89)	24.8 (±1.68)

In **Great Field** no effect of management type (control, defoliation, or trap crop) was evident for feeding injury on the OSR plants in September ($F_{2,4}$=0.44, *P*=0.651, Figure 12) or October samples ($F_{2,4}$=0.72, *P*=0.502, Figure 13). A significant effect of seed rate was apparent in September ($F_{2,4}$=5.04, *P*=0.021) with decreasing injury levels as seed rate increased. This was not significant in the October assessment ($F_{2,4}$=0.72, *P* =0.072). Reductions in the amount of injury on OSR within the 1m trap crop border was observed (September: $F_{2,4}$=30.76, *P*<0.001 and October: $F_{2,4}$=7.43, *P*=0.006).

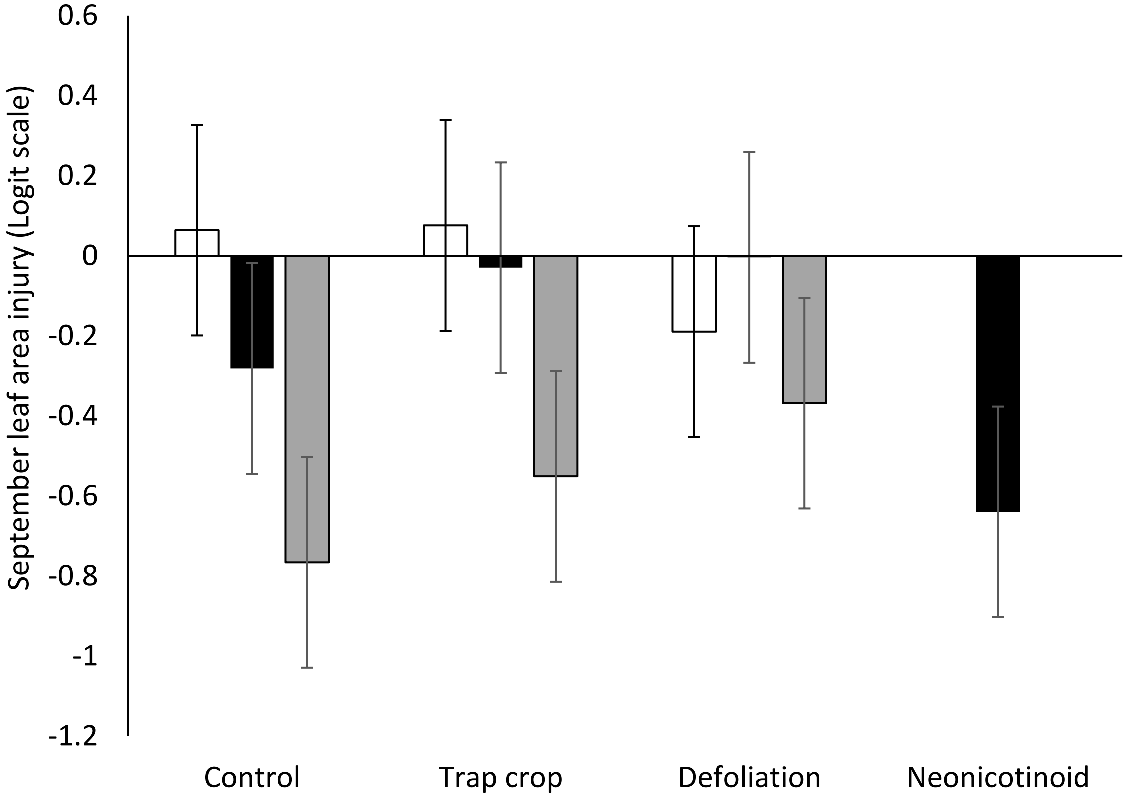


Figure 12. Great Field: Mean (+/-SE, on Logit scale) leaf area injury (attributed to *P. chrysocephala*) in September to central oilseed rape (OSR, Brassica napus) sown at 3 seed rates and under different pest protection methods. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR seed treated with neonicotinoid (Cruiser). OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2.

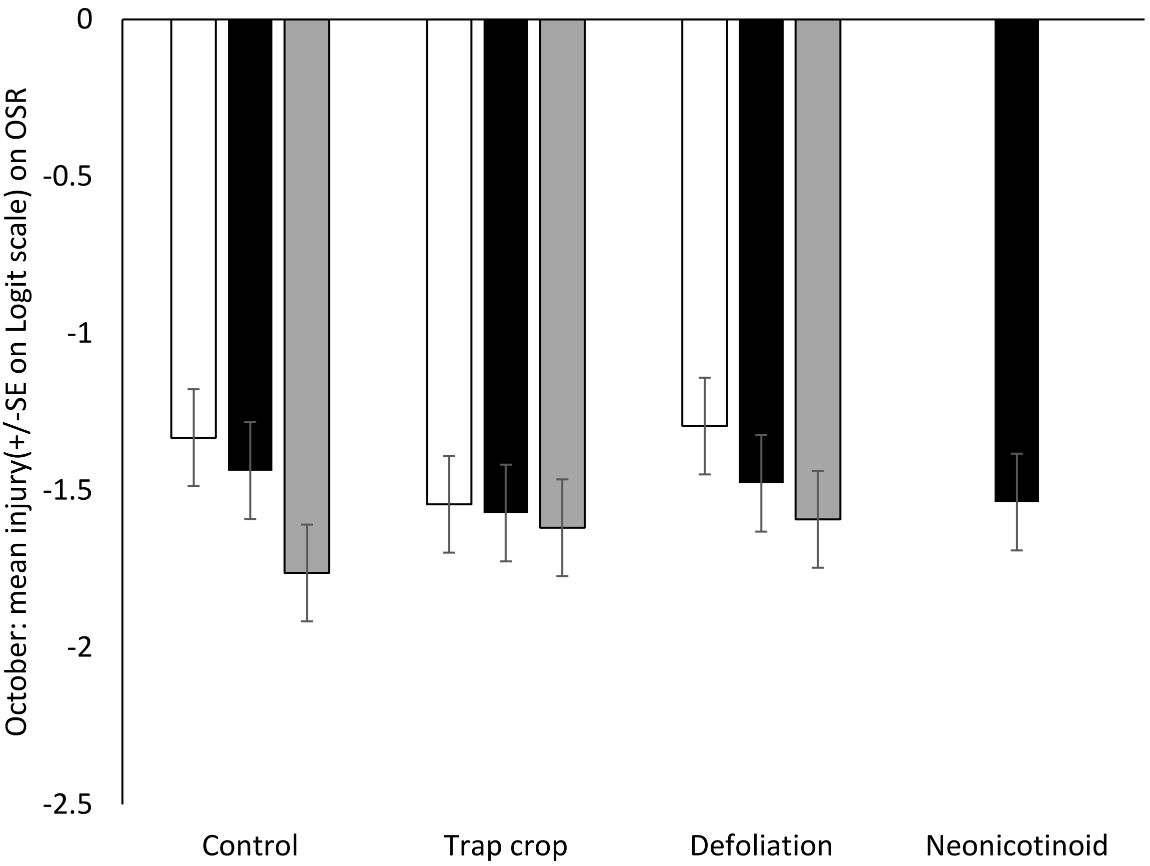


Figure 13. Great Field: Mean (+/-SE, on Logit scale) leaf area injury (attributed to *P. chrysocephala*) in September to central oilseed rape (OSR, *Brassica napus*) sown at 3 seed rates and under different pest protection methods. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR seed treated with neonicotinoid (Cruiser). OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2.

Growth stage (GS) of the OSR plants in the plot centres in Great Field was not affected by treatment or seed rate in either sample (treatment September $F_{2,18}$=1.19, *P*=0.326 and October $F_{2,18}$=0.26, *P*=0.776 and seed rate September $F_{2,18}$=2.12, *P*=0.149; October

$F_{2,18}$=0.08, *P*=0.920, Table 7). No effect on the GS of OSR within the border metre of plots was observed in September ($F_{2,4}$=0.57, P=0.387). However, in the October assessment OSR plants within the trap crop border were at an earlier GS than other treatments ($F_{2,4}$=47.10, *P*<0.001).

Table 7 Growth stage of oilseed rape (OSR, *Brassica napus*) on the BBCH scale (Lancashire *et al.*, 1991) in the central area of experimental plots (see Table 1). Mode values have been rounded to the nearest whole value.

	September	October
West Barnfield	12	15
Great field	12	14-15

4.4.4 *Psylliodes chrysocephala* larval infestation:

West Barnfield: A total of 20 OSR plants were not infested with *P. chrysocephala* larvae, with a maximum of 54 in a single plant. A total of 258 plants exceeded the 5 larvae/plant threshold level for spray application (total n=470). In the November assessment turnip rape trap crop borders did not affect larvae loading in the OSR ($F_{1,8}$=1.79, *P* =0.191) neither did the nurse crop ($F_{1,8}$=0.34, *P* =0.566). Pyrethroid sprays applications did not reduce larvae loading ($F_{2,6}$=2.40, *P* =0.109) neither did neonicotinoid spray application ($F_{1,6}$=1.36, *P* =0.252). Seed treatment with Lumiposa™ did not affect larvae numbers ($F_{2,6}$=0.69, *P* - =0.509), whereas neonicotinoid seed treatment did reduce the number of larvae ($F_{1,6}$=4.70, *P* =0.038). In the February assessment the number of larvae per plant was significantly different between treatments ($F_{12,180}$=19.06, $P<0.001$) with higher numbers of larvae in both nurse crop and trap crop treatments and reduced in neonicotinoid seed treatments (figure 14).

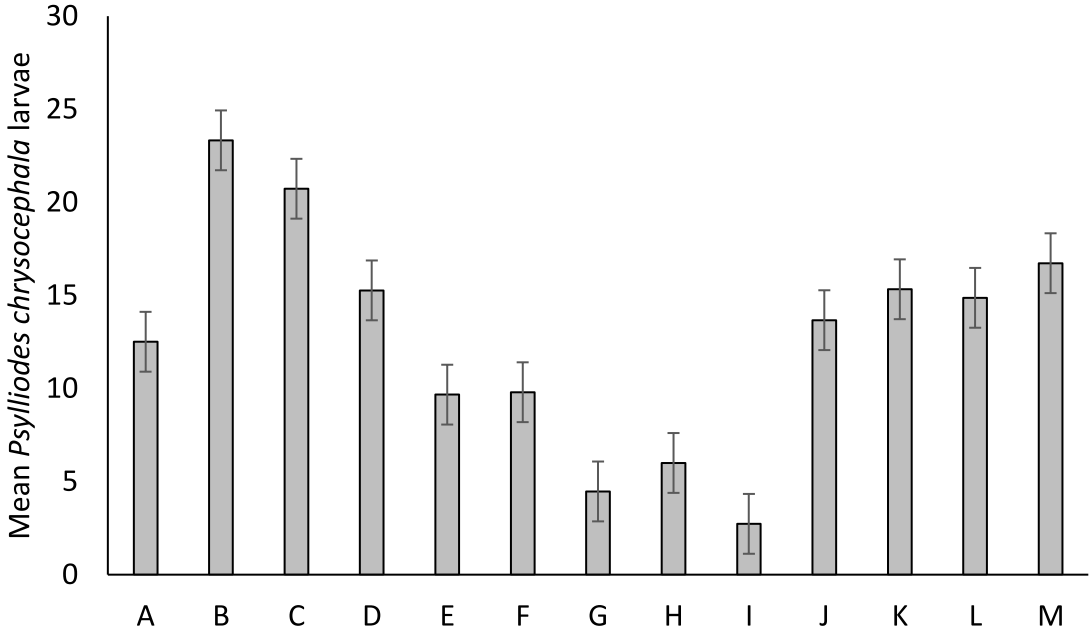


Figure 14. West Barnfield: Grand means (+/-SE) of *Psylliodes chrysocephala* larvae extracted from oilseed rape (OSR, *Brassica napus*) plants from the central area of plots with different control measures against P/ chrysocephala: Treatment code: (A) OSR – untreated control, (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™ seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya).

Great Field: In the November assessment, the larval numbers were reduced with increasing OSR seed rate but were not significantly different ($F_{2,4}$=2.62, *P*=0.103) and the presence of a trap crop also had no effect on larval numbers ($F_{2,4}$=0.46, *P*=0.640, see Figure 14).

At the February assessment only six individual OSR plants avoided infestation with *P. chrysocephala* larvae (total dissected N= 481). Reductions in larval numbers were seen as seed rate increased ($F_{2,4}$=14.18, *P* <0.001, Figure 15) there was no effect from the different control measures ($F_{2,4}$=0.81, *P*=0.445, Figure 15).

At the final assessment in March seed rate once again showed significant effects on total larval numbers per plant with numbers reducing as seed rate increased (Table 8, $F_{2,4}$=9.74, *P*=0.002). The defoliation treatment showed a significant reduction in larvae infection numbers in plots ($F_{2,4}$=90.37, *P*<0.001). With defoliation reducing the per plant larvae to below the action threshold whereas all other treatments were much higher (Figure 16).

The number of *P. chrysocephala* larvae extracted from OSR plants increased between each assessment with highest numbers in the March assessment (Figure 17). Defoliation had clear effects, reducing the numbers within the defoliated plants on the March assessment (Figure 17). When looking at the numbers of larvae in relation to the OSR seed rate, no consistent pattern was observed with larval number decreasing as seed rate increased in November which was reversed in February and not continuous in March but showing lower numbers of larvae at higher seed application rate (Table 9).

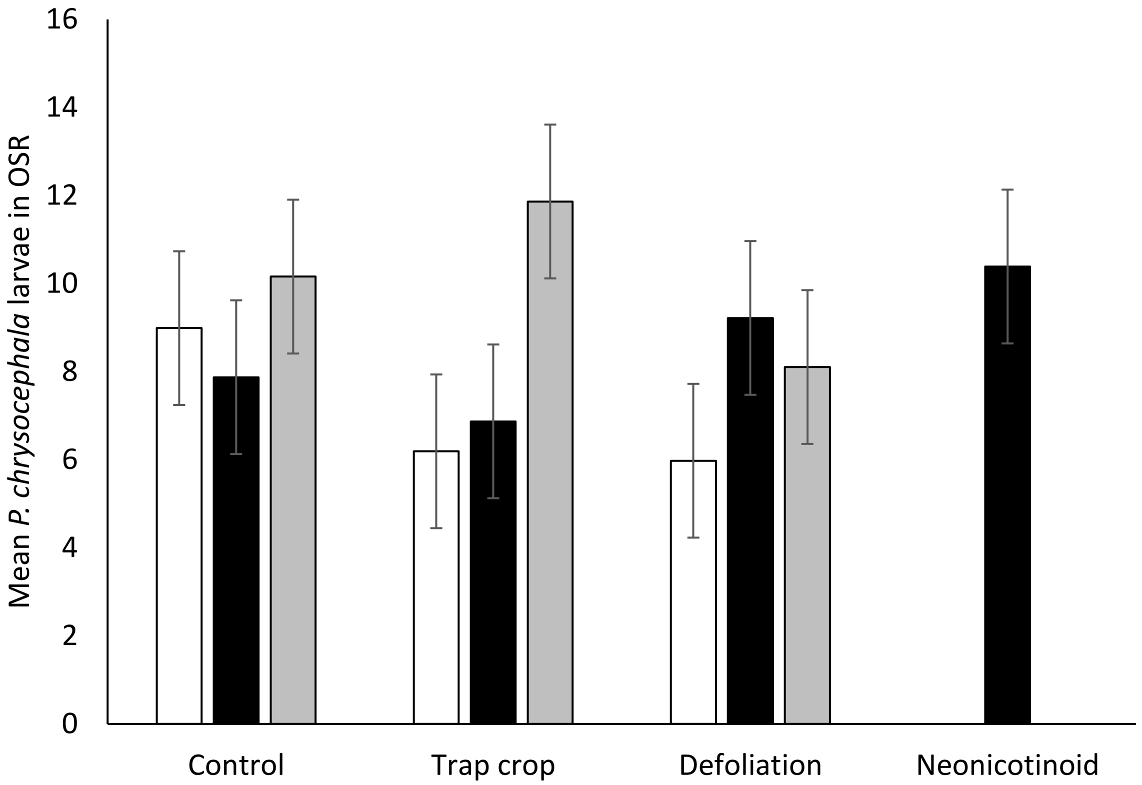


Figure 15. Great Field: Mean number of *Psylliodes chrysocephala* larvae dissected in November from oilseed rape (OSR, *Brassica napus*) sown at 3 seed rates and under different pest protection methods. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR seed treated with neonicotinoid (Cruiser). OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2.SE shown.

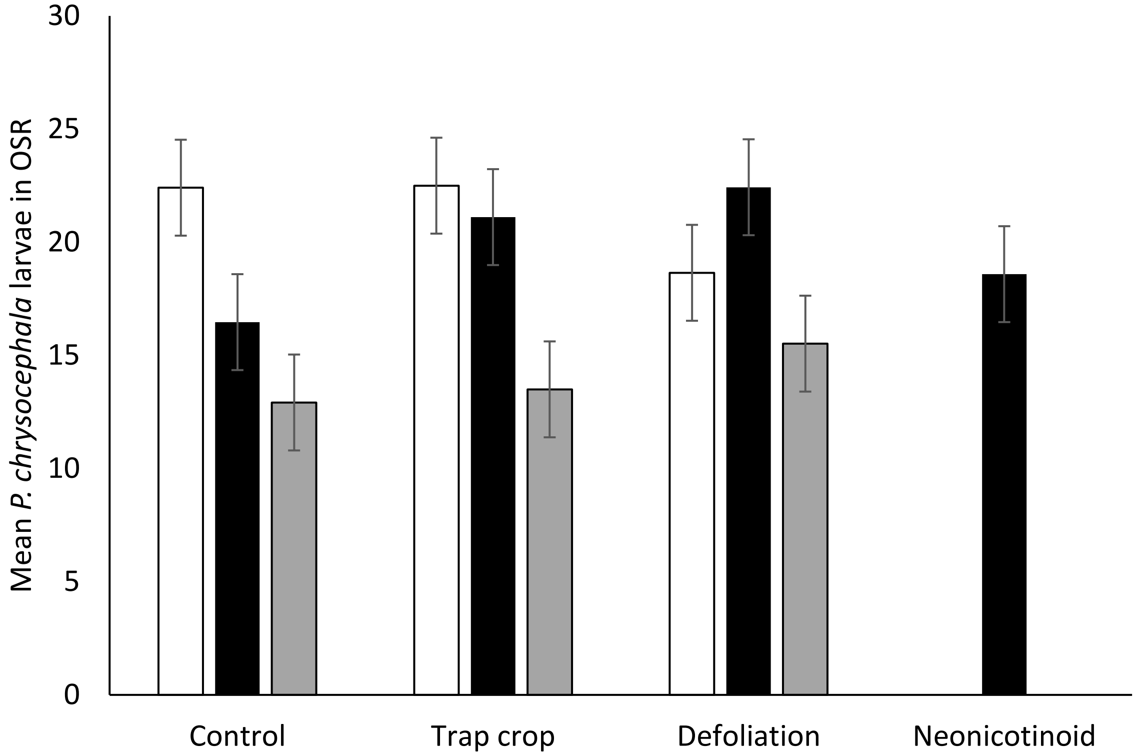


Figure 16 Great Field: Mean *Psylliodes chrysocephala* larvae dissected in February from oilseed rape (OSR, *Brassica napus*) sown at 3 different seed rates and under different pest protection methods. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR seed treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

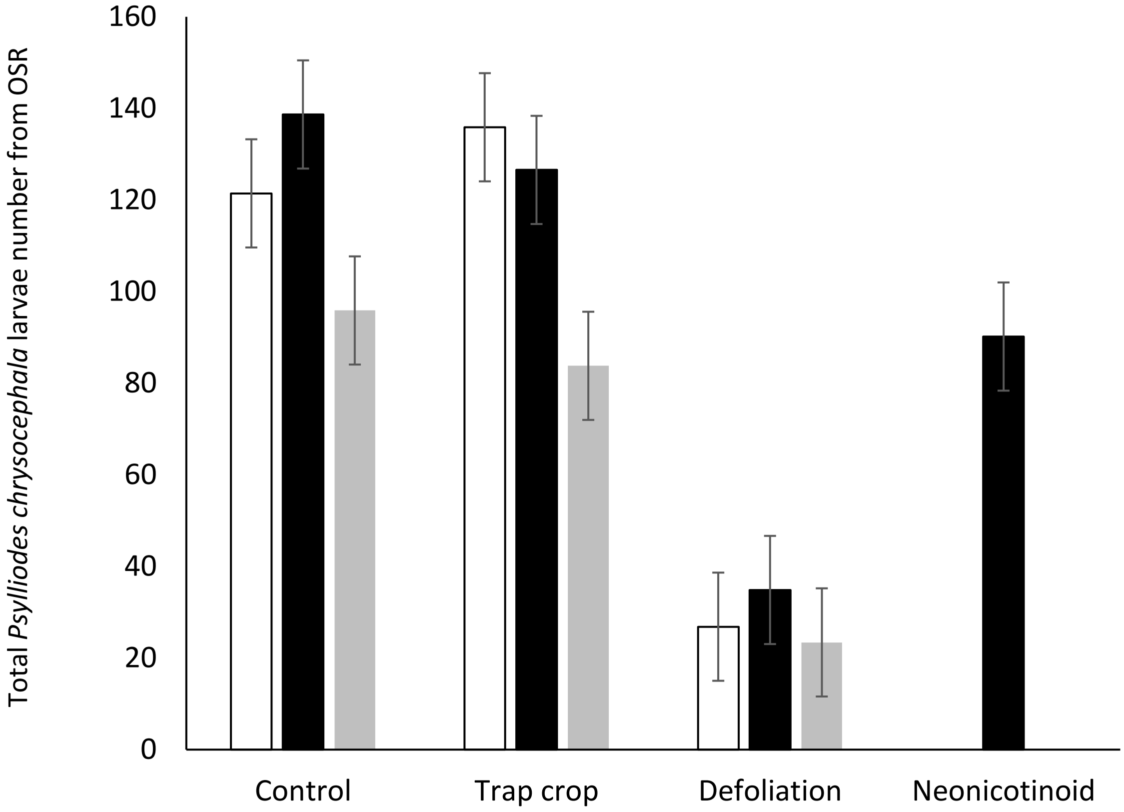


Figure 17. Great field: Mean *Psylliodes chrysocephala* larvae removed from 10 dissected oilseed rape (OSR, *Brassica napus*) plants in March 2016. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. Data shown is for plot totals larvae counts from 10 dissected plants with SE shown.

Table 8 Great field: mean (+/- SE) *Psylliodes chrysocephala* larvae dissected from 10 oilseed rape (OSR, *Brassica napus*) plants. Values are means of all replicates and all seed rates of each treatment, n=9 for control, defoliation cut and turnip rape trap crop boarder and n=3 for neonicotinoid.

Treatment	November	February	March (post cutting)
Untreated	45 (+/- 9.85)	88.3 (+/-12.74)	118.3 (+/- 11.43)
Defoliation	41.8 (+/- 9.85)	94.7 (+/-12.74)	28.3 (+/- 11.43) (*)
Turnip rape trap crop border	38.6 (+/- 9.85)	91.6 (+/-12.74)	115.1 (+/- 11.43)
Neonicotinoid seed treatment	52.7 (+/- 9.85)	91.3 (+/-12.74)	92.3 (+/- 11.43)

Table 9 Great field: mean numbers of *Psylliodes chrysocephala* larvae dissected from oilseed rape (OSR, *Brassica napus*) plants at three seed rate applications: low 60s/m2, medium 100s/m2 and high 120s/m2. Values are means of all replicates, control, defoliation cut and turnip rape trap crop border.

Treatment	November	February	March
Low seed rate	34.8 (+/- 9.85)	107 (+/- 12.4)	93.3 (+/- 11.43)
Medium seed rate	41.1 (+/- 9.85)	97.2 (+/- 12.4)	101 (+/- 11.43)
High seed rate	49.9 (+/- 9.85)	70.3 (+/- 12.4)	67.4 (+/- 11.43)
Neonicotinoid seed treatment (always medium)	52.7 (+/- 9.85)	91.3 (+/- 12.4)	92.3 (+/- 11.43)

4.4.5 Larval loading in nurse and trap crop species:

Due to concerns from Rothamsted farm all nurse and trap crop plants were removed in May (04/05/2017) before they could flower. Therefore, the only measurement of *Psylliodes chrysocephala* in the companion plants was taken in November.

West Barnfield: Larvae were retrieved from all Chinese Cabbage (n= 35) and Pac Choi (n= 30) nurse crops dissected and in all but 2 of the turnip rape trap crop plants (n= 34), there was no evidence of any infestation in Linseed (Figure 18).

Great Field: Comparable numbers of larvae were extracted from both the OSR crop and the Turnip rape trap crop plants (Figure 19).

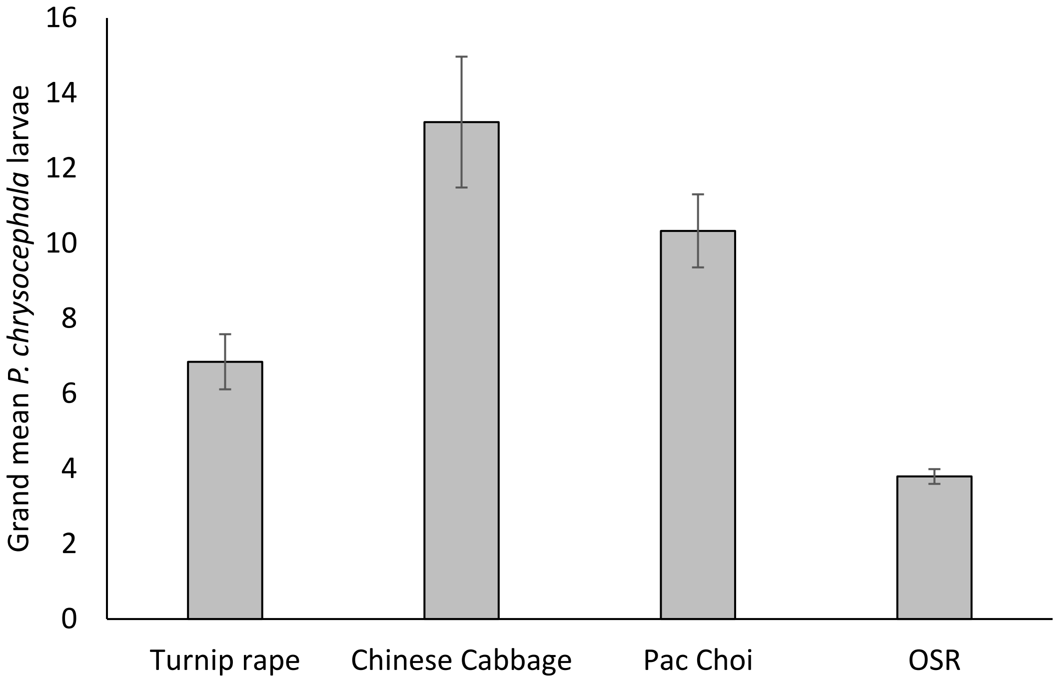


Figure 18. West Barnfield. Plant dissections for *Psylliodes chrysocephala* larvae. Showing grand mean (+/SE) for the trap crop (Turnip rape, *Brassica rapa*), nurse crops Chinese Cabbage (*Brassica rapa* var. chinensis *cv. Joi choi*) and Pac Choi (*Brassica rapa subsp. pekinensis* Lour.) alongside OSR (Oilseed rape, *Brassica napus*).

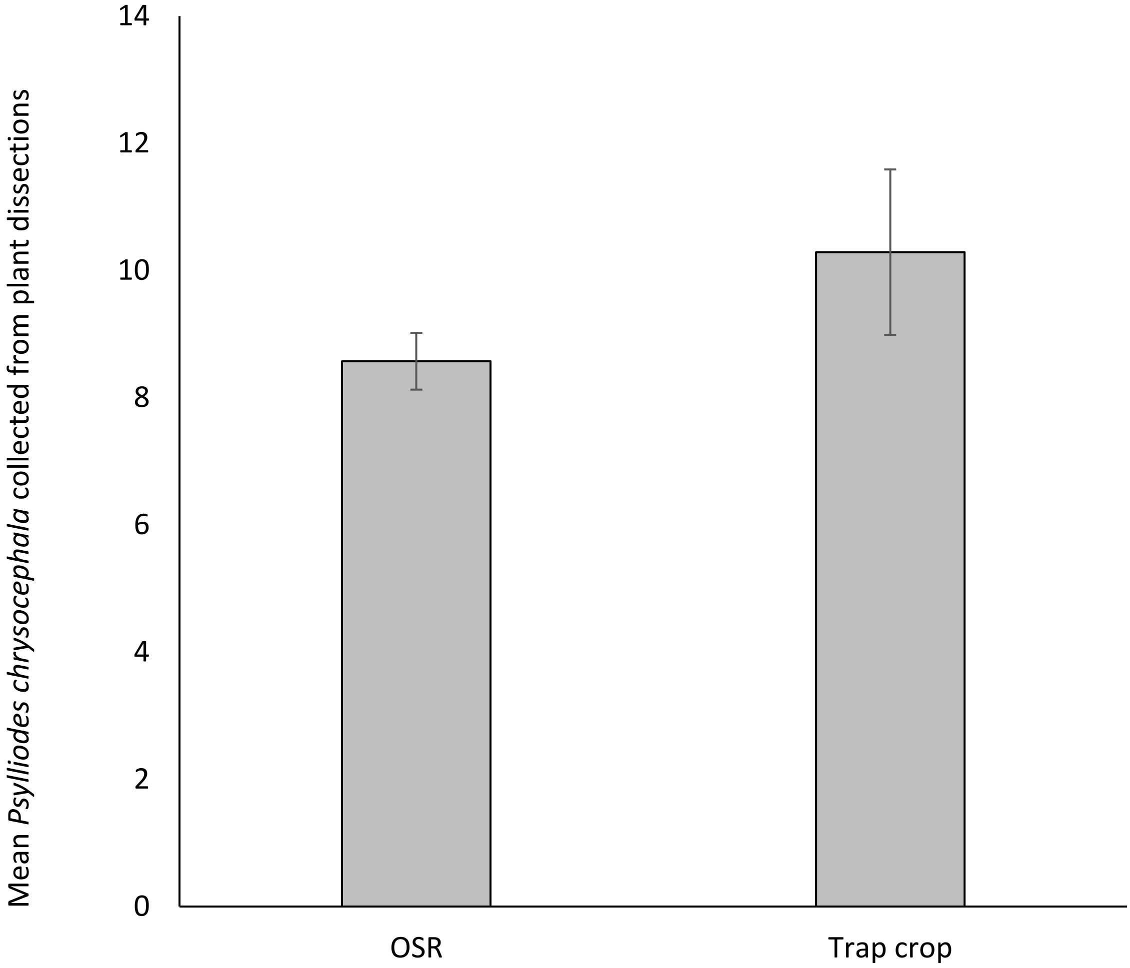


Figure 19. Great field. *Psylliodes chrysocephala* larvae dissected from Oilseed rape (OSR, *Brassica napus*) and trap crop (Turnip rape, *Brassica rapa*) plants. SE shown.

4.4.6 Pitfall trapping for ground-active arthropod diversity.

West Barnfield: No difference in the total numbers of *P. chrysocephala* was observed ($F_{12,30}$=1.18, *P*=0.339). Carabidae numbers were not influenced by trap crop borders ($F_{1,8}$=0.45, *P* =0.506) or nurse cropping ($F_{1,8}$=2.41, *P* =0.132) but greater numbers were caught in plots which received pyrethroid applications ($F_{2,6}$=5.77, *P*=0.008) but did not differ between any other chemical treatment ($F_{2,6}$=1.31, *P* =0.287). Staphylinidae numbers did not vary between treatments ($F_{12,30}$=0.80, *P*=0.649) neither did numbers of Linyphiidae ($F_{12,44}$=0.83, *P*=0.621, see Table 10).

Table 10. West Barnfield: grand means of pitfall traps within oilseed rape (*Brassica napus*) under various autumn pest protection practices. Treatments: (A) OSR – untreated control, (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa® seed dressing, (K) OSR treated with Lumiposa® seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE shown.

Treatment	*P. chrysocephala* (+/-SE 19.87)	Carabidae (+/- SE 18.52)	Staphylinidae (+/- SE 5.15)	Linyphiidae (+/- SE 11.88)
A	146.3	118.8	18.0	59.1
B	124.7	144.0	18.0	83.7
C	120.7	123.7	17.0	60.6
D	147.0	111.3	17.0	64.0
E	148.7	122.7	19.0	73.3
F	150.3	162.3 (*)	20.3	58.7
G	126.3	172.0 (*)	22.3	52.3
H	137.7	116.7	2.3	69.0
I	118.0	158.0	25.7	58.7
J	170.3	112.3	17.0	61.7
K	180.7	145.7	26.0	73.7
L	140.0	136.7	17.7	60.7
M	145.7	122.7	24.0	54.7

Great field: When comparing the grand total counts of *P. chrysocephala* caught in pitfall traps there were no effects of treatment from seed rate ($F_{2,4}$=0.25, *P*=0.783) or management type ($F_{2,4}$=1.06, *P* =0.370). Carabidae numbers were greater in defoliation plots than other treatments ($F_{2,4}$=4.40, *P* =0.033). No effect of seed rate was apparent ($F_{2,4}$=0.16, *P* =0.855). Staphylinidae numbers were also greater in defoliation plots but not significantly different from the control ($F_{2,4}$=3.27, *P* =0.063) with no other treatments showing variations ($F_{2,4}$=2.44, *P* =0.137). No effect of seed rate ($F_{2,4}$=0.94, *P*=0.413) or management ($F_{2,4}$=2.19, *P* =0.149) was seen on Linyphiid although numbers were lower in neonicotinoid treated plots and higher in defoliation treatments than in OSR control (Table 11).

Table 11 Great field: grand means (+/- SE) of pitfall catches according to treatment in oilseed rape (*Brassica napus*). * denote significant differences.

Table of means	*P. chrysocephala* (+/-10.97)	Carabidae (+/- 19.45)	Staphylinidae (+/-3.94)	Linyphiidae (+/-4.24)
Control	99.7	189	16.9 (*)	45.1
Defoliation	89.9	209.6 (*)	25.0 (*)	51.3
Trap crop	93.4	167.6	20.2	44.7
Neonicotinoid seed treatment	102.3	175.7	14.7	40.7

4.4.8 Crop plant density at harvest:

West Barnfield: There was no effect of treatment on crop plant density shortly prior to harvest ($F_{10,26}$=0.41, *P*=0.932, grand mean: 6.22 range: 5.11-7.00 plants per quadrat).

Great Field. There was an increase in plant density in plots sown with increased seed rate, as would be expected, but perhaps surprisingly, the difference was not significant ($F_{2,4}$=1.56, *P*=0.238, Figure 20). There was no significant effect of the neonicotinoid seed treatment on crop plant density compared with the density in the agronomic treatments ($F_{1,4}$=0.06, *P*=0.945). No effect of defoliation was observed on the number plants surviving to harvest ($F_{1,4}$=0.03, *P* =0.876).

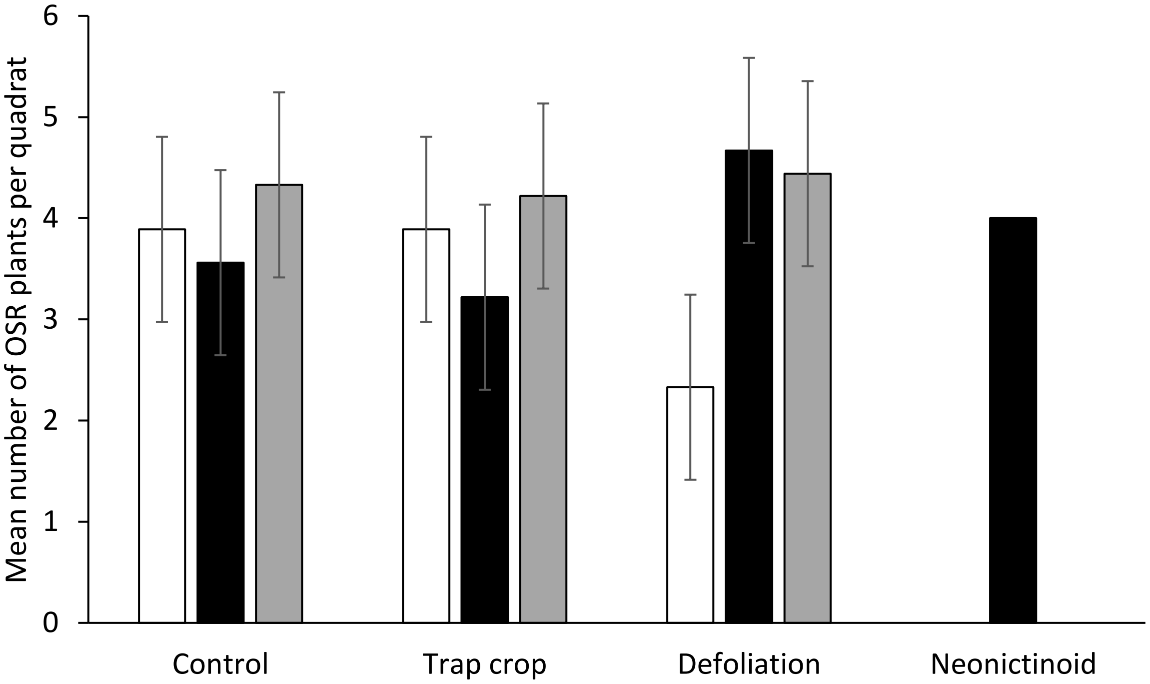


Figure 20. Great field mean number of oilseed rape (OSR, *Brassica napus*) sown at three different seed rates and with different control measures for Psylliodes chrysocephala: Control – OSR monoculture; trap crop – OSR with turnip rape trap crop border; Defoliation – OSR cut to 5cm before stem elongation; neonicotinoid – OSR treated with neonicotinoid (Cruiser). Oilseed rape seed rate: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

4.4.8. Plant growth (height, branching and pod production).

West Barnfield: The only treatment to show a difference from the control treatments was the application of Lumiposa combined with a neonicotinoid spray (treatment K); these plants were shorter than those in all other treatments ($F_{2,4}$=3.76, *P* =0.037, Figure 21). Branching was not affected by treatment ($F_{10,26}$=1.06, *P*=0.429, Figure 21 and Figure 22) neither was the total number of pods produced per plant ($F_{10,78}$=1.14, *P* =0.370, Figure 22).

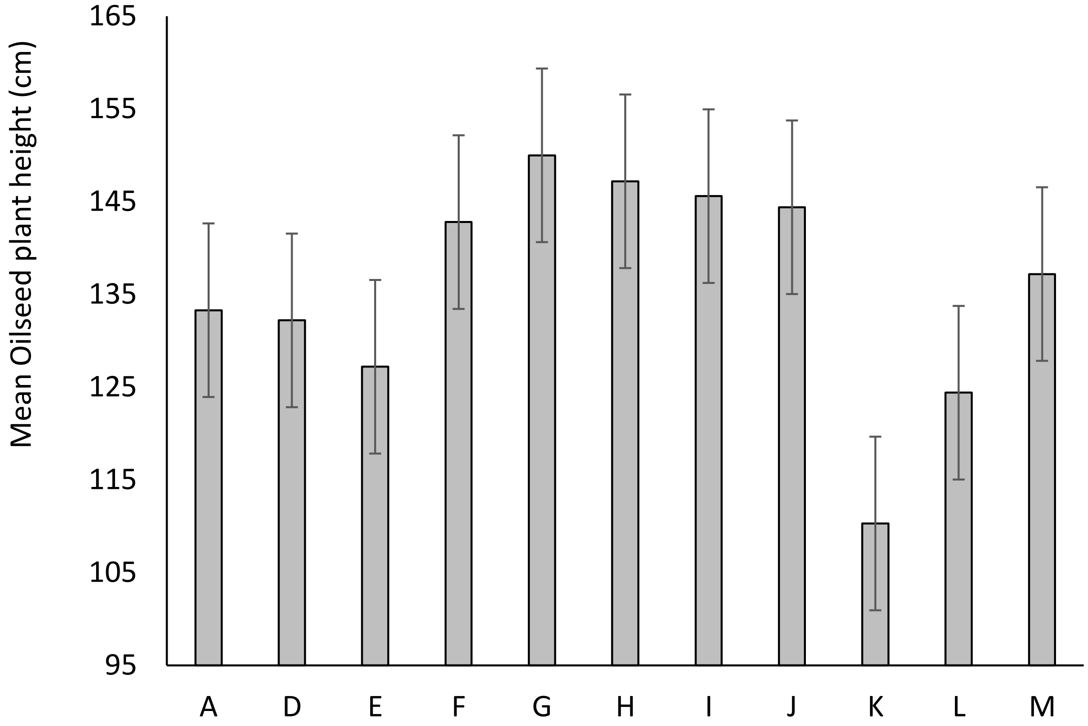


Figure 21. West Barnfield: Mean oilseed rape (*Brassica napus*) plant height (cm) under different pest protection methods Treatment code: (A) OSR – untreated control, (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa® seed dressing, (K) OSR treated with Lumiposa® seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with standard error.

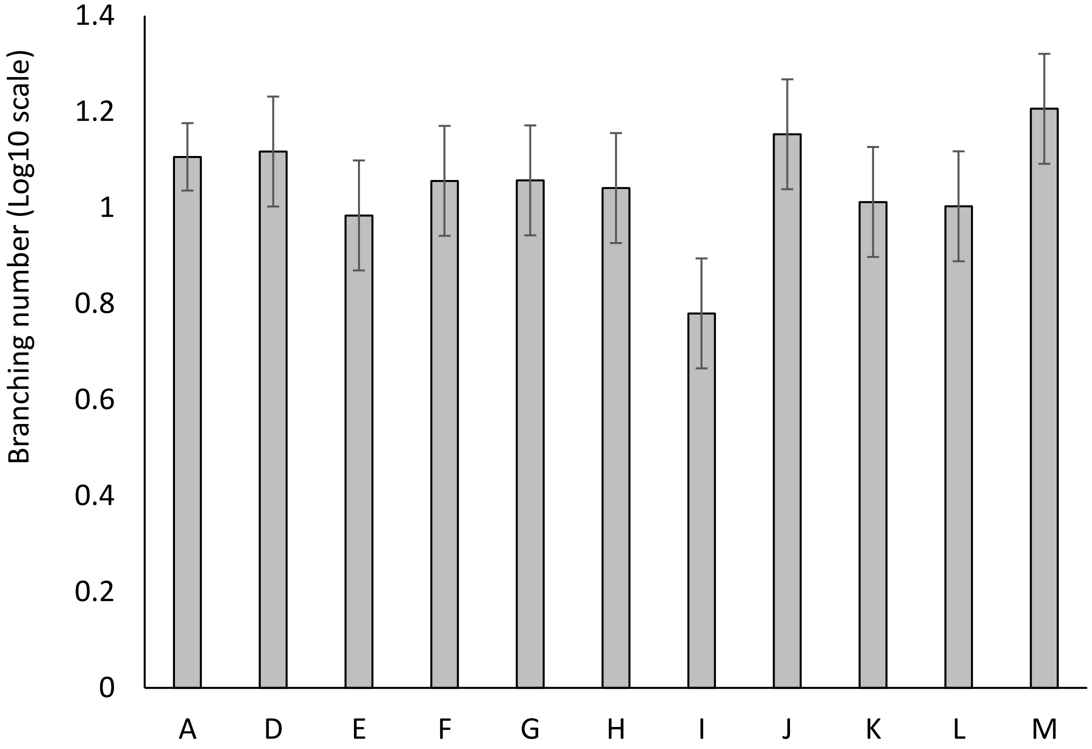


Figure 22. West Barnfield: Branching of oilseed rape (Brassica napus) under different pest protection methods. Treatment code: (A) OSR – untreated control, (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa® seed dressing, (K) OSR treated with Lumiposa® seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data presented on Log10 scale to account for plants which did not produce side branches with standard error.

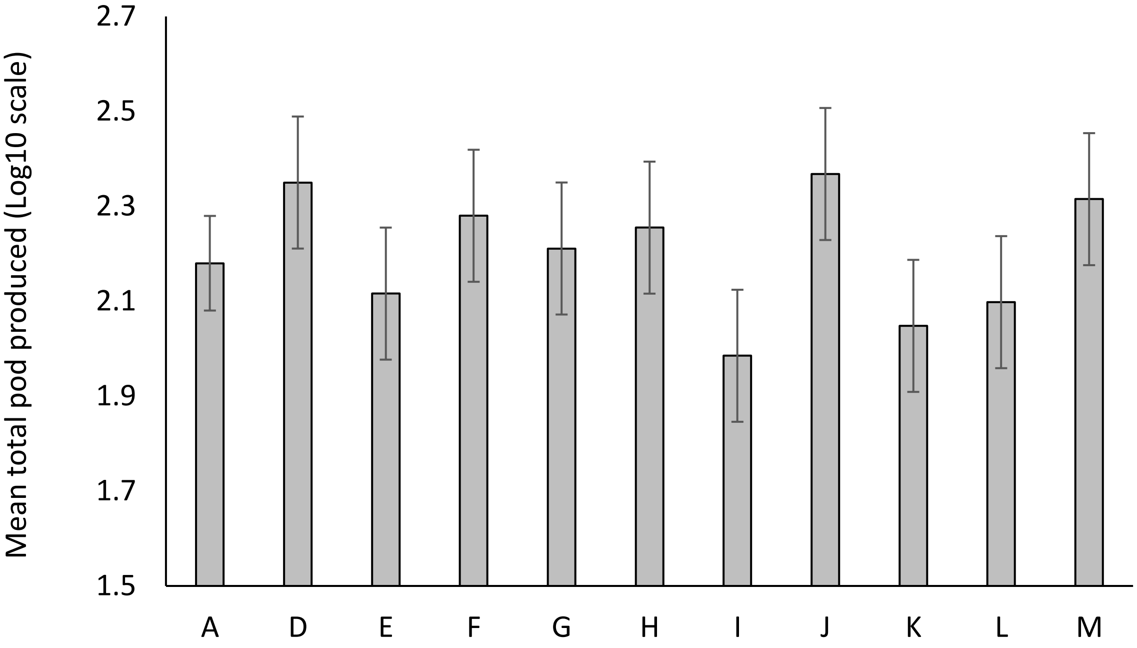


Figure 23. West Barnfield: Mean total pod production of oilseed rape (*Brassica napus*) under different pest protection methods Treatment code: (A) OSR – untreated control, (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa® seed dressing, (K) OSR treated with Lumiposa® seed dressing + neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE shown.

Great field: Plant height at maturity was not affected by seed rate (F2,4=0.86, *P* =0.422) but was reduced by defoliation ($F_{2,4}$=4.43, *P* =0.012, Figure 24). Neonicotinoid seed treatment did not differ from control plants ($F_{1,18}$=0.1, *P*=0.754, Figure 24). There was no effect of management on the amount of branching ($F_{2,18}$=0.99, *P*=0.394, Figure 25) nor was there any effect of seed rate ($F_{2,18}$=0.23, *P*=0.794, Figure 25).

The total number of pods set were reduced by defoliation ($F_{2,18}$=4.68, P =0.023, Figure 26) but was unaffected by seed rate ($F_{2,18}$=0.16, P =0.856. Figure 26).

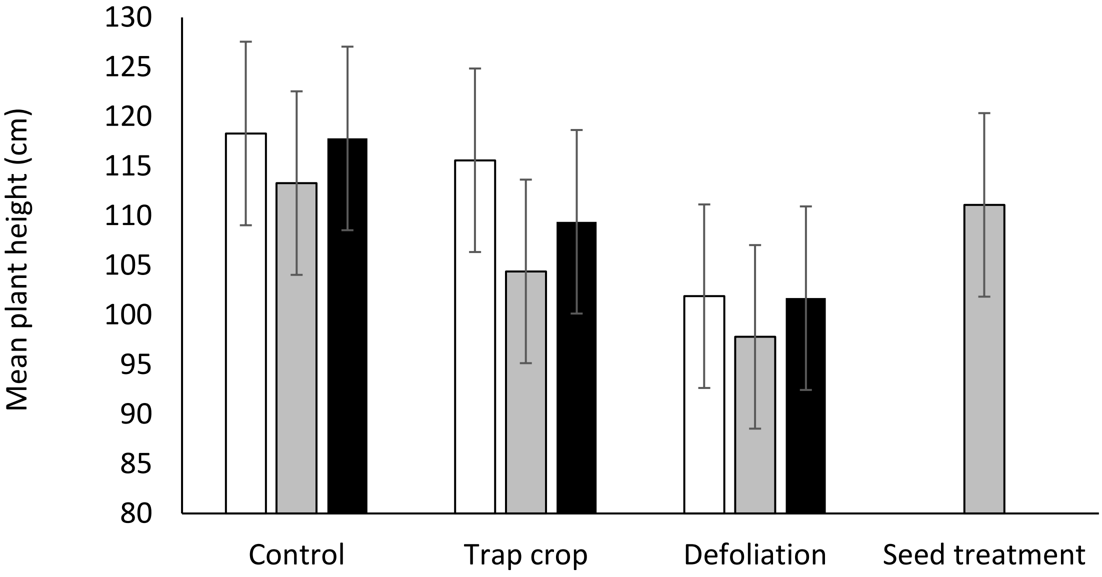


Figure 24. Great field: Mean height of oilseed rape (OSR, *Brassica napus*). Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

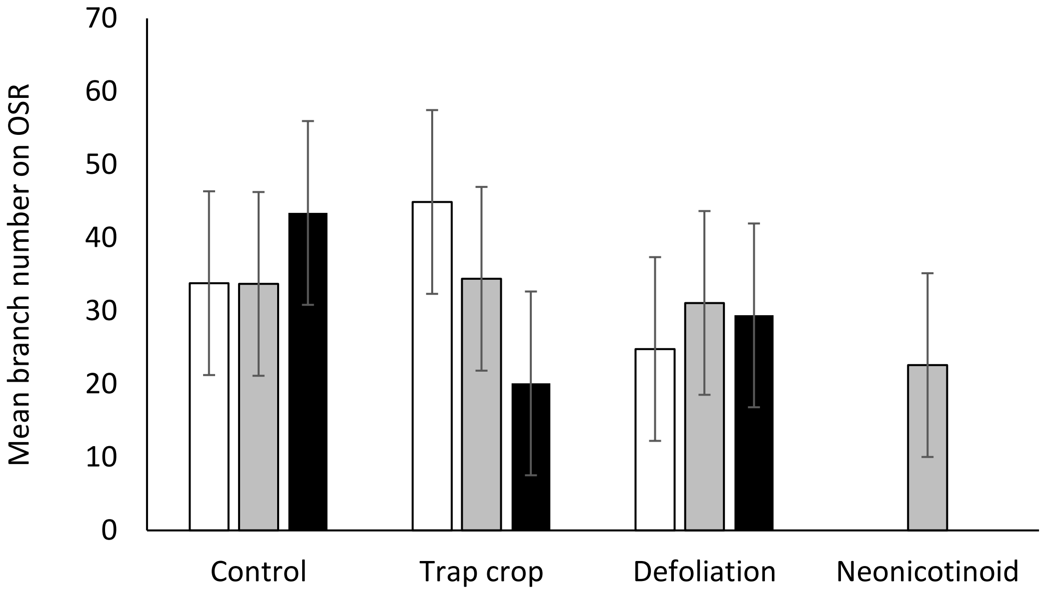


Figure 25. Great Field: Mean branching number of oilseed rape (OSR, *Brassica napus*) under differing pest protection methods. Control – OSR monoculture; Trap crop – OSR with turnip rape (Brassica rapa) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

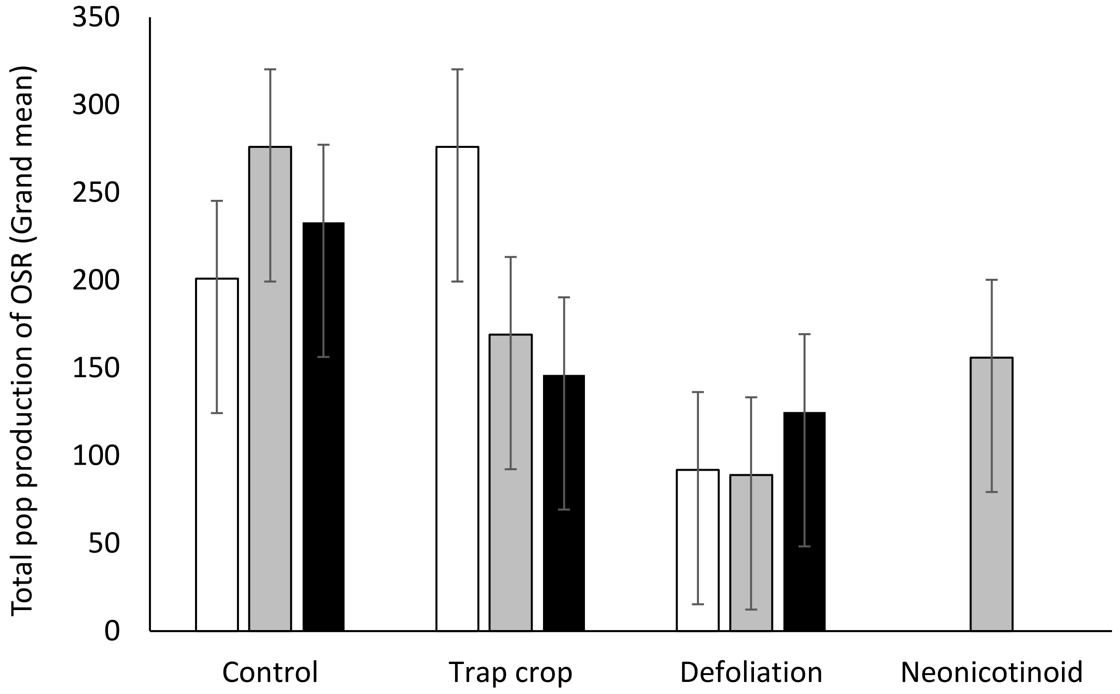


Figure 26. Great field average total pod set on oilseed rape (OSR, *Brassica napus*). Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

4.4.9. Crop Harvest and yield measurements:

4.4.9.1 Grain yield:

In **West Barnfield** the trap crop treatment showed increased grain yield compared to the control (tons/ha, $F_{1,8}$=14.09, P <0.001). No effect was apparent from any insecticide applications (pyrethroid sprays: $F_{2,4}$=2.05, P =0.151, neonicotinoid seed dressing: $F_{1,4}$=0.13, P

=0.723, neonicotinoid spray: $F_{1,4}$=0.01, *P* =0.904, Lumiposa™ seed treatment: $F_{2,4}$=2.51, *P* =0.103, Lumiposa™ and neonicotinoid spray $F_{2,4}$=0.37, *P* =0.694). See Figure 27.

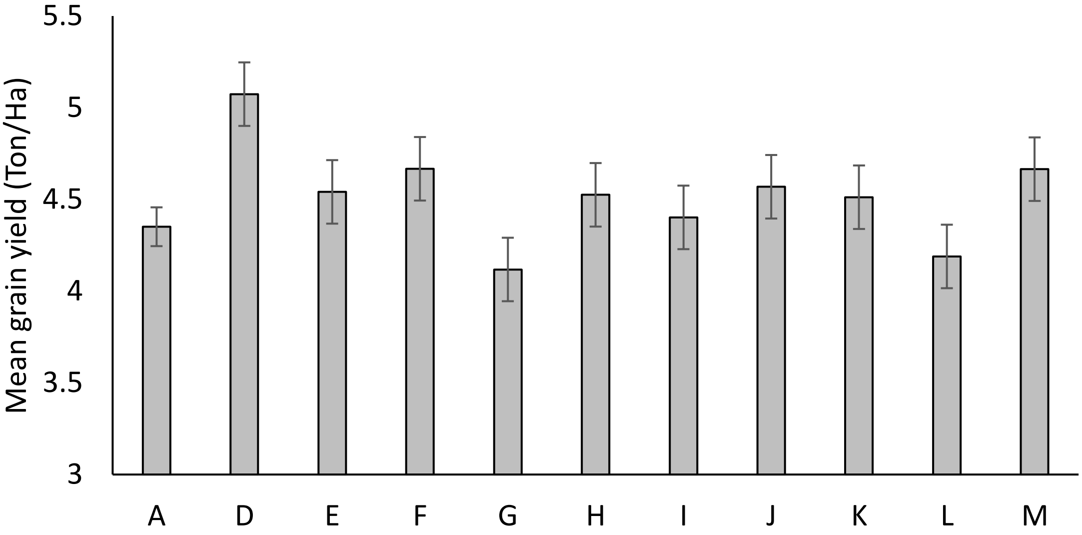


Figure 27. West Barnfield: Mean grain yield of oilseed rape under different treatments for control of *Psylliodes chrysocephala*. Treatment code: (A) OSR – with nurse crop mix, (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment – Cruiser, (F) OSR treated with neonicotinoid seed dressing - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR treated with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™ seed dressing + neonicotinoid spray (Biscaya), (L) OSR no seed dressing + Neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Treatment (B) OSR - with nurse crop and (C) OSR – with nurse crop and trap crop border are not represented due to abandonment of treatments before harvest. SE shown.

Great Field: Reductions in grain yield were observed for the defoliation treatments (Grain tons/Ha: $F_{2,18}$=80.95, *P* <0.001 see Figure 28). Seed rate did not affect the amount of grain produced ($F_{2,18}$=0.49, *P* =0.622).

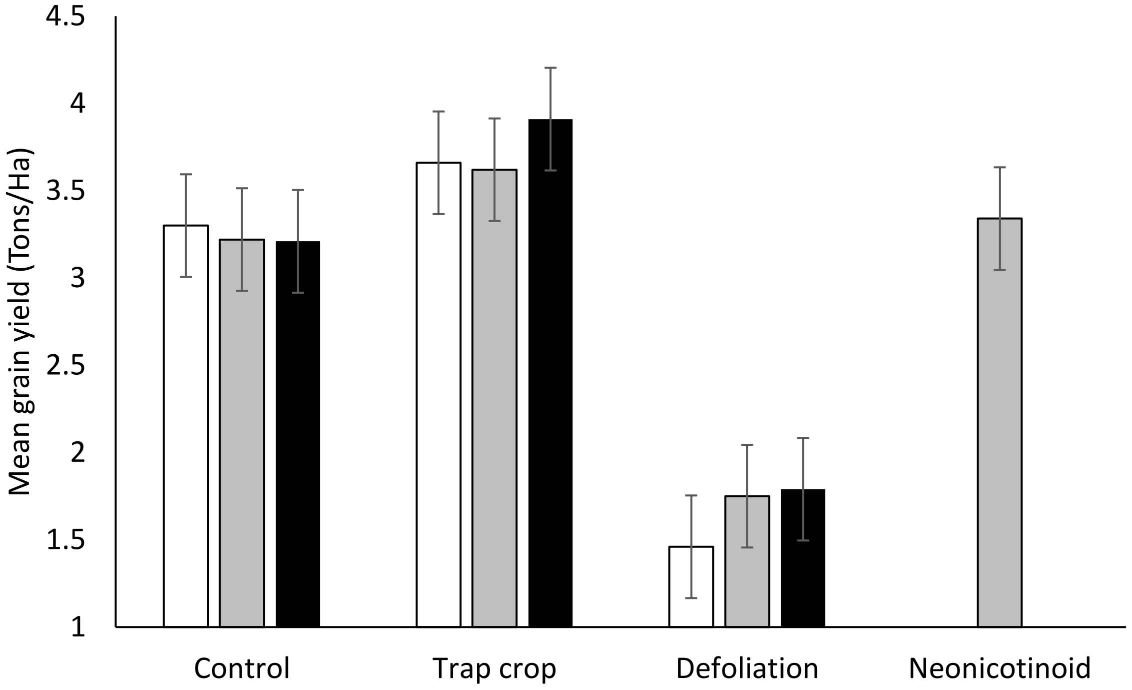


Figure 28. GreatField: Mean grain yield expressed as tonnes per hectare. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

4.4.9.2 Oil percentage in grain:

West Barnfield: The oil percentage in grain was higher in the trap crop treatment ($F_{1,8}$=6.77; *P*=0.019) and tended towards higher in the neonicotinoid seed dressing ($F_{1,4}$=4.14, *P* =0.061). There was no difference between control and pyrethroid spray ($F_{2,4}$=2.59, *P*

=0.109), neonicotinoid spray ($F_{1,4}$=2.80, *P* =0.114) or the use of Lumiposa™ seed treatment ($F_{2,4}$=3.02, *P* =0.080) was observed (Figure 29).

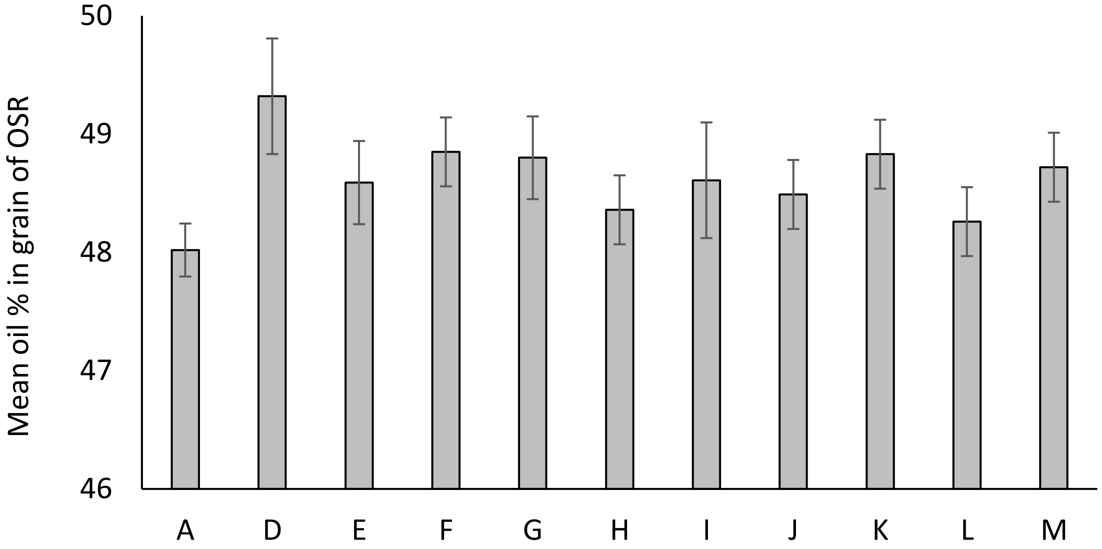


Figure 29. West Barnfield, mean (± SE) oil percentage of oilseed rape seeds for each treatment. Treatment code: (A) OSR – with nurse crop mix, (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment – Cruiser, (F) OSR treated with neonicotinoid seed dressing - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR treated with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™ seed dressing + neonicotinoid spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Treatment (B) OSR - with nurse crop and (C) OSR – with nurse crop and trap crop border are not represented due to abandonment of treatments before harvest. SE shown.

Great Field: Defoliation showed a reduction in the oil percentage in grain (F2,18=4.01, *P* =0.036). There was evidence of inverse relationship between seed rate and oil % in grain; with the lower seed rate showing significantly greater yield ($F_{2,18}$=4.01, *P*=0.036, Figure 30).

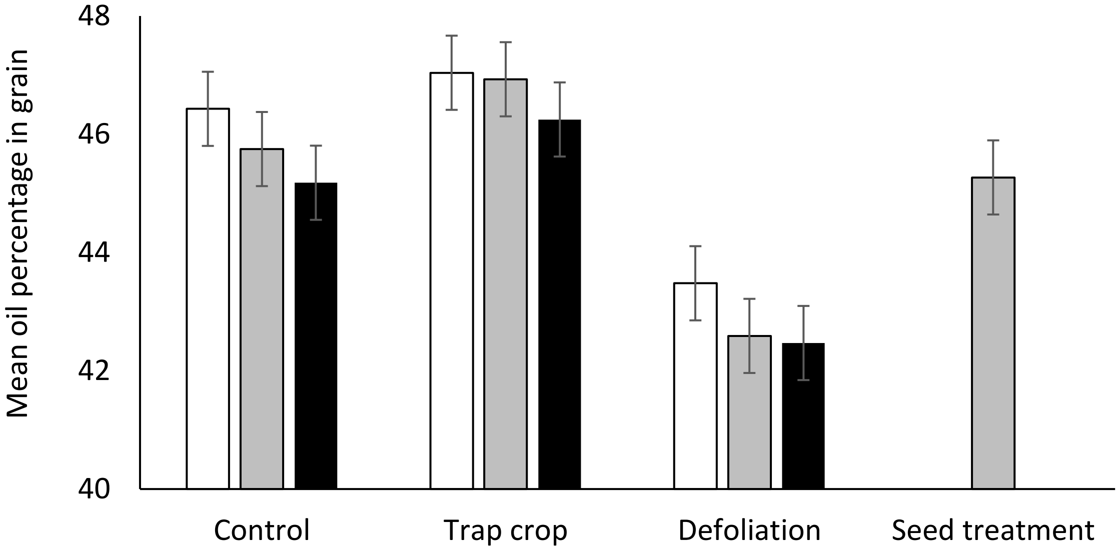


Figure 30. Great field mean oil % in grain of oilseed rape (OSR, *Brassica napus*). Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

4.4.9.3 Oil yield tonnes per hectare:

West Barnfield: The total oil yield when expressed as tonnes per hectare was unaffected by any treatment (Trap crop: $F_{1,8}$=1.90, *P* =0.186, pyrethroid spray: $F_{2,4}$=0.76, *P* =0.484, neonicotinoid seed dressing: $F_{2,4}$=0.30, *P* =0.592, neonicotinoid spray: $F_{1,4=}$0.01, *P* =0.943, Lumiposa™: $F_{2,4}$=1.93, *P* =0.175, or Lumiposa and neonicotinoid spray: $F_{2,4}$=0.12, *P* =0.884, Figure 31).

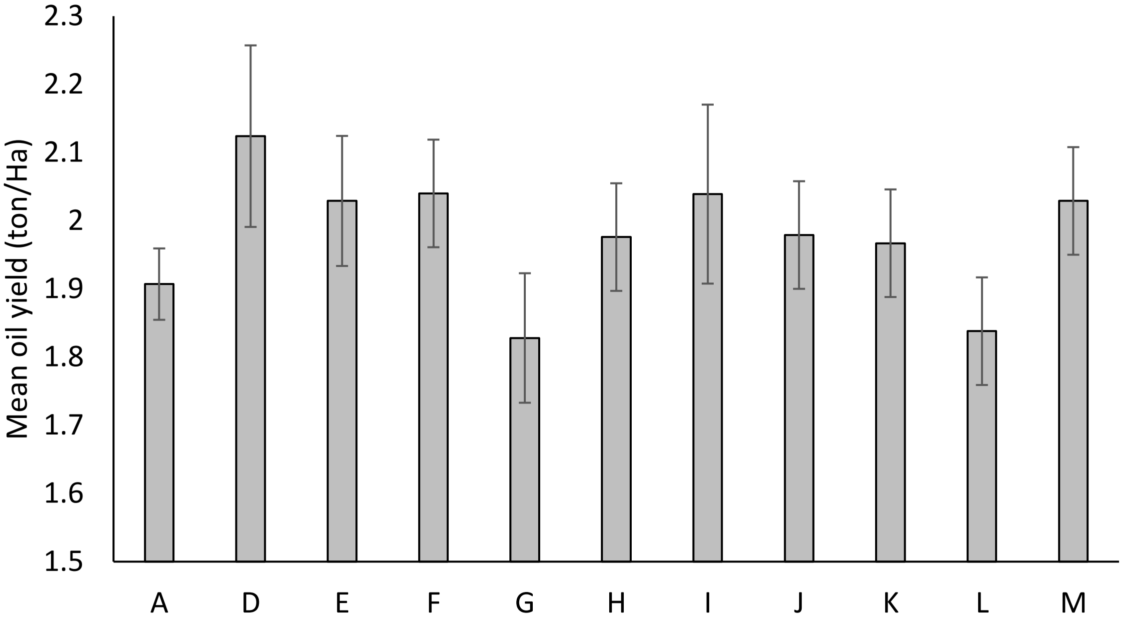


Figure 31. West Barnfield: Mean oil yield in terms of tons per hectare. Treatment code: (A) OSR – with nurse crop mix, (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment – Cruiser, (F) OSR treated with neonicotinoid seed dressing - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR treated with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™ seed dressing + neonicotinoid spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Treatment (B) OSR - with nurse crop and (C) OSR – with nurse crop and trap crop border are not represented due to abandonment of treatments before harvest. SE shown.

Great Field: The oil yield expressed as tonnes per hectare was not affected by seed rate ($F_{2,4}$=0.56, P =0.588) but was reduced by defoliation ($F_{2,4}$=183.57, P <0.001), see Figure 32.

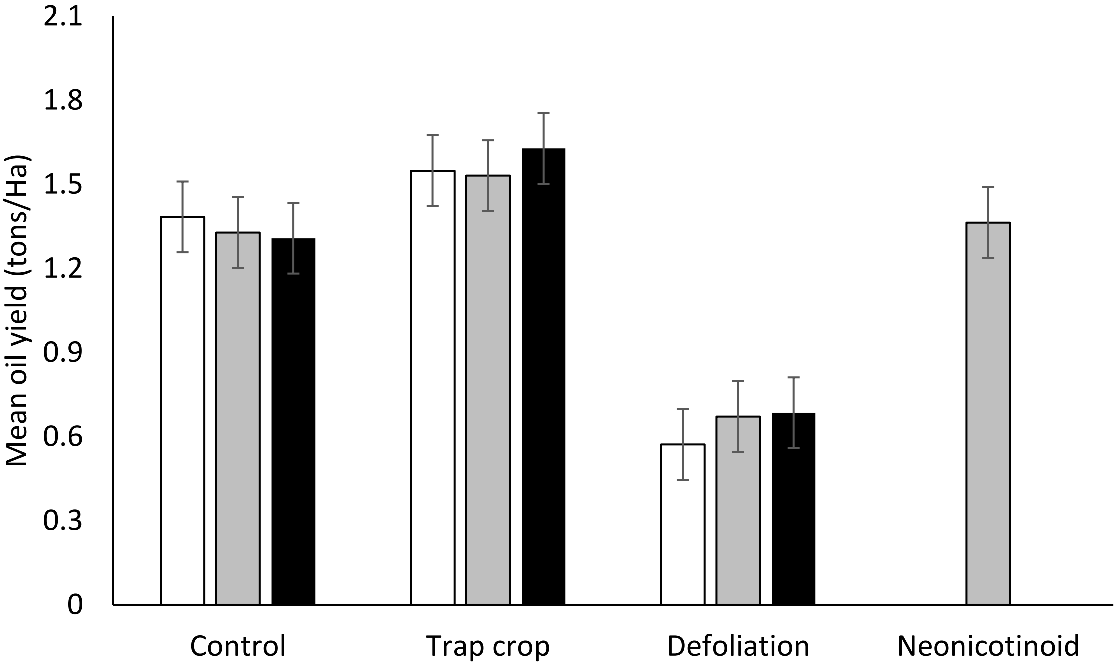


Figure 32. GreatField: Mean oil yield expressed as tonnes per hectare. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

4.4.9. 4. Thousand Grain Weight:

West Barnfield: There was a significant effect of treatment on thousand grain weight ($F_{10,26}$=2.22, P=0.05) with E, L and M (OSR – neonicotinoid seed treated, OSR neonicotinoid spray and OSR – neonicotinoid seed treated and neonicotinoid spray) showing lower weights than the other treatments (Figure 33).

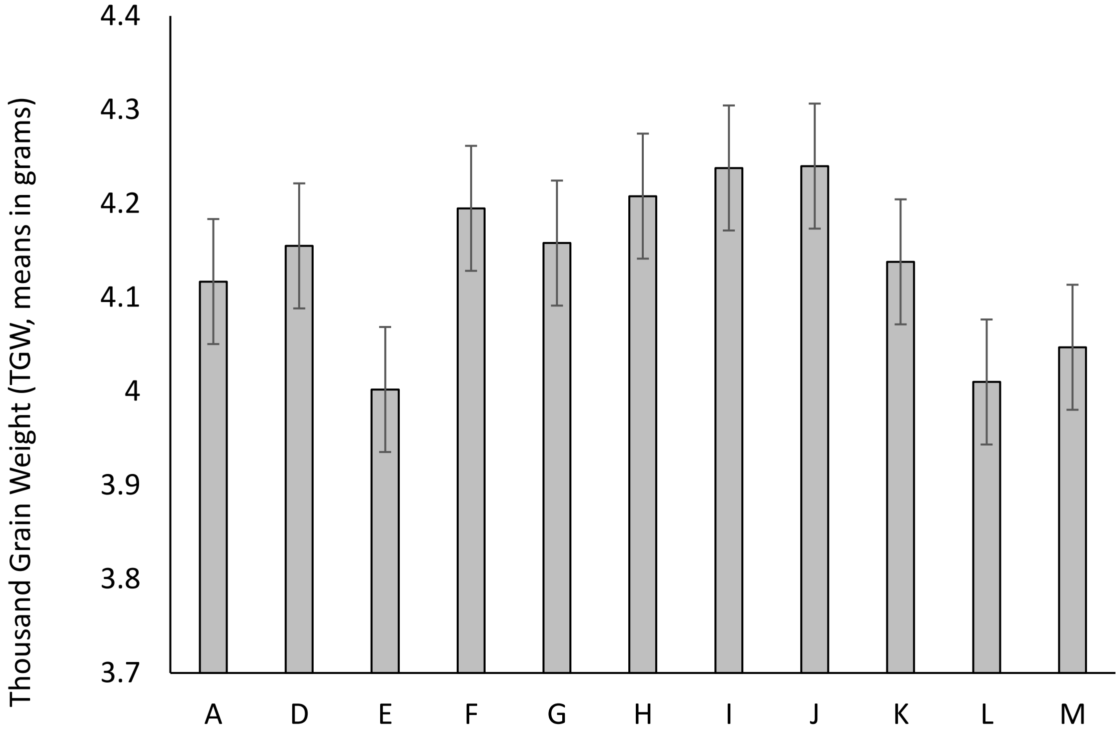


Figure 33. West Barnfield: Mean Thousand Grain Weight (TGW) for oilseed rape (OSR). Treatment code: (A) OSR – with nurse crop mix, (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment – Cruiser, (F) OSR with neonicotinoid seed dressing - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR with Lumiposa™ seed dressing, (K) OSR with Lumiposa™ seed dressing + neonicotinoid spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Note: Treatment (B) OSR - with nurse crop and (C) OSR – with nurse crop and trap crop border are not presented due to abandonment of nurse crop treatments before harvest.

Great Field: There was a significant effect of management on TGW with reduction in defoliation treatments ($F_{2,4}$=95.22, *P*<0.001). There was no significant effect from seed rate ($F_{2,4}$=2.60, *P*=0.134) although a trend of reducing TGW with increasing seed rate was observed (Figure *Figure 3*34). There was no effect of neonicotinoid seed treatment ($F_{1,4}$=0.00, *P*=0.989).

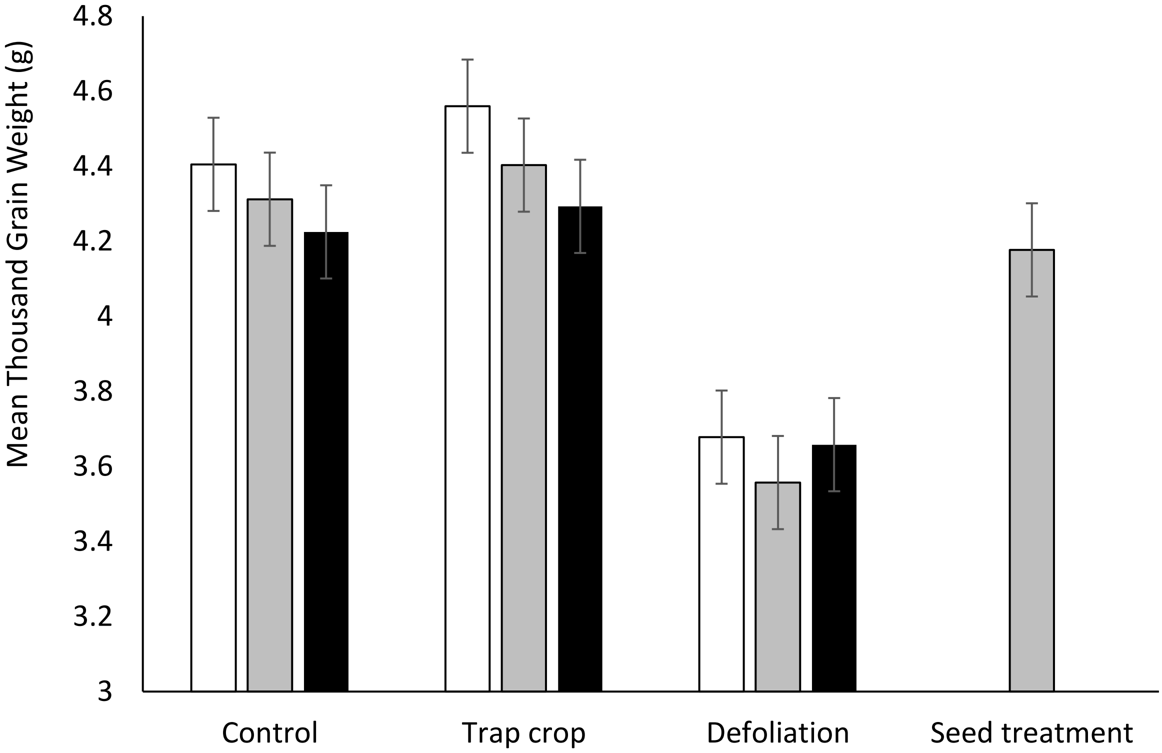


Figure 34. Great field Thousand Grain Weight (TGW) of oilseed rape (OSR). Control – OSR monoculture; Trap crop – OSR with turnip rape trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/3/2017); Neonicotinoid – OSR treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m^2; grey – 100 seeds/m^2 and black – 120 seeds/m^2. SE shown.

4.4.10. Ranking treatment:

West Barnfield: To better interpret the findings of the West Barnfield experiment, each measurement (leaf injury, larval loading, and yield measurements) was given a relative score for each treatment. Using the treatment means derived from the statistical analyses, the relative rank for each treatment was calculated. The level of pest control was ranked lowest to highest leaf area injury and number of larvae per plant. All harvest measurements were ranked from highest to lowest. This produced a relative rank for each treatment whereby the lower the score the lower the pest pressure and the higher the yield (Table 12).

Table 12. West Barnfield. Relative ranking of treatment effects on pest control and end oilseed rape (OSR, *Brassica napus*) when under multiple autumn pest protection practices. Values taken from data and set-in numerical ranking from best performance (lowest pest injury and pressure and highest yield return). In each case 1 is the best performer and 10 the worst. Sections with a dash (–) are nurse crop treatments which were destroyed prior to harvest.

Treatment	September leaf injury	October leaf injury	Larval loading (November)	larval loading (February)	Total score pests	**Rank (Pest control)**	Grain yield	TGW	oil % in grain	oil yield	Total score (yield)	**Rank (yield)**
A	1	1	2	1	5	best	9	8	11	9	37	Next worst
B	12	9	9	13	43		-	-	-	-		-
C	13	13	6	12	44	worst	-	-	-	-		-
D	9	7	11	9	36		1	6	1	1	9	best
E	8	2	13	5	28		5	11	7	4	27	
F	11	8	4	6	29		2	4	2	2	10	
G	10	12	3	3	28		11	5	4	11	31	
H	6	10	8	4	28		6	3	9	7	25	
I	4	11	1	2	18	2nd	8	2	6	3	19	
J	5	5	5	7	22	3rd	4	1	8	6	19	
K	2	3	10	10	25	4th	7	7	3	8	25	
L	3	6	12	8	29		10	10	10	10	40	worst
M	7	4	7	11	29		3	9	5	5	22	

4.5 Discussion:

4.5.1 Overview:

No method of pest protection used in this study was significantly different in the end yield to the untreated control monoculture. This included the use of neonicotinoid seed dressing and would suggest that the ban on its use in the EU is justified as it provided little actual crop protection. The use of pyrethroid sprays did reduce the larval infestation levels in February, suggesting there is still some level of larval control if not control of the adults of *P. chrysocephala.* The defoliation of OSR did show reductions in larval numbers and did not reduce the number of harvestable plants, however timing issues lead to secondary pest problems in this study, but there is evidence that this method can reduce larvae numbers without increasing plant mortality and further work is needed to avoid secondary pest issues.

4.5.2 *Psylliodes chrysocephala* migration:

When measuring migration of *Psylliodes chrysocephala* into the field a bias towards greater catches of beetles further into the field was observed (Figure 8 and Figure 9). The within field distribution of *P. chrysocephala* has been shown to be patchy within the central area of the crop (Thioulouse, Debouzie and Ballanger, 1984). Suggesting that *P. chrysocephala* migrated into the central area of the crop before distributing within the crop (Ferguson *et al.*, 2006; Warner *et al.*, 2003).

It was also apparent in both fields that the beetles do not enter the crop from all sides equally (Figure 8 and Figure 9). A clear bias in terms of number of beetles captured along the southern edge in GreatField and the southern and eastern edges in West Barnfield. From the data collected during this experiment it is not possible to determine if this directional bias to migration is consistent between years. With multiple-year sampling of OSR fields and directionality of migration it would be possible to determine if in a given field the beetles enter on a known edge each year. With this information a more informed decision on the location of a trap crop border could be applied along the edges of main migration routes. A single trap crop border may be able to be placed in the direct line of primary migration and at a larger block area and not a strip boarder. Further recordings on *P. chrysocephala* migration into crops is required to develop appropriate trap cropping systems. The field migration of pollen beetles (*Brassiocgethes aeneus*) has been shown to be heavily effected by wind direction (Mauchline *et al.*, 2017). This knowledge can allow trap crop boarders to be located in the best location to disrupt pests entering the crop, as a boarder functions with *B. aeneus* (Cook *et al.*, 2007).

4.5.3 Past: neonicotinoid seed treatment and foliar spray:

In the trial reported here there was no benefit to treating seed either with a neonicotinoid (AI: thiamethoxam) or with Lumiposa™ (AI: cyantraniliprole) as there was little to difference when compared to untreated control plants in terms of autumn pest levels and cropping yield. The level of protection bestowed on OSR by neonicotinoid seed treatment was not enough to protect OSR in 2015 at Rothamsted (chapter 3 this thesis). The concerns of farmers that OSR cannot be grown without neonicotinoids (UK, 2020) are unsubstantiated by this trial. With no yield increase or pest protection increases seen compared to controls the extra cost of insecticide application would be unnecessary in the 2016-17 season. Recent reviews on the loss of neonicotinoid from the OSR highlights the concerns of farmers on the issues regarding implementation and seed bed preparation of OSR being inhibited by *P. chrysocephala* (Kathage *et al.*, 2018). Here the perceived efficacy of neonicotinoid seed dressing is brought into doubt and any further examination of its use should be based on empirical evidence of actual pest control. With the current ban imposed primarily to limit exposure to pollinating insects (Goulson, 2013) the limited benefit to the crop reported here requires further investigation.

4.5.4 Current: Pyrethroid foliar spray insecticides:

The level of resistance in both experimental fields was shown to be high (49% and 85%). However, these measures were taken after pyrethroid application in the fields and suggest that non-resistance beetles were killed during applications. The application of spray insecticides (pyrethroid) did not significantly affect the level of leaf area injury during the autumn. However, reductions in *P. chrysocephala* larvae loading were observed for pyrethroid spray applications in the following year. This reduction in a field which was shown to have up to 84.9% resistance in adults (Table 54) suggests that pyrethroids are less affective against adult beetles but do still show some control of larval stages of *P. chrysocephala*.

4.5.5 Current: Seed application rate:

A significant effect of seed rate was apparent in September ($F_{2,4}$=5.04, *P*=0.021) with decreasing injury levels as seed rate increased. This was not significant in the October assessment ($F_{2,4}$=0.72, *P* =0.072). The effect of seed rate has shown to be highly variable between space and time (White *et al.*, 2020). Increases in yield were apparent as the seed rate applied reduced, recording declining yield return with increasing seed rate. The mechanism behind this observation may be due to competition between conspecific plants (Berry and Spink, 2006). These data are in line with current recommendations of increasing seed rate as a pest prevention measure (AHDB, 2019). However, in years of lower *P. chrysocephala* pressure the benefit of increased seed rate may be outweighed by the increase in plant-plant competition. In years of hight *P. chrysocephala* reductions in individual plant injury can be reduced by increased seed application (chapter 2 this thesis). In the season of these experiments the *P. chrysocephala* population was not as large or as damaging as seen in other years (chapter 2 and 3, this thesis). It is suggested here that improved yields can be achieved at low seed rate and that recommendations of increasing seed rate as a pest protection measure can reduce levels of per plant injury as reported here. However, it is not enough in years of high pest abundance as seen in 2015 (chapter 2 this thesis). In years where pressure is not so severe as in that reported here the increase in seed rate did not show a significant yield penalty. This may be partly down to reductions in later pest pressure from *B. aeneus* from increased plants/m^2 (AHDB, 2021).

4.5.6: Future cultural: Defoliation:

Reductions in larval loading from defoliation has been reported when the crop is topped and when grazed by sheep (White *et al.*, 2020). In the trial reported here the defoliation did result in a significant reduction in larvae number but reduced the end yield. The defoliation did not affect the final plant density, supporting the hypothesis that OSR can tolerate high levels of injury. The OSR defoliation treatment plots were mown in March (14th March 2017) just before stem extension. This was to maximise the number of larvae removed by the treatment but could have been too late and may have contributed to the yield reduction in this treatment (Clarke, 1978; Freyman, Charnetski and Crookston, 1973; Kirkegaard *et al.*, 2008; Seymour *et al.*, 2015). In other studies, defoliation was performed earlier and did not result in yield loss (Kirkegaard *et al.*, 2012; McCormick, Virgona and Kirkegaard, 2013; Spink, 1992; Susko and Superfisky, 2009).

The treatments which were defoliated exhibited a delay in the start of flowering compared to the un-cut treatments and surrounding crop. This delay led to the defoliated plants being at bud stage when the rest of the crop was in full flower. *Brassiocgethes aeneus* are known to enter flower buds to access pollan and that this can cause bud abortion (Seimandi-Corda, Jenkins and Cook, 2021). The yield reductions reported here may be due to high levels of pollen beetle causing greater pod aborting in the defoliated plants. Previous studies on the impact of defoliation to OSR yield have been carried out on a larger scale (White *et al.*, 2020; Syrovy, Shirtliffe and Zarnstorff, 2016; Seymour *et al.*, 2015). The hypothesis of reducing *P. chrysocephala* larvae through defoliation and the crop surviving the injury has been shown here and in other studies in the UK (White *et al.*, 2020) and on spring OSR in Canada and Australia (Kirkegaard *et al.*, 2012). Further examination on the timings on grazing in spring and winter OSR in Australia demonstrate the importance of livestock removal before stem elongation (Sprague *et al.*, 2014). The method of defoliation was also an important consideration for farmers surveyed on their opinions of novel pest protection methods; these farmers raised concerns about using machinery on the crop in winter/ early spring on wet soil and damaging the soil surface (chapter 8 this thesis); farmers tending to prefer the method of sheep grazing to reduce damage to soil structure (chapter 8 this thesis).

4.5.7 Future cultural: trap cropping and nurse cropping:

The nurse crop treatments were removed after winter (May) due to a mild winter and limited effect of the Clearfield herbicide to remove the nurse species. The farm management were concerned about nurse species and the turnip rape trap crop seeding and causing potential problems with weed contamination of the field site in future years. The nurse crops did provide a level of crop protection at early growth with reductions on the level of leaf area injury on the OSR crop when in association with a nurse crop. This provided evidence that nurse cropping can reduce levels of early growth injury to OSR crop plants as shown in chapter 2 (this thesis). However, the difficulty to successfully control the nurse crops without removing the OSR crop highlights the complexity of nurse cropping with Brassicas. Prior to its removal the nurse crop did demonstrate that Pak choi and Chinese cabbage are suitable host plants for *P. chrysocephala.* However, this did result in higher numbers of larvae in the nurse crop treatment.

The trap crop borders did reduce the levels of *P. chrysocephala* injury to the OSR crop. They were shown to contain more larvae than the OSR confirming the findings of Barari *et al.,* (2005). This supports the hypothesis that turnip rape can be used as a means of manipulating *P. chrysocephala* feeding and interactions with the OSR crop. However, the boarders were removed in early spring, due to farm concerns about seed drop and subsequent volunteers. Therefore, any benefit from the trap was only conferred during the autumn and winter. This reduced the potential positive effect of trap crops on reducing infestation in the OSR plots by subsequent pests such as pollen beetle (Cook and Denholm, 2008; Cook *et al.*, 2007; Gotlin Čuljak *et al.*, 2016). This increased the open area (1m strip) around the central treatment area which were then exposed to higher levels of sunlight. The increased yield observed in this treatment may be due to increased photosynthetic potential, a primary factor in yield formation (Diepenbrock, 2000), which cannot be confirmed or discounted in this study.

4.5.8. Future synthetic control:

Lumiposa™ did not significantly reduce leaf area damage or infestation of *P. chrysocephala* larvae over untreated-seed control. Larval infestation was actually found to be higher in Lumiposa™ treated seed than in plants sown using untreated seed. No significant improvement in yield was observed over untreated seed. However, like all treatments in this study effects may have been masked due to the small plot size used. Larger field trials are required to understand the levels of protection Lumiposa™ can provide an OSR crop. The use of a neonicotinoid foliar spray also converted little benefit to the crop in levels of pest control and in the end crop yield.

4.5.7 Conclusions:

Data presented here suggests that alternative non-synthetic chemical-based pest protection methods are equal to the task of defending OSR from *P. chrysocephala* and show comparable yield returns to both types of synthetic insecticides used in this experiment. This suggests that further research into the functionality of trap cropping as an autumn pest protection method should prove fruitful. Issues with pollen beetle restricted any meaningful measurements to be taken from the cutting treatments tested here. However, *P. chrysocephala* larval levels supports previous studies showing OSR can recover via compensatory growth from cutting with reduced internal larvae loading. The data presented here suggest that growing OSR in the post-neonicotinoid era is possible in the UK.

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5.0 The impact of injury caused by *Psylliodes chrysocephala* L. adults and larvae on flower and yield production in *Brassica rapa* L:

5.1 Abstract:

Protecting crops from pest attack is a major undertaking in modern agriculture. Under current EU legislation the use of neonicotinoid seed dressing is banned in oilseed rape (OSR, *Brassica napus* L.), an important oil crop and break crop in the UK. This restriction and the reduction in efficiency of other synthetic chemical alternatives has exacerbated pest pressure from the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) a pest which can threaten establishment in OSR. In two experiments, the ability of OSR to compensate for injury caused by adult and larval stages of *P. chrysocephala* was assessed. Leaves on experimental plants were injured by either simulated shot holing or inoculating plants with larvae at different densities. Both experiments showed that leaf area loss at early growth of OSR can be compensated for. Whilst there was no effect of inoculating with <5 larvae, significant impacts were observed when 25 were introduced. The data presented here suggest that higher numbers of larvae reduce plant productivity in terms of number of flowers produced and the harvestable yield. The implications for flower visiting insects and farming productivity are discussed.

5.2 Introduction:

Oilseed rape (OSR, *Brassica napus* L.) is the second most produced vegetable oil crop globally (Shahbandeh, 2020) and the primary oil crop in Europe (Commission, 2020) where it forms an important component of the agricultural rotation with cereals (Redman, 2019). *Psylliodes chrysocephala* L. (cabbage stem flea beetle) is one of the most important autumn pests of winter OSR in the UK and coastal areas of Europe causing direct injury in two distinct ways: (i) adult beetles feeding on the cotyledons and leaves of plants in the early stages of crop establishment cause characteristic 'shot-holing' injury which can threaten crop establishment, and (ii) the stem-boring larvae which feed within the leaf petioles and stem, weakening the plant and increasing its susceptibility to secondary infections, e.g. stem canker *Leptosphaeria maculans* Ces & Not (Williams, 2010).

Since the new millennium *P. chrysocephala* has typically been controlled through the use of neonicotinoid insecticides; these act systemically, entering all plant tissues (Bass and Field, 2018), and can be applied as seed coatings to protect the crop from germination. Neonicotinoid seed treatments are considered to confer effective pest control during the early growth stages of the crop (Dewar *et al.*, 2016) and were designed to protect crop establishment (Bass and Field, 2018). If pest pressure is still evident after the efficacy of seed treatment has reduced the application of spray insecticide in the pyrethroid group was recommended for further protection from *P. chrysocephala* pressure. However, due to the systemic nature of neonicotinoids there has been growing global concern about their impact on non-target species (Blacquiere *et al.*, 2012; Pereira, Diniz and Takasusuki, 2020). In 2013, this led the EU to impose restrictions in their use on crops used by bees, including OSR. This was extended (2018) to a blanket ban on their use outside (EU, 2018) and calls to review of their use in the USA, Canada, Australia, New Zealand (Goverment, 2019; Zealand, 2018) and some parts of Africa (NASAC). In the absence of neonicotinoids, growers are increasingly relying on the use of the pyrethroid insecticides alone (Nicholls, 2016). This overdependence of a single insecticide group has in effect stalled any insecticide resistance management strategy and exacerbated the development and spread of resistance to pyrethroid insecticides, a phenomenon already being reported in *P. chrysocephala* across the EU (Bothorel *et al.*, 2018; Brandes and Heimbach, 2018; Foster and Williamson, 2015; Gavloski *et al.*, 2000; Højland and Kristensen, 2018; Højland *et al.*, 2015; Zimmer *et al.*, 2014; Willis *et al.*, 2020). This originally raised concerns that OSR cultivation may be threatened by injury from *P. chrysocephala* in the UK and on the European continent without adequate synthetic control options (Zhang *et al.*, 2017). This has been realised in the UK with reductions in the area of winter OSR grown from 621,000 ha in 2014 before the ban (DEFRA, 2014a) to 337,000 ha in 2020 (DEFRA, 2020), with reluctence to grow OSR attributed to *P. chrysocephala* now causing potential loss of OSR from rotations (Dyer, 2019). With no alternative synthetic control options, it is crucial to better understand what level of injury the crop can compensate for to ensure the minimum level of application and thus reduce exposure of the beetle reducing the pressure leading to resistance. The decision for application is often based on an economic action threshold, i.e. the abundence of a pest above which yield deficit is greater than the cost of management implimentation (Pedigo, Hutchins and Higley, 1986).

Economic action thresholds for pyrethroid insecticides against *P. chrysocephala* exist in Europe and vary slightly between countries; of these, the UK has the most conservative thresholds (14). Growers are advised to apply insecticide: (i) against adult injury at the first sign of attack if the risk is high during emergence of cotyledons or when >25% of leaf area has been lost between cotyledons unfolding and the 2 leaf stage or when 50% of the leaf has been eaten at the 3-4 leaf stage or when the crop is growing more slowly than it is being eaten (Oakley, 2003; AHDB, 2019) or (ii) against larvae when the mean number exceeds five larvae per plant; this threshold was recently increased from two larvae per plant and was initially set due to the low cost of application (Green, 2008) but was increased due to the high incidence and spread of resistance (AHDB, 2019).

Data on the level of larval infestation of OSR in the UK has shown an increasing trend in occurrence and abundance of *P. chrysocephala* larvae in the UK following the ban on neonicotinoid seed treatments in 2013, ranging from 88% of plants with scaring (related to larval density) in the East of England 59% in the North, although the national average remains below the five larvae per plant action threshold (FERA, 2020). This trend for increasing abundance has raised the concern for the pest and requires more research to understand the carrying capacity of OSR for *P. chrysocephala* larvae without suffering loss in yield.

Oilseed rape has been shown to have high levels of compensation capacity to leaf area loss or defoliation, given time (Ellis, 2015a; Nowatzki. T and Weiss. M, 1997). Research on early growth OSR tolerance to injury has focused on rate of biomass accumulation (Nowatzki. T and Weiss. M, 1997; Ellis, 2015a; DEFRA, 2014b; White *et al.*, 2020) or production of glucosinolate as a response to injury (Döring and Ulber, 2020; Koritsas, Lewis and Fenwick, 1991; Koritsas, Lewis and Fenwick, 1989). These studies did not take the OSR to yield and have not recorded any long-term compensation capacity. It has been shown that OSR can tolerate high levels of defoliation (from grazing by sheep or mowing) if the injury occurs prior to stem elongation (Kirkegaard *et al.*, 2008a; Kirkegaard *et al.*, 2012).

The threshold of 5 larvae is derived from Purvis (1986) where the efficacy of organophosphates to control *P. chrysocephala* was tested, however, as noted by the author, the yield responses seen at a mean of 5 larvae/plant may have been due or partially due to the benefits from insecticidal action on reducing virus vectors (aphids). More recent studies have been done assessing larval development (Döring and Ulber, 2020) and OSR glucosinolate production (Koritsas, Lewis and Fenwick, 1989; Koritsas, Lewis and Fenwick, 1991), but none have taken the OSR to maturity and direct relationship between injury and larval infection on yield is still lacking from the literature.

Previous studies on *P. chrysocephala* larval feeding have used complex collection techniques and specialised equipment to rear the larvae (Döring and Ulber, 2020) or have not provided detailed information on how the larvae were collected (Koritsas, Lewis and Fenwick, 1991). This study is the first the authors are aware of that manipulates in a controlled way *P. chrysocephala*- adult-induced leaf injury and infestation using field collected larvae to quantify their combined effects on flower production, floral rewards, and seed production in OSR.

5.2.1 Aims of study:

This study aimed to quantify the direct impact of *P. chrysocephala* injury on the productivity of OSR in terms of seed quantity and quality. The effects were assessed on potted plants in semi-field conditions over two harvest years. In experiment 1 (2017 cropping season), the leaves of the OSR plants were manually injured to varying degrees using a hole punch to simulate adult *P. chrysocephala* feeding in a consistent manner. In experiment 2 (2018 cropping season), in addition to controlled leaf area injury, plants were inoculated with known numbers of *P. chrysocephala* larvae to test the interaction between the level of leaf area loss and levels of larval infestation. In experiment 2, measurements were made to test whether injury or larval infestation impacts flowering time, abundance, or floral resource quality. In both experiments the OSR was pot-grown and kept in pest-free mesh cages outdoors to maturity in order to assess the impact of injury on final yield.

5.3 Methods:

5.3.1 OSR pot experiment 1 (2017): Leaf injury:

For experiment 1 (harvest: 2017) Winter OSR (cv. DK Imperial) plants were sown in staggered batches in individual plant plugs (19mm^2, 30mm depth) using a standard compost mix (Petersfield Products, Leicester, UK) and kept in an unlit, unheated glasshouse until germination had occurred. The plants were then transplanted to 18cm pots (13/02/2017), ensuring all plants had equal amounts of substrate, then placed in an outdoor net cage (4m x 4m x 2m, with a mesh gauge of 2mm (Garratt *et al.*, 2018)) to exclude pests and pollinators.

Four levels of simulated leaf injury were applied (0%, 25%, 50% or 90% leaf area loss) to plants at two early growth stages (GS) expressed according to the BBCH scale (Lancashire *et al.*, 1991): cotyledons expanded (GS9) and the first true leaf extended (GS10). Simulated injury was applied 25 days after sowing (18/02/2017). A hole punch (3mm diameter) was used to remove a controlled amount of leaf area from plants, applying simulated injury as comparable as possible to actual *P. chrysocephala* feeding i.e. shot hole injury, as opposed to the cutting action caused by caterpillars; it has been shown in spring OSR that defoliation by cutting produced less biomass re-growth and less pods then hole-punch defoliated plants (Susko and Superfisky, 2009). Simulated injury was used as a proxy for adult feeding to allow careful control of the level of leaf area removal and to ensure standardization between replicates and treatments.

Following plant injury, two grids of 100 plants were set out; each comprised 10 randomized blocks of 10 plants, with one plant of each level of leaf area loss (25%, 50% and 90%) at both growth stages (GS9 and GS10) and two un-injured (0% injury) control plants for each GS in each block. There were therefore eight treatments overall (Table 1), with each injury level replicated 20 times for each growth stage group (GS9 and GS10) and the two control treatments (0% injury) each replicated 40 times. The extra control treatments were added to aid blocking structure and achieve the desired plant density. Plants were equally distributed within each grid over an area of 2m^2, thus simulating a density of 50 plants/m^2, common for OSR crops (Roques and Berry, 2016), with a 1m gap between the grids. The plants were placed on a metal mesh supported ~10cm from the ground and slug pellets were spread on the ground underneath to prevent slug feeding. Plants were hand watered until 19th of May 2017, when automatic irrigation was set up. Plants were maintained until harvest (see section 2.3).

Table 1 Treatments to test the effect of *Psylliodes chrysocephala* feeding injury on oilseed rape (*Brassica napus* OSR) plants in 2017. Feeding injury was simulated using a hole punch to remove varying amounts of leaf material (0, 25, 50 and -90%) at two different growth stages (GS09 and 10).

Treatment number	Growth Stage	%Leaf area injury
1	9	0
2	9	25
3	9	50
4	9	90
5	10	0
6	10	25
7	10	50
8	10	90

Figure 1. Experiment in 2017, layout of treatments inside field cage at University of Reading, nearside 'grid 1' and far side 'grid 2'. All plants were laid out to a plant density of 50 plants/m^2. Plant pots were supported off the ground on a metal grid to prevent slug injury.

Table 2 Layout of pots with treatments 1 – 8 in the 2017 experiment. The treatments are (1) GS 9; 0% injury, (2) GS 9; 25% injury, (3) GS 9; 50% injury, (4) GS 9; 90% injury, (5) GS 10; 0% injury, (6) GS 10; 25% injury, (7) GS 10; 50% injury, and (8) GS 10; 90% injury. Randomized blocks (columns) with two controls (treatments 2 and 5) for each growth stage tested. Layout produced 50plants/m^2.

Grid 1										Grid 2									
4	7	3	3	6	2	3	1	1	2	2	4	2	1	2	8	2	4	1	3
1	4	6	8	1	5	2	1	3	1	8	1	3	2	1	1	1	1	2	2
6	6	8	1	4	6	8	5	7	7	7	1	1	6	5	5	8	8	5	5
5	5	4	6	3	7	5	6	5	4	5	6	5	7	6	3	6	6	4	1
3	3	5	2	5	8	4	2	1	1	1	8	6	1	7	5	4	7	6	7
8	5	2	5	8	1	5	7	2	3	3	7	4	3	5	7	5	1	8	1
5	8	5	4	2	5	7	8	8	8	6	3	5	4	8	6	5	2	5	8
2	1	1	1	1	3	1	5	4	5	5	5	8	5	4	2	7	5	3	5
1	1	1	7	7	4	6	4	5	6	1	5	7	5	3	1	3	5	7	4
7	2	7	5	5	1	1	3	6	5	4	2	1	8	1	4	1	3	1	6

5.3.2 Oilseed rape pot experiment 2 (2018): Leaf injury and larval infestation:

For experiment 2 (harvest 2018), Winter OSR (cv. DK Imperial) plants were grown in the same conditions as experiment 1 to reduce variation between years. Plants were sown on 5/10/2017. Leaf area loss treatments were combined with subsequent controlled infection with *P. chrysocephala* larvae. As experiment 1 showed no effect of growth stage on effects of leaf area loss (see section 3.11), plants at the 1-2 true leaf stage were used (GS11-12) as these plants are more likely to infested with larvae than cotyledons (personal observation).

Adult *P. chrysocephala* feeding was simulated using the same method as experiment 1 with 0%, 25% and 90% leaf area removed, 55 days post sowing (29/11/2017). These were each followed 48 hours later by infection with *P. chrysocephala* larvae at zero, low or medium (current action threshold) levels of infestation (0, 1 and 5 larvae, respectively). Non-injured plants were also subjected to a high level of infestation (25 larvae). This sequence of simulated adult injury followed by larval infestation mimics the usual order of injury as it occurs in the field. Each of the resulting 10 treatments (Table 3) was replicated 12 times, with plants arranged in randomized blocks split into neighbouring grids comprising six blocks of 10 plants. Guard rows of additional OSR plants were grown around the experiments to allow each of the experimental plants to be within a density of 50 plants per m^2 and to allow comparable growth restrictions across all blocks, i.e., no experimental plants were located on the edges of the grids.

Table 3 Treatments in 2018 to test the impact of varying amounts of adult *Psylliodes chrysocephala* feeding injury (0, 25, and 90% leaf area injury), varying larval infestation (0, 1, 5 and 25 larvae/plant) and their interactions.

Treatment number	# larvae introduced	% leaf injury
1	0	0
2	1	0
3	5	0
4	25	0
5	0	25
6	0	90
7	1	25
8	1	90
9	5	25
10	5	90

Table 4 Layout of pots for second experiment (2018). Numbers represent the treatment type; (1) 0 larvae/ 0 injury, (2) 1 larvae/ 0 injury, (3) 5 larvae/ 0 injury, (4) 25 larvae/ 0 injury, (5) 0 larvae/ 25 injury, (6) 0 larvae/ 90% injury, (7) 1 larvae/ 25% injury, (8) 1 larvae/ 90% injury, (9) 5 larvae/ 25% injury, and (10) 5 larvae/ 90% injury.

Grid 1						Grid 2					
5	6	4	2	6	3	9	8	2	2	9	3
9	7	7	5	1	10	8	5	5	1	2	5
8	4	5	9	3	6	3	7	7	6	1	7
6	9	6	3	10	2	1	1	9	10	8	2
3	10	9	7	9	4	2	4	6	4	4	1
2	5	8	8	2	7	7	9	4	9	10	4
7	1	3	6	7	9	10	6	10	5	5	8
4	3	1	4	5	1	5	2	3	3	6	9
10	2	10	10	4	5	6	10	1	8	7	10
1	8	2	1	8	8	4	3	8	7	3	6

Figure 2. Second experiment (2018) layout of treatments inside field cage at University of Reading, nearside Grid 1 and far side Grid 2. All plants were laid out to a plant density of 50 plants/m^2. Plant pots were supported off the ground on a metal grid to prevent slug injury.

5.3.3 Simulation of shot holing leaf injury (experiments 1 and 2):

In both experiments 1 and 2 the OSR plants were injured using a leather punch to simulate the effects of *P. chrysocephala* feeding activity (3mm diameter hole punch, Figure3). The required level of injury was estimated by eye and all injury was done by the same individual (myself) to ensure consistency between treatments and comparability with assessment of adult feeding injury in the field (Chapters 2, 3 and 4, this thesis). It was important to ensure that the simulated injury was as comparable as possible to real insect feeding as it has been shown that the method of defoliation can have significant impacts on OSR growth (Susko and Superfisky, 2009).

In experiment 1 for group 1 (GS: 10), the simulated feeding injury was applied as a total area of both the cotyledons, whereas for group 2 (GS: 11) the injury was applied to the first true leaf.

In experiment 2 the simulated feeding injury was applied to the first two true leaves (GS 10-11) on 29th November 2017, and this was 48 hours prior to larval inoculation. This sequence of adult feeding injury of leaves followed by larval infestation of the stems mimics the usual order of injury as it occurs in the field.

Figure 3. Simulated injury to winter oilseed rape (*Brassica napus*) (A) and the leather punch (B) set to 3mm to mimic *Psylliodes chrysocephala* adult feeding injury.

5.3.4 Infestation of plants with *Psylliodes chrysocephala* larvae (experiment 2):

Larvae of *P. chrysocephala* were obtained from an untreated crop of OSR (cv. Campus) on Rothamsted Farm, Harpenden, UK in December 2017. Live larvae were carefully extracted from the plants by cutting open the stems and petioles using a scalpel under a light microscope and removing the larvae with a paint brush. The 2nd instar larvae (determined using the key by Ebbe-Nyman (1952)) were transferred to Petri dishes lined with damp filer paper and kept in a dark fridge at 3-4°C prior to plant infection. Second instar larvae were selected as they were more robust (less prone to desiccation) and had a higher infection success rate than either 1st or 3rd instars (unpublished preliminary trials); they were also caught most frequently in pitfall traps set in OSR crops (personal observations), suggesting that they actively move between plants.

Plants were infested with known numbers of larvae (0, 1, 5 or 25 per plant) by carefully placing them at the base of the stem (hypocotyl); larvae were left to locate and enter the plant naturally. To make the rate of infestation comparable between treatments, and more realistic of field conditions, the larvae were introduced over a nine-day period with 20% of the total number of larvae added every other day (1-9 December 2017). This allowed time for the collection of larvae and ensured that larvae were added to experimental plants within 24 hours of being extracted from field-collected plants. Staggered infestation also simulates the infestation process in the field, where it occurs over time. Plant fleece was placed over the plants during the infection period as freezing conditions were forecast. No larval mortality was observed.

Figure 4. Introduction of *Psylliodes chrysocephala* larvae to the base of oilseed rape, *Brassica napus*. A total of four larvae can be seen close to the OSR stem.

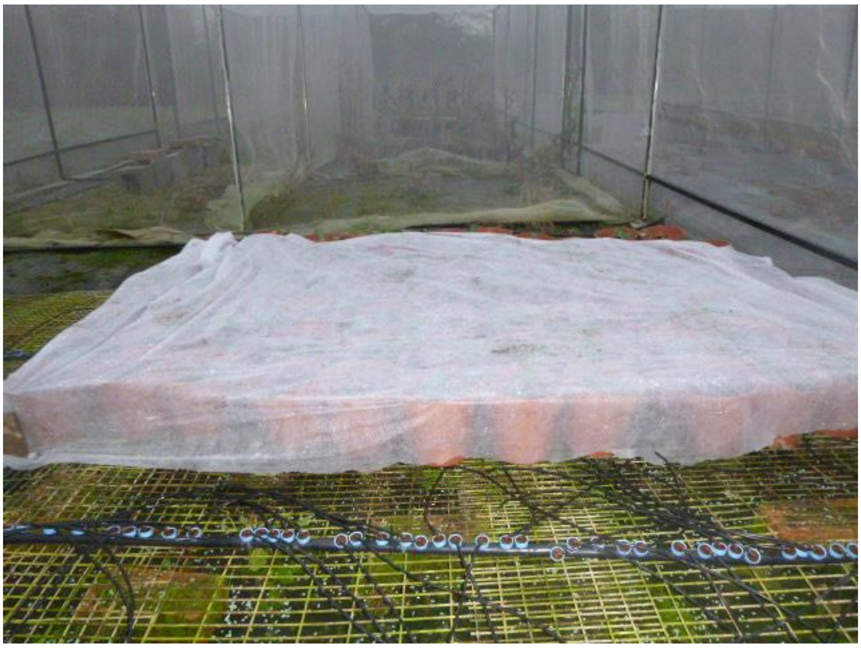

Figure 5. Experiment 2 (2018) showing plants covered with horticultural fleece to protect from frost damage and to reduce mortality of *Psylliodes chrysocephala* larvae that were artificially introduced.

5.3.5 Confirmation of larval infestation rates in the plants (experiment 2):

To confirm that larvae had entered the plants and to assess the proportion of larvae which entered the plants successfully, destructive sampling was performed on two of the blocks in spring (103 days after introduction, 21-23 March 2018; GS14), and plants were dissected to locate the larvae. A further four blocks were left to flower and were removed before pod ripening (GS77-83) to assess larval survival and development to adulthood. The plants were dissected (154 days after infection, 21-22 May 2018) as described above and scaring and the number of larvae found in the stems was recorded. The plant pot (with the compost) was bagged to capture emerging adults after pupation. Bagged plants were observed every 2-3 days for 5 weeks until 27th June; any adult *P. chrysocephala* captured in the bags following emergence were recorded and removed.

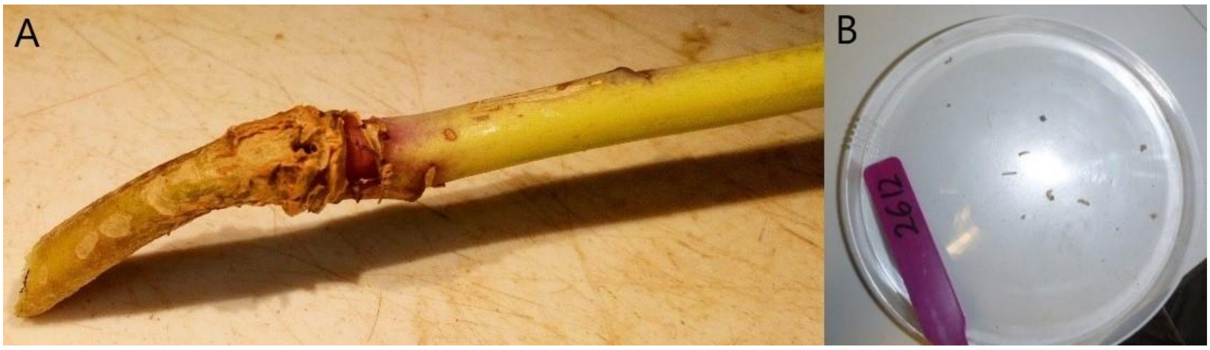

Figure 6. A) Evidence of stem scaring on OSR; this injury is indicative of *Psylliodes chrysocephala* larvae entering the stem to feed. B) *Psylliodes chrysocephala* Larvae extracted from one oilseed rape plant (treatment 4; 25 larvae introduced) on 21st March 2018 - confirming that larvae did actively enter the plants after inoculation.

5.3.6 Floral longevity (experiment 2):

The dates of the start (first flower open) and end (last flower senesced) of flowering were recorded for whole plants and the duration of flowering (days) was calculated for each plant in experiment 2. The longevity of flowering is affected by levels of pollinator activity (Bell and Cresswell, 1998), and, as flower-visiting insects were excluded from the cage, the flowering period would expected to be longer than it would be in field conditions (Carruthers *et al.*, 2017). Therefore, assessments were only considered as relative measures of treatment effects.

5.3.7 Nectar and Pollen measurements (Experiment 2):

Six flowers were removed from each plant in experiment 2 for analysis of nectar (3 flowers) and pollen productivity (3 flowers). All six flowers were collected from the main raceme and were of approximate age i.e., between flower number 20-35 counting from the first flower up, to maximise comparability between flowers and plants. Flowers produced early were chosen for analysis so not to affect the yield; removal of later-produced flowers (but not early-produced flowers) has been shown to affect yield (Tommey and Evans, 1992). Whole flowers were cut at the stem (base of pedicle) with sharp scissors to minimise plant injury, disturbance and leave a clean uniform wound. This was done 24 hours after first opening to allow nectar secretion to occur and the pollen to dehisce (Nedic *et al.*, 2013), and between 11:00 -13:00 hrs to ensure consistency in daily fluctuation of these resources (Carruthers *et al.*, 2017).

Nectar was extracted by inserting a micropipette (10µL, Drummond, Broomall, PA, USA) into a single nectary and the percentage of sugar was measured using an eye refractometer (Bellingham and Stanley Ltd, Tunbridge Wells, UK. 0-50% and 40-85%). Due to time constraints during flowering, it was not possible to accurately record nectar volume and as such the values obtained are relative measures of nectar sugar concentration, expressed as a relative treatment effect.

Flowers collected to measure pollen were stored immediately after collection in 99.9% ethanol for later analysis. Quantification of the number of pollen grains per m/l was determined based on methods adapted from Hicks *et al* (2016). Anthers were removed from the flower using fine scissors and placed in an Eppendorf tube. Pollen was extracted by sonication and vortex spinning samples. Once all the pollen grains were in suspension, the anthers were removed, and the tubes were spun in a centrifuge to form a pollen pellet. The samples were dried at 60^{o}C overnight. Each dry pellet was re-suspended in ethanol (60-120µl), vortex spun and sonicated to evenly distribute the pollen grains. A subsample was then transferred to a haemocytometer and the grains counted under a light microscope. The counts were converted to estimated number of pollen grains per ml using the following equation:

*pollen grains per ml = ((pollen count/cell number) *cell volume) * dilution factor*

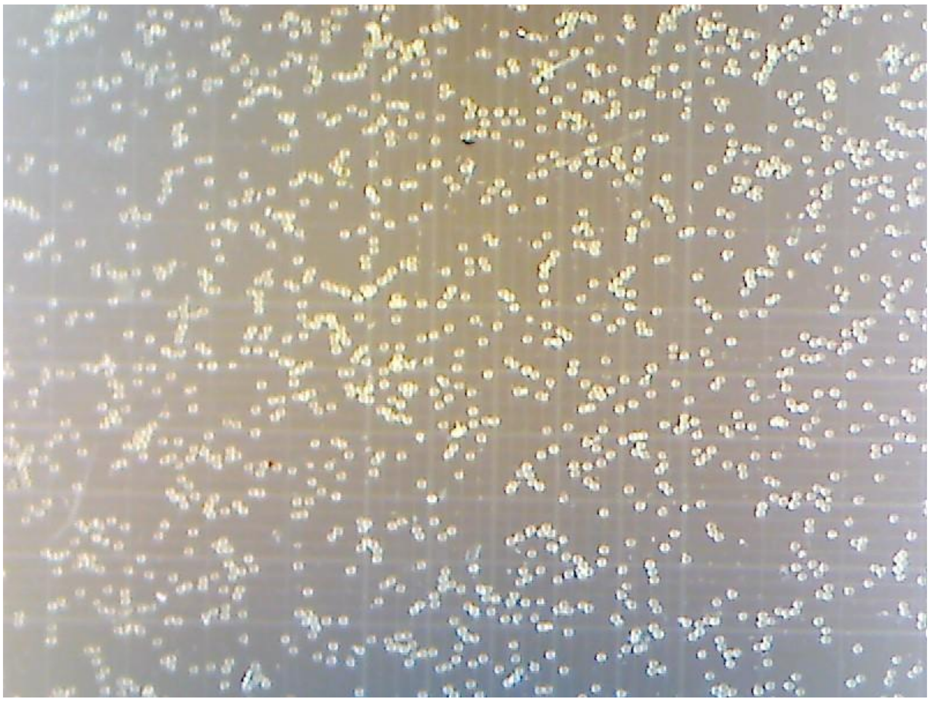

Figure 7. Sample of *Brassica napus* pollen on haemocytometer.

5.3.8 Harvest and yield measurements (experiments 1 and 2):

All plants were hand-harvested, when pods were dry to the touch (GS 89), on the 1st of September 2017 for experiment 1 and on 12th of July 2018 for experiment 2, and all set pods stored in paper bags, one for the main raceme and one for the side branches. The bags were stored in dry conditions prior to seed analysis. Plant height (to nearest cm) was recorded using a tape measure, and the number of side branches, pods set, and number of blind stalks were counted and recorded. Blind stalks are pod-less stalks with remnants of flowers that failed to set pods (Williams, Martin and White, 1986; Seimandi-Corda, Jenkins and Cook, 2021). By adding the number of pods and number of blind stalks, an estimated flower number per plant was calculated.

From each plant, a set of 10 pods were randomly selected from the main raceme and split to extract the seeds. The seeds were analysed using a Near InfraRed Analyser (Perten DA 7250 NIR Analyser) to measure the oil percentage. The same set of seeds were then processed through an electronic seed counter (Elmor applied electronics, C3 Counter) to determine the number of seeds per sample and the thousand grain weight as measures of seed yield.

5.3.9 Statistical Analyses:

All statistical analyses were performed using Genstat for Windows 18th Edition (International, 2016).

All responses from Experiment 1 were analysed using multi-stratum ANOVA (with strata corresponding to whole grids, blocks within grids, and pots within blocks within grids). After adding an offset of 1 to account for zeros, the number of secondary branches was transformed to logarithms (base 10), and the number of flowers to square roots, to remove variance heterogeneity; all other responses were untransformed. Plant height, number of secondary branches, primary pods, thousand grain weight and percentage oil content were unrecordable for 8, 9, 16, 16, 12 and 10 plants (i.e. a maximum of only 8% of the total of 200), respectively, and were hence set to missing and estimated using the method of Healy & Westmacott (Healy and Westmacott, 1956) as implemented in Genstat. Experiment 2 was analysed using a combination of multi-stratum ANOVA and where responses were zeros REML was used with transformation on LOG scale where necessary. For nectar a Wald test was performed due to restrictions in sample numbers. The pollen analysis was done using a REML to take missing samples into account and retain the blocking structure.

5.4 Results:

5.4.1 Confirmation of larvae introduction:

Dissections carried out in Experiment 2 showed that larvae successfully entered the experimental plants. Although the target infestation levels were not achieved, especially for the 25 larvae/plant treatment, increased introduction rates led to increased infestation, with significant differences between treatments ($F_{6,8.54}$; *P*=0.027, Table 5). Therefore, each treatment returned low (<1), medium (c. 2), and high (>5) larval infestation.

Table 5. Mean numbers ±SE of *Psylliodes chrysocephala* larvae re-captured 103 days post introduction to oilseed rape plants. No larvae were found in 0 plants with SE derived from transformation.

Number of larvae introduced	Mean number located in test plant dissections	-SE	+SE
0 (n=6)	0	0.685	0.396
1 (n=6)	0.33	0.685	0.396
5 (n=6)	2.33	0.685	0.396
25 (n=2)	6.5	0.685	0.396

Larval development and survival assessment in summer (GS 77-83, 154 days post introduction, 21-22nd/05/2018) showed a total of 19 out of 28 plants exhibited evidence of *P. chrysocephala* feeding activity (external scaring and internal feeding tunnels) with 13 adults collected from eight plants (range 1-3) with the highest capture from treatment with 25 larvae introduced). This provides evidence that *P. chrysocephala* larvae were capable of surviving the artificial infestation method and completing their development to adults.

5.4.2 Plant Mortality (experiments 1 & 2):

A total of eight plants died in experiment 1 (leaf area loss): five controls and three from the 90% leaf area loss treatment. In experiment 2 (leaf area loss x larval infestation) a total of five plants died from different treatments with no clear trend with treatment (Table 6). All other plants survived and produced harvestable pods.

Table 6. Plants which did not survive to harvest in experiment 2: experiment to determine effect of varying leaf area loss (0, 25 or 90%), varying larval infestation (0, 1, 5 or 25 larvae) and their interaction. Details show treatments of plants that died (n=1 plant each).

Treatment	Larvae introduced	Level of injury applied
3	5	0
5	0	25
7	1	25
8	1	90
9	5	25

5.4.3 Flowering period (experiment 2):

There was no effect of leaf injury level on the period of flowering ($F_{4,55}$=1.27; *P*=0.29, mean: 22 days, range: 4-33 days Figure 8). However, there was a statistically significant reduction in flowering duration for the 25 larvae treatment (treatment 4, $F_{2,55}$=3.81; *P*=0.028, Figure 8).

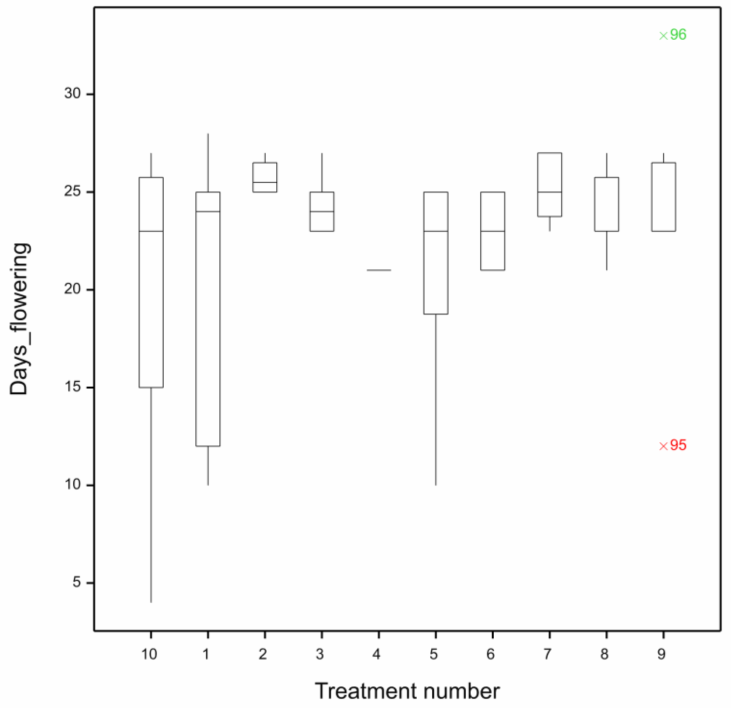

Figure 8. Experiment 2 (2018). Number of days the OSR plants were in flower after receiving leaf area injury and introduction of *Psylliodes chrysocephala* larvae. Treatment numbers: (1) 0 larvae/ 0 leaf injury, (2) 1 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury, (10) 5 larvae/ 90% leaf injury.

5.4.4 Nectar and pollen (experiment 2):

A total of 13 plants did not produce flowers in the desired location for sampling according to the protocol (See section 2.3.2) in grid 1 to measure pollen or nectar. Of these, five plants had died prior to flowering (Table 6). Of those which were alive but did not produce flowers in the right place on the main raceme, five were from treatment 4 (25 larvae introduced) and the other three plants were from different treatments (2, 5 and 10). Of these only 2 (treatment 4) produced any pods on the main raceme, while all surviving plants produced pods on secondary branches. In grid 2, 16 plants could not be measured for pollen and nectar, four from treatment 4 (25 larvae introduced) three from 10, two from treatments 9, 3 and 6 and one from treatment 2. All plants in grid 2 were destructively sampled for evidence of *P. chrysocephala* activity and were not measured for yield. Pod set data was collected and showed pods were produced on all except one plant but outside of the pollen and nectar sampling window.

In treatment 4 (25 larvae introduced) only two flowers were available at the correct age on the main raceme for analysis (desired n=24). The same was true for pollen, with only three samples being achieved, all from the same plant.

The percentage of sugar in the nectar was highly variable (mean: 63.39%, range: 29-75% sugar) with no effect of leaf area loss (χ^2_2 =0.88, P=0.644) or larval infestation (χ^2_2=3.03, P=0.220) and there was no interaction (χ^2_4=7.91, P=0.095, Figure 9). The same was found for numbers of pollen grains (mean: 30,0081, range: 53,333- 9,666,666 grains/ml), with no effect of variation in leaf area loss ($F_{2,50.7}$=0.71, P=0.496) or larval infestation ($F_{2,52}$=0.91, P=0.410), with no interaction ($F_{4,51}$=0.38, P=0.821, Figure 9). It should be noted that no pollen beetles (*Brassicogethes aeneus* F.) or other insects were observed on collected flowers, thus confirming the efficacy of the mesh cages in excluding insects.

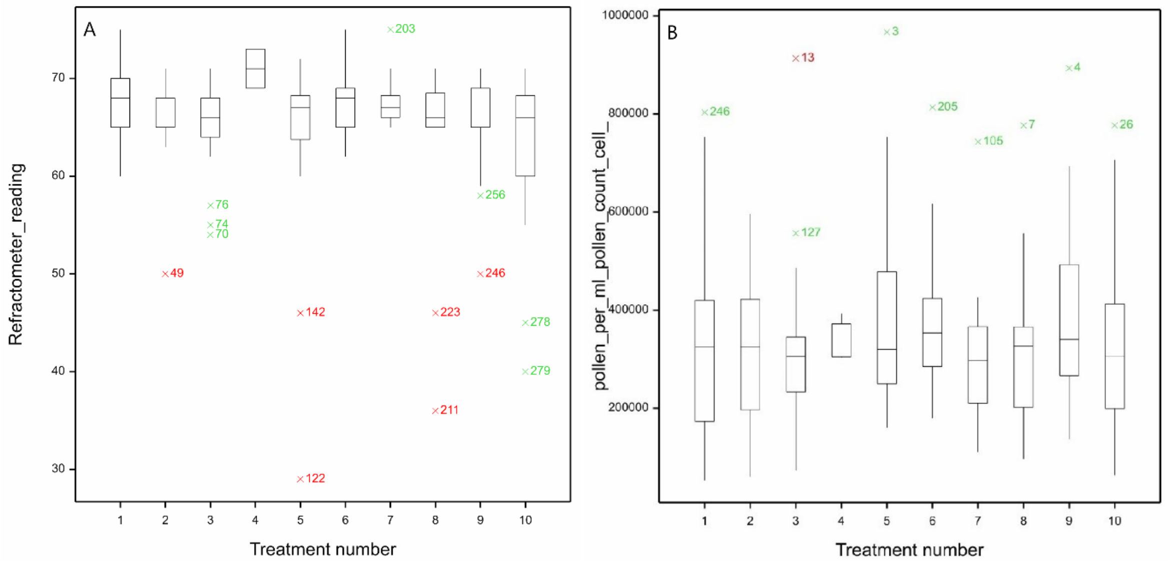

Figure 9. Experiment 2 (2018). (A) Refractometer readings of percentage sugar content of nectar and (B) number of pollen grains per ml. Treatments: (1) 0 larvae/ 0 leaf injury, (2) 1 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury, (10) 5 larvae/ 90% leaf injury.

Summing the total number of pods and the number of blind stalks gave the total number of flowers produced by each plant. In Experiment 1 (analysis on square root scale), the total number of flowers produced (range 0 – 542, overall mean (square root scale): 9.51 (back-transformed mean: 90.44)) was not affected by injury level ($F_{3,172}$=1.26, *P*=0.291) or growth stage ($F_{1,172}$=1.51, *P*=0.221), and there was no interaction between these factors ($F_{3,172}$=2.24, *P*=0.085). In Experiment 2 the only treatment to differ from controls was the 25 larvae introduction showing significant reductions in flower potential ($F_{1,42}$=8.15, *P*=0.007, Table 7 and Figure10). All other treatments did not differ from un-injured controls ($F_{2,42}$=0.89, *P*=0.419, Table 7 and Figure 10).

Table 7. Average total flower potential of oilseed rape (*Brassica napus*) when exposed to early growth leaf area injury and *Psylliodes chrysocephala* larvae introduction. Estimates of flower potential taken from final pod and blind stalk counts. * donates significant difference (P<0.05).

		Percentage leaf area injury (+/-SED=8.926)		
		0	25	90
No. of *P. chrysocephala* larvae introduced	0 (n=18)	53.17	54.32	30.67
	1 (n=18)	40.33	39.23	40.52
	5 (n=18)	40.32	54.72	51.67
	25 (n=6)	25.00 *	n/a	n/a

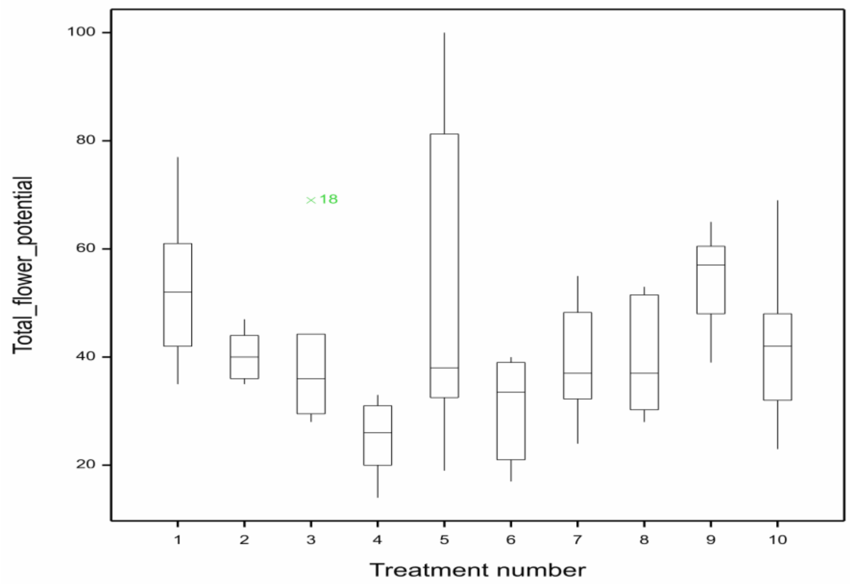

Figure 10 Total flower potential of oilseed rape (*Brassica napus*) plants when exposed at early growth stage to leaf area loss (simulated feeding injury by *Psylliodes chrysocephala* adults) and infestation with P. chrysocephala larvae. Treatments were: (1) 0 larvae/ 0 leaf injury, (2) 1 larva/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larva/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury (10) 5 larvae/ 90% leaf injury. Values calculated from combining total pod production and blind stalks at harvest.

5.4.5 Plant height and branching:

In Experiment 1 (leaf area loss), plant height was unaffected by the amount of leaf area loss ($F_{3,165}$=1.9, *P*=0.132, mean 116.49cm, range; 80-150cm) for both growth stages ($F_{1,165}$=0.09, *P*=0.765), with no interaction between the two treatment factors ($F_{3,165}$=0.07, *P*=0.976). However, in Experiment 2 (leaf area loss x larval infestation), plants in treatment 4 (25 larvae introduced) were significantly shorter compared to all other treatments (whole treatment mean: 83.8cms, range; 40-125cm, treatment 4: mean: 52.1cm, range: 45-64cm, $F_{1,40}$=27.59, *P*<0.001, Figure 11), all other treatments did not differ in height from the control.

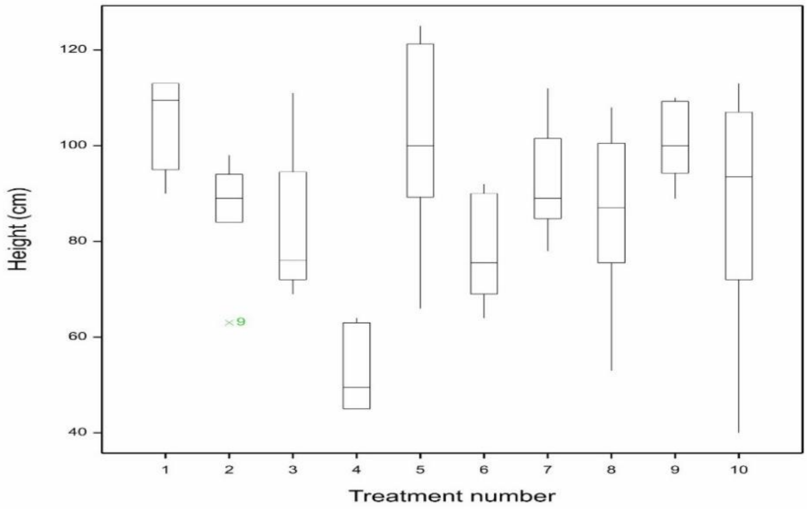

Figure 11. Experiment 2 (2018). Effect of leaf area loss and infestation with *Psylliodes chrysocephala* larvae on plant height (cm) in oilseed rape (*Brassica napus*). Treatment numbers: (1) 0 larvae/ 0 leaf injury, (2) 1 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury, and (10) 5 larvae/ 90% leaf injury.

Production of secondary branches was low in Experiment 1 (analysis on log scale with offset) with only 8% of plants producing more than 10 side branches (range: 1-49; overall mean (log scale): 0.701 (back-transformed mean: 4.023)). No effect of either leaf area loss ($F_{3,164}$=0.70, P=0.552) or growth stage ($F_{1.164}$=1.14, P=0.288) was observed and there was no interaction between these treatment factors ($F_{3,164}$=1.61, *P*=0.188). In Experiment 2 (leaf area loss x larval infestation), production of secondary branching was also low (16 out of 39 plants produced secondary branches range:1-4, mean: 2.43) but plants in the 25 larvae per plant treatment produced a significantly higher number of branches than all other treatments ($F_{1,40}$=7.37; *P*=0.010, see **Error! Reference source not found.**12).

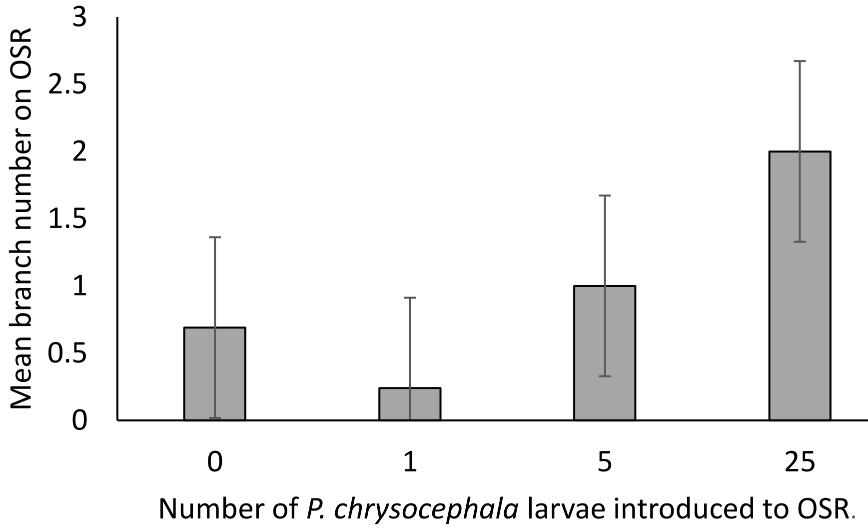

Figure 12. Branching in Oilseed rape (OSR, *Brassica napus*). When inoculated with larvae of *Psylliodes chrysocephala*).

5.4.6 Pod production:

In Experiment 1, primary pod production was not affected by the amount of leaf area loss (pod production range: 7-169, overall mean 51.78, $F_{3,157}$ = 0.79; P=0.501, Figure13) at either growth stage ($F_{1,157}$=0; P=0.956, Figure13). There was an interaction between growth stage and injury level ($F_{3,157}$=2.88, P=0.038). The same was found when the total number of pods per plant were considered; this was unaffected by injury level ($F_{3,172}$=1.49, P=0.218, Table 8) at either growth stage ($F_{1,172}$=0.02, P=0.892, Table 8) and there was no interaction ($F_{3,172}$=1.59, P=0.194, Table 8).

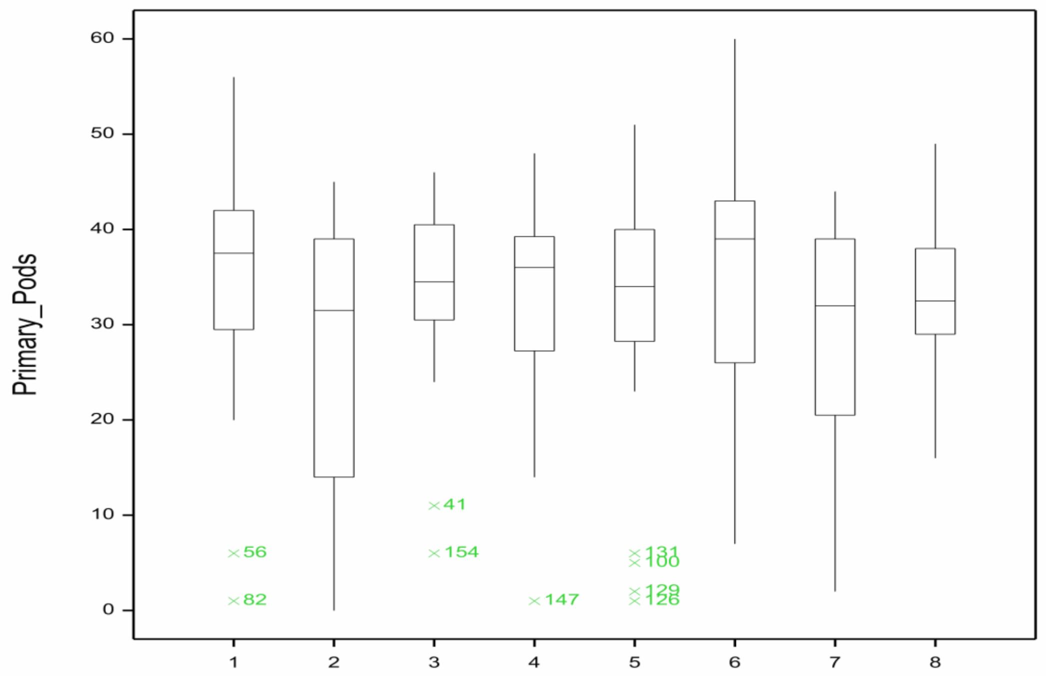

Figure 13. Number of primary pods produced by of oilseed rape (*Brassica rape*) when exposed to early growth leaf area injury at two Growth Stages (GS, 9 – cotyledon and 10 – first true leaf unfurled). Treatments: (1) GS9/0 injury, (2) GS9/25% injury, (3) GS9/50% injury, (4) GS9/90% injury, (5) GS10/0 injury, (6) GS10/25% injury, (7) GS10/50% injury, (8) GS10/90% injury.

Table 8. Mean total pod production of oilseed rape (*Brassica napus*) exposed to early growth leaf area loss Growth Stages (GS, 9 – cotyledon and 10 – first true leaf unfurled). +/- SE=5.29.

	Percentage leaf area removed			
Growth stage when injury applied	0 (n=80)	25 (n=40)	50 (n=40)	90 (n=40)
9	55.2	54.7	57.2	46.0
10	49.6	64.0	53.4	53.8

In experiment 2, all surviving plants produced harvestable pods (mean: 18.16, range: 3-51 pods/plant, Figure 14). Plants in the 25 larva per plant treatment produced significantly fewer pods than the other treatments (treatment 4: mean: 9.83, range: 3-16 other treatments mean: 21.42, range: 8-51 pods/plant, $F_{1,40}$=16.65, *P*<.001, Table 9 and Figure 14). No effect was seen for any other larval level ($F_{2,40}$=1.13, *P*=0.332) or leaf area loss ($F_{2,40}$=0.69, *P*=0.393).

Table 9. Average number of total pods produced from whole plant of winter oilseed rape (*Brassica napus*) exposed to leaf area injury (0, 25 and 90% leaf area loss) and *Psylliodes chrysocephala* larvae introduced (0, 1, 5 and 25). * donates significant difference.

		Percentage leaf area removed (+/-SED=4.308)		
		0	25	90
No. of *P. chrysocephala* larvae introduced	0 (n=18)	24.00	29.99	16.33
	1 (n=18)	19.83	21.58	18.59
	5 (n=18)	18.79	24.99	18.67
	25 (n=6)	9.83*	n/a	n/a

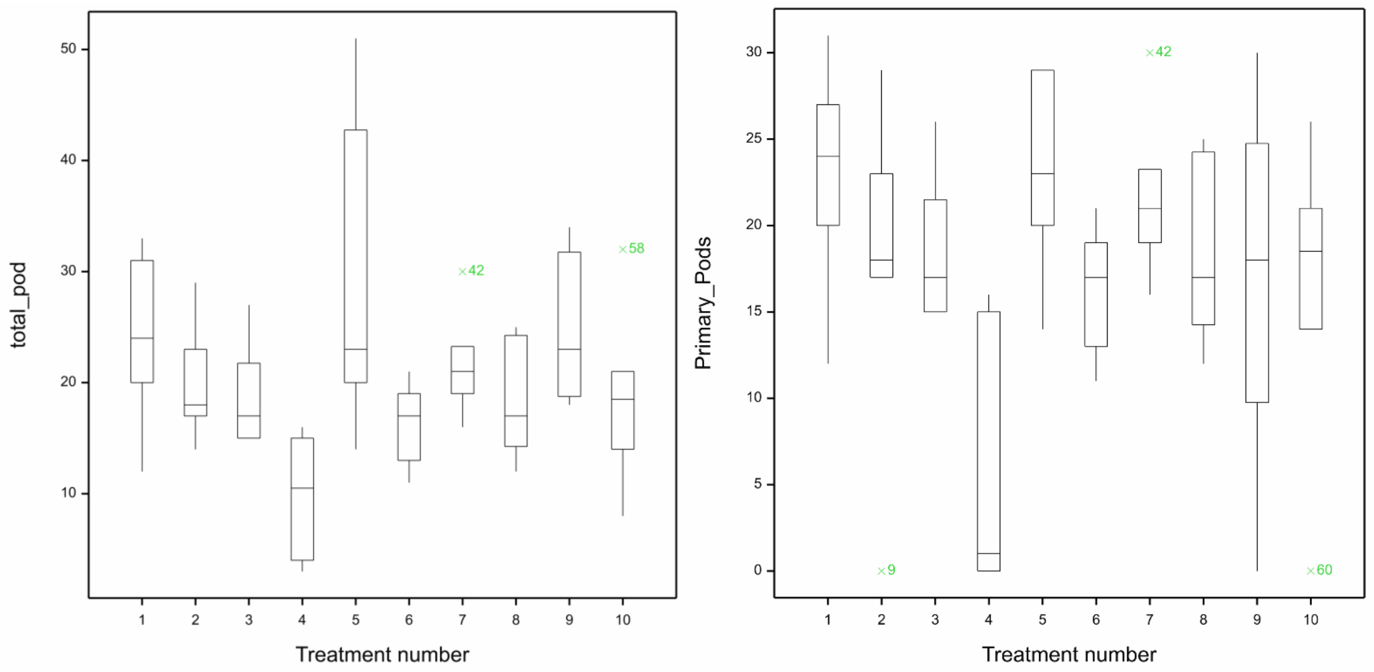

Figure 14. Number of primary pods on the main raceme and total pods from whole plants of oilseed rape (*Brassica napus*) plants when exposed at early growth stage to leaf area loss (simulated feeding injury by *Psylliodes chrysocephala* adults) and infestation with *P. chrysocephala* larvae. Treatments were: (1) 0 larvae/ 0 leaf injury, (2) 1 larva/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larva/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury (10) 5 larvae/ 90% leaf injury.

5.4.7 Seeds per pod:

In Experiment 1 there was no effect of level of leaf area loss ($F_{3, 162}$=0.30, *P*=0.829) or growth stage ($F_{1,162}$=0.22, *P*=0.642) on the average number of seeds per 10 pods (range: 13-275; overall mean: 198.7) and there was no interaction between the two factors ($F_{3, 162}$=0.12, *P*=0.947, Table 10).

Table 10. Mean number of seeds/10 pods of oilseed rape (*Brassica napus*) exposed to early growth leaf area removal at Growth Stages (GS, 9 – cotyledon and 10 – first true leaf unfurled).

	Percentage leaf area injury (+/-SED=11.03)			
Growth stage when injury applied	0 (n=40)	25 (n=20)	50 (n=20)	90 (n=20)
9	198.5	195.1	199.2	195.4
10	204.9	194.8	197.3	198.7

In Experiment 2 the average number of seeds per pod was significantly reduced on plants with 25 *P. chrysocephala* introduced ($F_{1,40}$=16.70, *P*<.001, Table 11). All other treatments did not differ from uninjured control plants ($F_{2,40}$=0.42, *P*=0.658, Table 11).

Table 11. Mean number of seeds in ten pods of oilseed rape (*Brassica napus*) exposed to early growth leaf area removal (0,25 and 90% removed) and introduction of *Psylliodes chrysocephala* larvae (0, 1, 5 and 25 individuals). * donates significant differences.

		Percentage leaf area injury (+/-SED=26.5)		
		0	25	90
No. of *P. chrysocephala* larvae introduced	0 (n=18)	188	175	171
	1 (n=18)	176	198	193
	5 (n=18)	181	195	150
	25 (n=6)	100*		

5.4.8 Thousand Grain Weight:

In experiment 1, the thousand grain weight of seed was not affected by leaf area lost ($F_{3,161}$=0.78; *P*=0.508, grand mean: 4.552g, range: 1.529 – 8.947g, Table 12) nor by the growth stage at which the injury was applied ($F_{1,161}$=2.17; *P*=0.143, Table 12) and there was no interaction between the two factors ($F_{3,161}$=1.38, *P*=0.251, Table 12).

Table 12. Mean Thousand Grain Weight (TGW, g) from 10 pods of oilseed rape (*Brassica napus*) exposed to early growth leaf area removal.

	Percentage leaf area injury (+/-SED=0.2331)			
Growth stage when injury applied	0 (n=40)	25 (n=20)	50 (n=20)	90 (n=20)
9	4.607	4.469	4.760	4.370
10	4.545	4.762	4.886	4.960

In Experiment 2 there was no significant effect on TGW from leaf area injury ($F_{2,38}$=0.41; *P*=0.67, Table 13 and Figure 15) or level of larval infestation ($F_{2,38}$=0.68; *P*=0.514, Table 13 and Figure 15).

Table 13. Mean Thousand grain weight of seeds (TGW, g) calculated from seed taken from 10 pods of oilseed rape (*Brassica napus*) exposed to early growth leaf area removal and infestation with *Psylliodes chrysocephala* larvae. +/-SED=0.515.

		Percentage leaf area removed		
		0	25	90
No. of *P. chrysocephala* larvae introduced	0 (n=18)	3.53	2.96	2.48
	1 (n=18)	2.87	2.85	2.66
	5 (n=18)	2.86	3.22	3.34
	25 (n=6)	2.80		

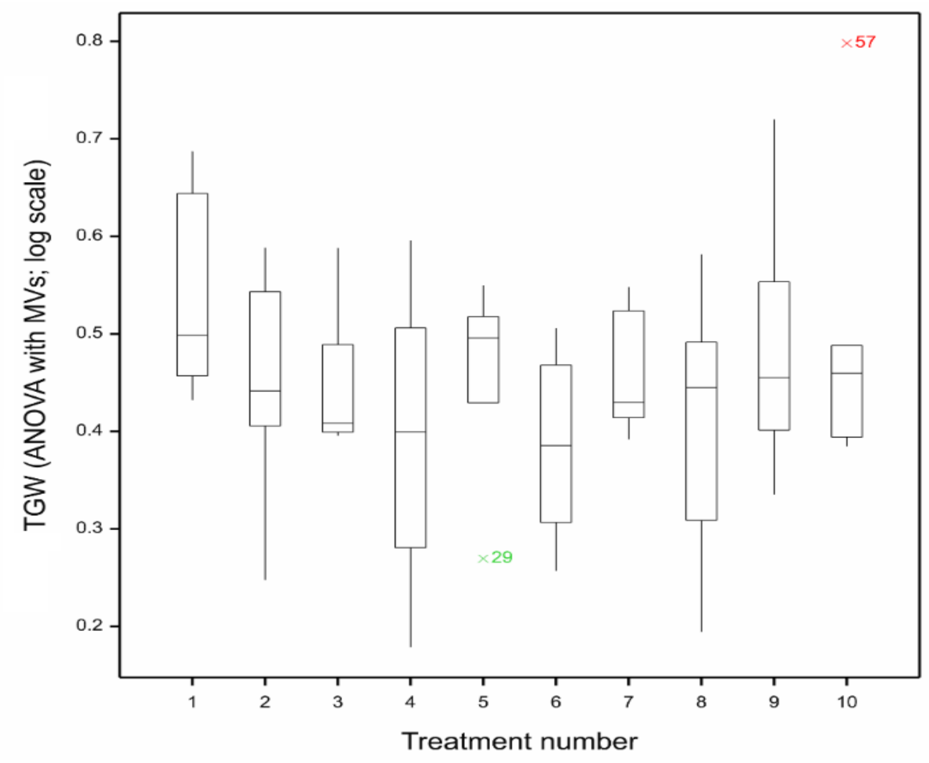

Figure 15. Experiment 2 (2018). Thousand grain weight for 10 pods on the primary raceme of oilseed rape (*Brassica napus*) when exposed to early growth leaf area injury and introductions of *Psylliodes chrysocephala* larvae. Treatment numbers: (1) 0 larvae/ 0 leaf injury, (2) 1 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury, and (10) 5 larvae/ 90% leaf injury.

5.4.9 OSR yield quality measures:

Oil content of the seeds was not affected by injury level ($F_{3,163}$=0.48, *P*=0.694) or growth stage ($F_{1,163}$=0.62, *P*=0.431, mean: 48.47%, range: 31.61% – 53.87%, Figure 16) in experiment 1 (interaction $F_{3,163}$=0.20, *P*=0.899). However, in experiment 2, although there was no effect of the leaf area lost ($F_{2,40}$=1.12, *P*=0.335, mean: 50.48%, range: 32.69 –

55.38%), there was a significant reduction in percent oil content of seeds from plants in the 25 larvae per plant treatment (treatment 4, $F_{1,40}$=21.84, *P*<0.001, mean: 44.61, range: 32.69 – 52.78%, Figure 16) , with only sample producing over 50% oil content.

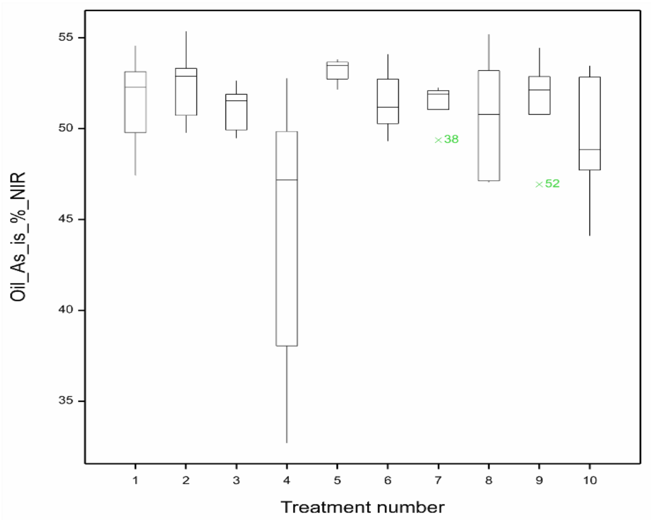

Figure 16. Seed oil percentage content from oilseed rape (*Brassica napus*) seeds exposed to early growth leaf area injury and infestation with *Psylliodes chrysocephala* larvae (Experiment 2). Measurements taken from all seeds from 10 pods from the main raceme. Treatments were: (1) 0 larvae/ 0 leaf injury, (2) 1 larva/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larva/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury (10) 5 larvae/ 90% leaf injury.

The levels of glucosinolate in seeds was also shown to be affected when plants were infected with high numbers of larvae. With levels recorded significantly higher in the treatment where 25 larvae were introduced ($F_{2,40}$=43.59, *P* <0.001, Figure17). All other treatments did not differ from uninjured control plants ($F_{4,40}$=0.69, *P* =0.600, Figure17)

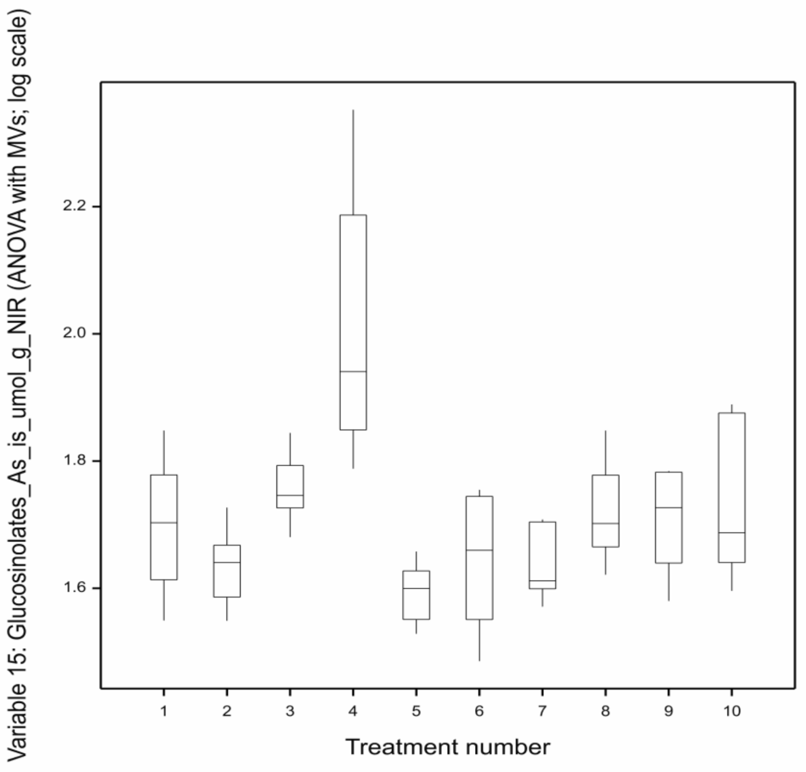

Figure 17. Total glucosinolates concentration (umol/g), on LOG scale, from oilseed rape (*Brassica napus*) seeds after exposure to early growth leaf area injury and introduction with *Psylliodes chrysocephala* larvae. Treatments were: (1) 0 larvae/ 0 leaf injury, (2) 1 larva/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larva/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury (10) 5 larvae/ 90% leaf injury.

5.5 Discussion:

The data reported here suggests that high levels leaf area loss at early growth stage, i.e. leaf injury as caused by adult *P. chrysocephala* feeding does not impact on final yield of oilseed rape and low levels of *P. chrysocephala* larvae infestations can also be tolerated. However, higher larval infestation resulted in negative effects on plant growth which would negatively affect both farmers and insects which use floral resources, such as bees.

The lack of significant effects on oilseed rape flower production and resource quality, plant architecture and yield from any of the leaf area loss treatments applied in this study are in line with studies showing little to no yield penalty from leaf area loss at early growth in both simulated injury experiments (Kirkegaard *et al.*, 2008b; Antwi, Olson and DeVuyst, 2008; Ramachandran, Buntin and All, 2000; Sunderland *et al.*, 1995; Syrovy, Shirtliffe and Zarnstorff, 2016) and field observations under natural pest infestations (Gavloski *et al.*, 2000). Susko & Superfisky (2009) demonstrated no significant impact from 50% leaf (simulated) area injury on early growth in spring OSR.

Actual pest feeding of another leaf flea beetle, *Phyllotreta* sp on spring canola exhibits reduction in seed production compared to simulated injury (Antwi, Olson and DeVuyst, 2008; Nowatzki. T and Weiss. M, 1997), potentially due to the absence in simulated injury experiments of biological factors such as pest saliva, which impacts the production of secondary chemicals, which entails a metabolic cost to the plant (Baldwin, 1990). Without the extra stimuli to create secondary metabolites the cost of compensation to injury may be reduced. Therefore, the lack of effect on later growth and yield parameters resulting from early growth leaf injury observed here may not truly represent the impact of *P.*

chrysocephala adult feeding activity in the field; where plants are exposed to all stimuli of feeding whilst also under potentially sub optimal conditions in terms of water and nutrient status and potential additional pest injury compared with the potted plants used in this study. Simulated injury was used here to control exactly and replicate sufficiently the amount of leaf injury, which is difficult to do with live insects (White *et al.*, 2020). The lack of cumulative injury may also account for the lack of effect from leaf injury seen here as the plant may be less able to cope with sustained attack than singular feeding bouts (Crawley, 1983). However, our results concur with field experiments that show that plants can respond to early growth stage defoliation with strong biomass recovery (Ellis, 2015b; Kirkegaard *et al.*, 2008a; Ellis, 2015a; Syrovy, Shirtliffe and Zarnstorff, 2016).

The results of this study suggest that once the crop is established, larval infestation can pose a significant effect to yield attainment. Other studies which have attempted to manipulate *P. chrysocephala* larvae have used complicated and time-consuming methods to obtain experimental larvae (Döring and Ulber, 2020; Ruck, Cadoux and Robert, 2018). This is the first attempt to experimentally manipulate numbers of stem boring *P. chrysocephala* larvae from field populations to quantify their effect on crop yield. The infestation success in achieving each of the treatment levels was not 100%, particularly for the high infestation treatment (25 larvae). This was potentially due to unknown factors such as intraspecific competition or variations in larvae robustness. However, larvae were successfully recovered from test plants and the capture of adult *P. chrysocephala* towards the end of the experiment demonstrates that manipulated infestation did not disrupt the development of the beetle and allowed the empirical test of the impact larval development on OSR growth and yield with limited equipment and facilities. This method can be used as a simple way of experimentally transferring *P. chrysocephala* larvae in further research e.g. into the tolerance of OSR cultivars to prolonged larval feeding or testing effects of exposure to novel insecticides.

The presence of *P. chrysocephala* larvae have been shown to effect the glucosinolate profile and concentration in leaf and stem material (Koritsas, Lewis and Fenwick, 1989; Koritsas, Lewis and Fenwick, 1991; Döring and Ulber, 2020). None of these studies have examined the chronic effect of larvae as done here and were sampled after 10 -21 days post simulated injury or larval introduction. All show an increase in GLS concentration as a response to injury and larvae infection. Here we show that this process is observable in the seeds; these showed higher levels of glucosinolates from plants when inoculated with high numbers of larvae.

Inoculating OSR with 25 larvae/plant returned an average of 6.5 larvae per plant when a subset of plants was dissected at a later stage. This level of actual infestation significantly reduced flower and pod production and the seeds produced contained lower oil content and higher concentrations of glucosinolate. There was no effect on these factors when lower numbers of larvae were introduced. This finding suggests that in this experimental set up the action threshold level i.e., the number pf *P. chrysocephala* above which there is a danger of yield loss if untreated for *P. chrysocephala* larvae is somewhere above 5.8 larvae/plant. Further study would be required to fully assess exact number in these conditions and experiments testing a range of OSR cultivars under varying management conditions are needed to develop truly accurate economic injury thresholds.

The total flower production was not affected by any leaf area injury in either experiment or by low or medium levels of larval infestation. However, when high levels of *P. chrysocephala* larvae were introduced the plant’s capacity to produce flowers was significantly reduced. Although the high larval infestation treatment plants produced fewer flowers no effect was seen in either nectar sugar concentration or the amount of pollen produced. However, as volume and total sugar concentration was not recorded in this experiment and pollen was not assessed for protein or amino acid content, other flower resource effects might have been missed. For instance, if the total volumes of nectar available were substantially lower in injured than in un-injured plants, then pollinator activity and pollination could be reduced with an impact on seed production and oil content (Bommarco, Kleijn and Potts, 2013). This requires further investigation as OSR is an important early flowering crop which is visited by a wide range of insects (Garratt *et al.*, 2014).

The artificial infection method detailed here can facilitate empirical testing of synthetic insecticides, alterative control methods and economic injury thresholds for *P. chrysocephala* and will aid the development of biologically relevant action thresholds which would inform farmers and policy makers to better calculate a cost: benefit of insecticide application based on empirical data and not economic costs of application.

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6.0 Feeding preference of *Psylliodes chrysocephala* L. on early true leaves of Oilseed Rape (*Brassica napus* L.):

6.1 Abstract:

With the EU ban on the use of neonicotinoid insecticides in oilseed rape (OSR, *Brassica napus*) protecting the crop from pest levels of damage from the cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala* L.) has become an increasing issue. For farmers to have reliable predictors of pest pressure an understanding of how the pest and crop interact ecologically is crucial for accurate predictions. It has been suggested that *P. chrysocephala* L. adults selectively feed on true leaves of oilseed rape (OSR, *Brassica napus* L.) over the cotyledons. In a whole plant choice experiment we support the hypothesis that adult *P. chrysocephala* preferentially feed on unfurled true leaves of OSR over cotyledons. The implications on crop establishment and pest protection practices for OSR which are discussed.

6.2 Introduction:

Protecting crops from pest pressure is a major aspect of agriculture and understanding how crops and pest species interact is critical to understanding the risks to the crop and production. The cabbage stem flea beetle, *Psylliodes chrysocephala* L., is a major pest of European oilseed rape (OSR, *Brassica napus* L.) production (Williams, 2010; Zhang *et al.*, 2017). The adult beetles enter the crop during early autumn and feed directly on the OSR leaves. After a period of feeding, the females begin to lay eggs at the base of the OSR stems and these develop into larvae which then tunnel into the stem and feed internally throughout the winter before exiting the plant to pupate in the soil in mid spring (Williams, 2010).

The importance of this pest has increased in recent years, particularly in the UK with populations increasing (Collins, 2017) at least in part, as the result of restrictions on the major synthetic chemical protection options (EU, 2018) and increasing pyrethroid resistance reported in France (Bothorel *et al.*, 2018), Germany (Heimbach and Brandes, 2016), Denmark (Castberg and Kristensen, 2018), Czech Republic (Jitka and Kocourek, 2019) and the UK (Foster and Williamson, 2015). As pest pressure has increased there have been reductions in the OSR cropped area in the UK (Scott and Bilsborrow, 2019). This is often attributed to *P. chrysocephala,* slugs and pigeons (Coston et al in prep). It has been shown that severe feeding damage by *P. chrysocephala* adults during the early growth stages of the crop can lead to crop failures (Chapter 2 and 3 this thesis).

With reduced pesticides options there is a need for better targeting of those available, including knowing which growth stages are preferred by the beetles and therefore in most need of protection. There is currently little data on the feeding preferences of adult *P. chrysocephala,* and information of this kind may be important in determining when the crop is most vulnerable; facilitating both the targeting of insecticide use and for developing time-specific trap or nurse crops that coincide with the preferred crop growth stage. Preliminary work by Diehl (2017) on *P. chrysocephala* feeding on leaf discs cut from leaves of varying ages of OSR demonstrated low feeding on cotyledons but higher and comparable levels on the first, second and third leaves after 48hours. This result may be due to experimental bias where the preparation of leaf discs breaks the leaf cuticle resulting in internal chemical cues the beetles may be utilising when selecting a suitable feeding site. To confirm the finding of Diehl (2017) while removing any effect of leaf cuticle damage, a whole plant choice experiment was used, avoiding any effect of previous leaf injury. Adult *P. chrysocephala* (2 male and 2 female) were introduced into enclosed propagator trays with OSR plants at four growth stages and the cotyledon/leaf area lost was assessed after 48 hours to compare feeding location. The experiment was concluded at 48 hours to ensure that all leaf material was not removed, and the initial feeding can be observed.

6.2.1 Aims of study:

This study was designed to confirm the finding of Diehl (2017) on leaf discs feeding preference in full plants. It is hypothesised that beetles selectively feed on older leaves over the cotyledons. This will be confirmed if feeding activity is recorded at higher levels on the true leaves over the cotyledons.

6.3 Methods:

6.3.1 Plants:

Winter oilseed rape (*Brassica napus,* cv. Falcon) were sown in seed trays using Levington L2+S compost. Trays of OSR were set up daily (01/11/2016 to 7/12/2016) to produce plants at Growth stage 10-13 based on the BBCH scale (Lancashire *et al.*, 1991) simultaneously. Where GS10 is cotyledons extended, GS11 is first true leaf unfolded, GS12 is two true leaves unfolded and GS 13 is 3 true leaves unfolded. Plants were kept under standard glasshouse conditions with no additional heat or lighting. Average temperature range was 12-15^{o}C with 9 daylight hours.

6.3.3 Experimental Arenas:

The experimental feeding arena comprised a plastic propagator with a clear lid and a plastic seed tray base (220 x 350mm) with a Rothamsted standard compost mix (Petersfield Products, Leicester, UK) and four OSR plants, one at each of BBCH Growth Stages GS10, GS11, GS12 and GS13 (Lancashire *et al.*, 1991), each equidistant from the centre of the tray (see Figure 1 and Figure 2). A total of 16 feeding arenas were established and set in four Latin square replicates with two replicate squares (‘end 1’ and ‘end 2’) placed directly below two lights blocks (to reduce differences between treatments in lighting), in a controlled environment room, (18^{0}C +/- 1^{o}C, L-D photoperiod 12-12 with strip fluorescent lights, (see Figure1 and Figure2). The environment conditions were set to allow activity of the beetle as they are observed to be highly active at these temperatures. The diel activity of *P. chrysocephala* is little understood (explored further in Chapter 7 this thesis) therefore, to ensure ample opportunity for feeding behaviour a 12:12 light: dark split was used. The distribution of plants within each tray formed a Latin square with each plant growth stage occurring once in each of the 4 positions within a block of 4 propagators (Figure1). After transplanting, all trays were watered and given 24hours for plants to recover from wilting stress before the beetles were introduced.

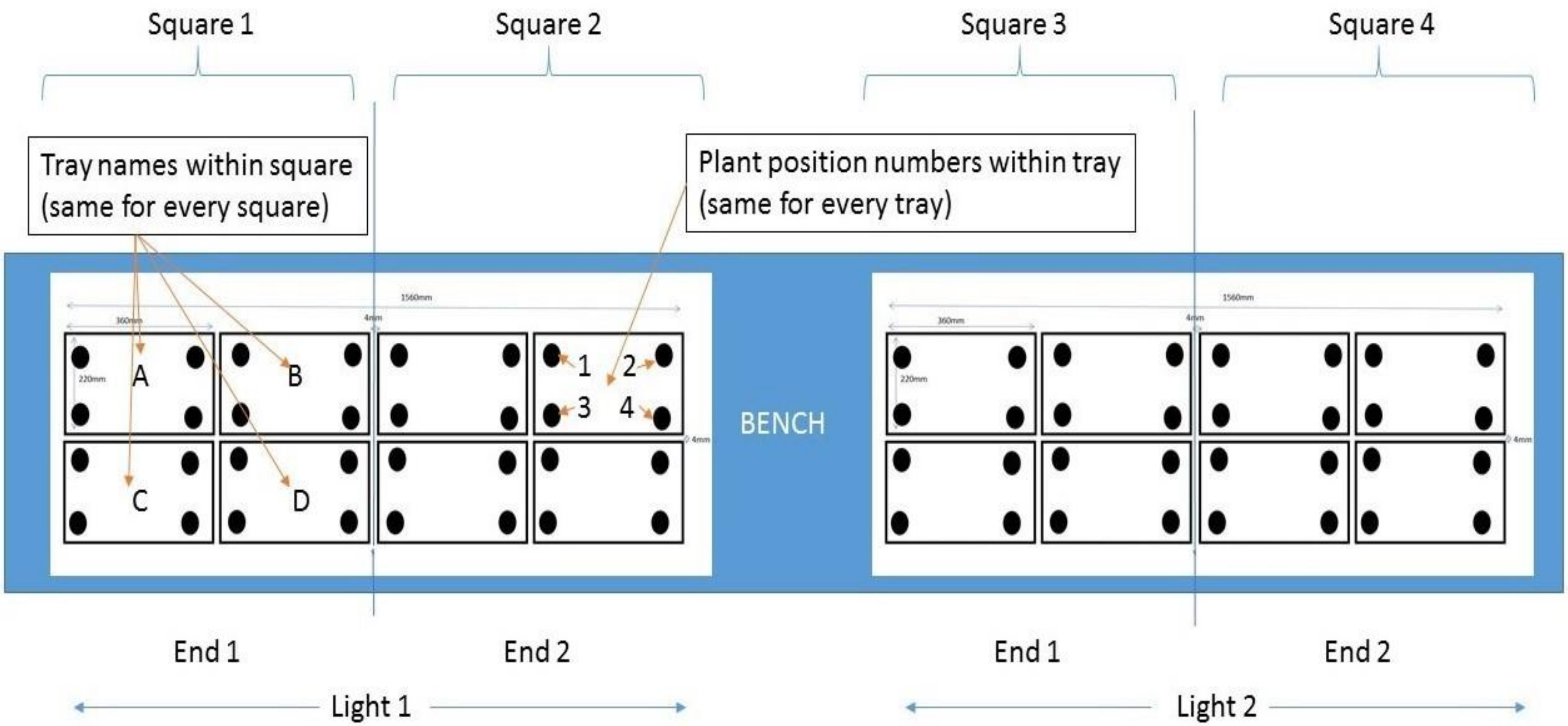


Figure 1. Schematic diagram of the layout of experimental arenas used to determine feeding behaviour of *Psylliodes chrysocephala* on *Brassica napus* plants of four different growth stages (1=GS10, 2 = GS11, 3=GS12 and 4=GS13). Black dots show the location of plants within arenas (rectangles labelled A, B, C D), arenas within Latin square replicates and the distribution of each replicate under two lights suspended above the bench in a controlled environment room.

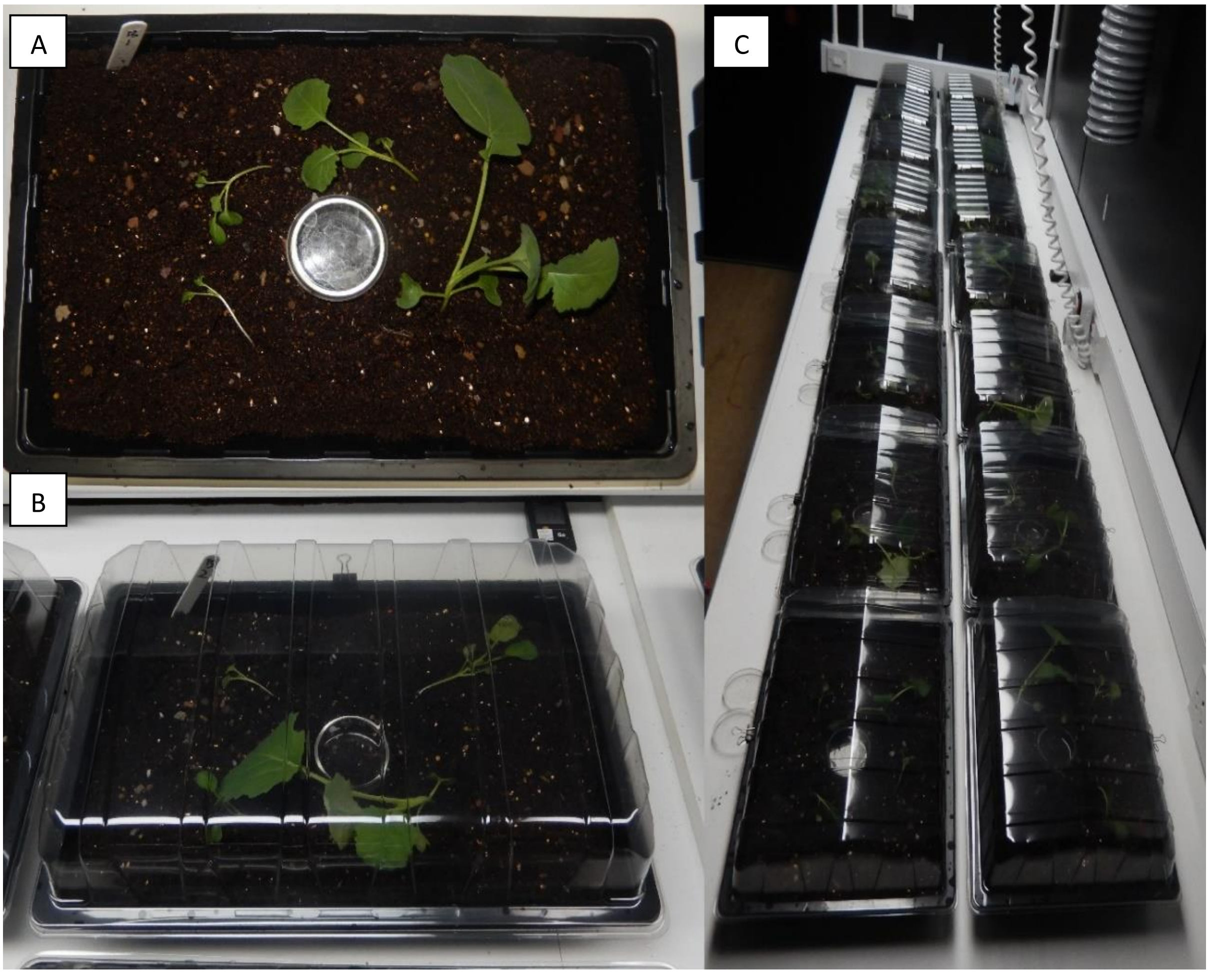


Figure 2. Photographs showing the experimental arena design and layout of the experiment to test feeding behaviour of *Psylliodes chrysocephala* on *Brassica napus* plants of four different growth stages. (A) Arrangement of the four plants inside the arena and the release point (glass petri dish) in the centre. (B) Experimental arena with propagator lid in place. (C) full layout of all arenas *in situ* during beetle exposure.

6.3.4 Feeding bioassay:

Four adult *P chrysocephala* (2 male and 2 female) were introduced into each tray. Littles is known about the behavioural differences of males and females therefore a 50:50 sex ratio was used throughout to ensure consistency and remove potential unknown variability. The sex was determined based on the structure of the front tarsal pads, which are flattened and larger on males than on females (Cook *et al.*, 2006). Beetles were collected from an area of OSR at Rothamsted Research, Harpenden, UK on the 13th of December 2016, the collection area had not been exposed to any insecticide treatment and the crop was at GS 17-18. Collected beetles were starved for a 24-hour period, in insect rearing cages at ambient laboratory conditions (20-25°C), prior to use. To allow easy introduction of beetles to arenas they were collected from cages using an electronic aspirator (Watkins and Doncaster Ltd) and put inside petri-dishes which were set centrally inside the arena equidistant from the plants (Figure3). When the trial was started the top of the petri-dish was removed and the propagator cover set in place, thus allowing the beetles to move freely around the arena and access all plants equally. The beetles were allowed to feed freely for 48 hours, after which time all remaining beetles were collected, using and electronic aspirator, and each leaf was photographed to measure the area which had been removed (see section 6.3.5).

6.3.5 Assessment of feeding activity:

Feeding activity was assessed by measuring the area of cotyledon/leaf lost using photographs of each leaf using ImageJ, an open-access image analysis software package (Schindelin *et al.*, 2015). In this experiment the cotyledon was considered as ‘leaf 0’ then each of the true leaves were leaf 1,2, 3 and 4. Where damage was present the total leaf area was calculated along with the area lost using the draw area function in ImageJ, where a polygon is drawn over an image and the internal area calculated (Figure 4). This allowed the proportion of area removed to be calculated for each leaf. Where the leaf edge was damaged and the original outline could not be determined, a ‘best fit’ was used to represent the outer edge both for the total area and the damaged area measurements (Figure). At the leaf petiole a straight line across the stem where the leaf green material branches out, was used to ensure consistency (Figure4).

6.3.6 Statistical analysis:

For each leaf, a total area was measured, and any feeding injury was also measured, these values were used to create a percentage area loss and these values were used for analysis. Due to high incidences of zero feeding injury all data was log transformed subsequent data was analysed using REML variant component analysis. All statistics were performed using GenStat statistical software package v 18 (International, 2016).

6.4 Results:

6.4.1 Beetles recovered at the end of the experiment:

After the 48h exposure time all the beetles were recovered alive from 12 out of the 16 replicate arenas (Table 1). However, in four of the arena's beetles were missing, in one case this was due to a male beetle being dead and the remaining were presumed to have escaped from the arena or to have burrowed deep into compost (see Chapter 7 this thesis).

Table 1 Total numbers of adult *Psylliodes chrysocephala* recovered following a 48h exposure of *Brassica napus* plants to 2 males and 2 females. Table shows the experimental design of the arena bioassay details and the sex ratio of beetles recaptured. * - one beetle was found dead in the arena. ** - missing beetles could not be located (timings unknown).

Square - Tray	Male	Female	Total
1 A	2	2	4
1 B	1	1	2**
1 C	2	2	4
1 D	1	2	3*
2 A	2	2	4
2 B	2	2	4
2 C	1	0	1**
2 D	2	2	4
3 A	1	2	3**
3 B	2	2	4
3 C	2	2	4
3 D	2	2	4
4 A	2	2	4
4 B	2	2	4
4 C	2	2	4
4 D	2	2	4

6.4.2 Numbers of leaves:

No leaves were completely eaten during the experiment and plant growth during the experimental period meant that there were more leaves present at the end of the exposure period than at the start (Table 2).

Table 2 Number of Oilseed rape (*Brassica napus*) leaves in each arena at the start and end of the experiment and number and proportions that were fed upon by the cabbage stem flea beetle (CSRB, *Psylliodes chrysocephala*).

Leaf number	GS10 cotyledons	GS11 1st true leaf	GS 12 2nd true leaf	GS 13 3rd true leaf	GS 14 4th true leaf
No. leaves present in each arena, according to experimental design	128	48	32	16	0
No. leaves at the end of the experiment	128	50	40	19	5
Total no. leaves with feeding damage	19	31	26	14	4
Proportion with feeding damage (%)	15	62	65	74	80

6.4.3 Leaf area loss:

Due to the difference in sizes of the differing ages of the leaves ($F_{4,57.9}=10.65$; $P<0.001$) the comparison of percentage area of leaf lost was not robust because the same area damaged on a small leaf will have a bigger proportion than on a larger leaf. However, this did not prevent comparisons of the incidences of feeding recorded at different leaf ages and the percentage of leaf area loss for the cotyledons and four leaves is shown in Figure 3. When comparing the absolute area loss between the leaf ages a difference was observed ($F_{3.35.6}$=12.9, P<0.001) with the cotyledons eaten less frequently than true leaves. There was no difference in area loss on any of the true leaves.

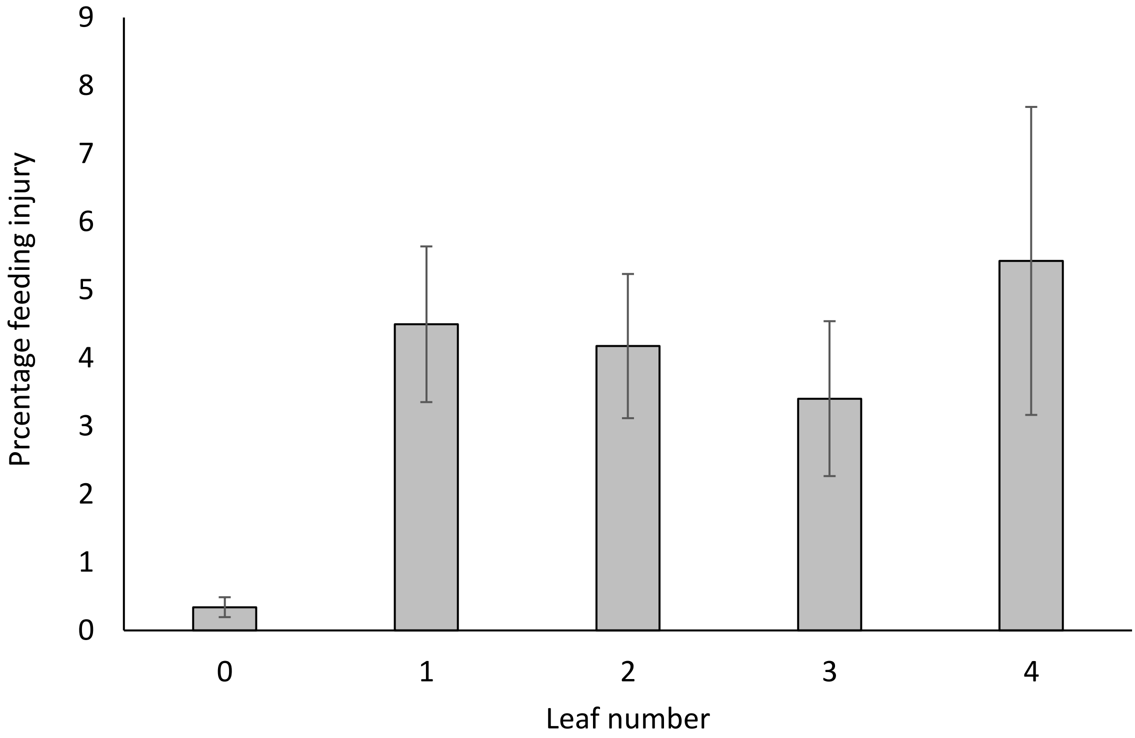
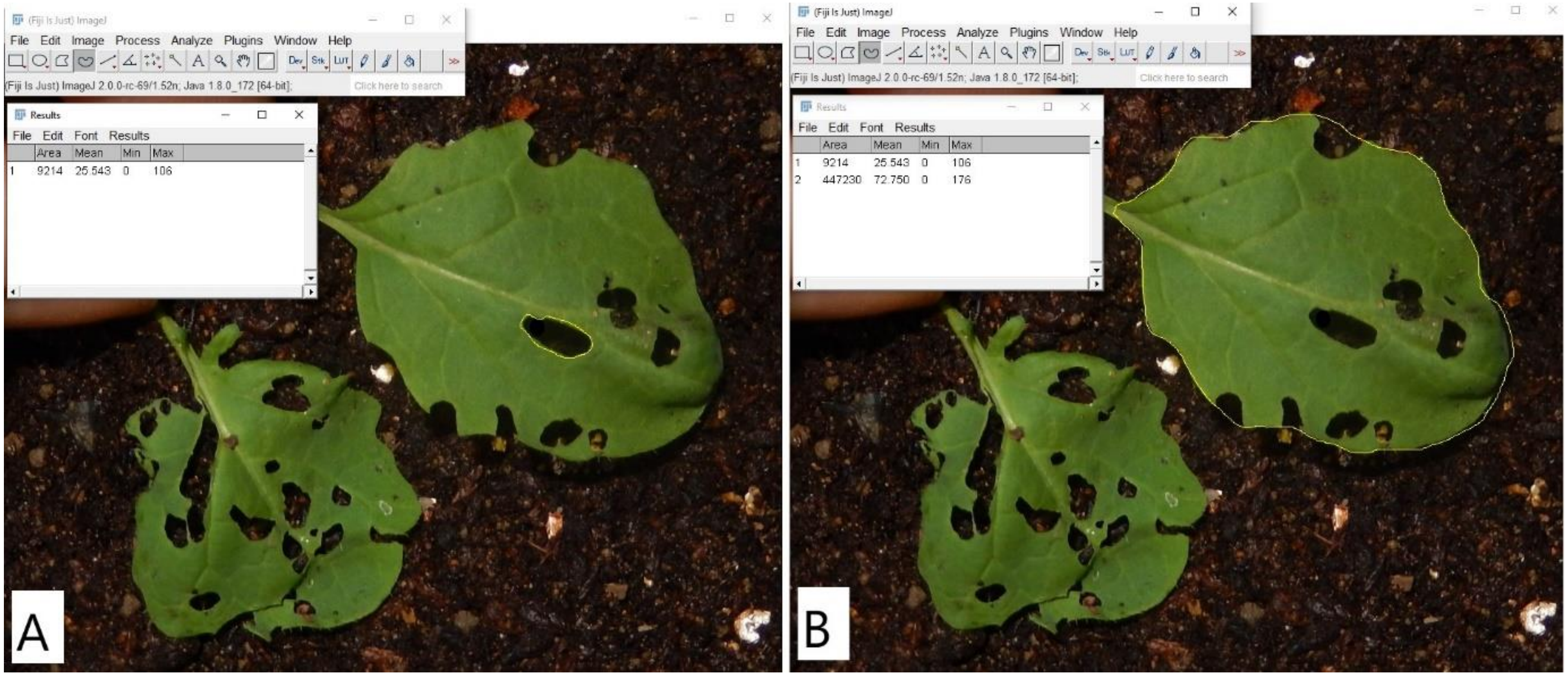


Figure 3. Percentage oilseed rape (*Brassica napus*) leaf area injured by the cabbage stem flea beetle (*Psylliodes chrysocephala*) for five different ages of leaves. Leaf 0 = cotyledons; Leaf 1 = first true leaf unfolded; Leaves 2-4 = 2nd -4th true leaves. Values are based on the total area removed from the leaf in relation to the total area of the leaf. Shown as an average of all leaves at each growth stage. SE shown.



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(Fiji Is Just) ImageJ 2.0.0-rc-69/1.52n; Java 1.8.0_172 [64-bit];
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A

```
   Area    Mean    Min  Max
1  9214    25.543  0    106
```

B

```
   Area    Mean    Min  Max
1  9214    25.543  0    106
2  447230  72.750  0    176
```

Figure 4. Photographs of *Psylliodes chrysocephala* feeding damage on *Brassica napus* leaves after 48 hours exposure. Representation of the photographic methods of measurements using ImageJ are (A) Here one internal area is marked (yellow border drawn around the edge of the hole) with the area of the hole shown in the ImageJ programme results box, (B) Here the area of the leaf is calculated by marking the leaf edge (yellow border drawn around the leaf) with the area of the hole shown in the ImageJ programme results box.

6.5 Discussion:

The data presented here provide evidence that *Psylliodes chrysocephala* avoid feeding on the cotyledons of OSR, in favour of feeding on true leaves. This confirms the findings of Diehl (2017) on leaf discs that *P. chrysocephala* feed primarily on true leaves. As OSR is known to release glucosinolate when injured by *P. chrysocephala* (Koritsas, Lewis and Fenwick, 1991) the finding of Diehl (2017) may have been an artifact of preparing the leaf disc. Here it is shown that the same pattern of feeding is also exhibited on whole plants thus removing any potential cues from previous injury. The low feeding on cotyledons has been shown in the under field conditions in Northern Serbia, feeding by *P. chrysocephala* was shown to be low on cotyledons and the first true leaf and was most intensive in November when the plants were at GS 16-18 (Sivčev *et al.*, 2016). Overall, levels of feeding were low with only five leaves having more than 20% leaf area lost. These low levels are to be expected due to the short exposure time (48 hours) and the low number of beetles (n=4).

The bias-towards true leaves reported here and shown by Diehl (2017) suggest that the cotyledons are less palatable or do not provide as strong a feeding response as true leaves. The lack of feeding on the cotyledons might be due to lower levels of glucosinolate, which may be present at higher levels in the true leaves (Rosa *et al.*, 1996) and have been shown to act as phagostimulants *P. chrysocephala* when added to agar (Bartlet *et al.*, 1994) but this was not the case for whole plant choice tests with cultivars of varying glucosinolate level (Bartlet, Mithen and Clark, 1996). The level of glucosinolate are known to vary depending on location (Bellostas, Sørensen and Sørensen, 2004) and growth stage (Rosa *et al.*, 1996) and it would have been interesting to measure the levels in the current experiments.

Although these findings would need to be corroborated with field observations to ensure the beetles feed in the same way in the field. The data presented here suggests that when given access to alternative leaves the cotyledons are left alone. Suggesting the efficacy of seed treatments to early establishment needs to be tested alongside the beetles feeding activity. Seed dressing insecticides such as Lumiposa (Active Ingredient: Cyantraniliprole (DuPont, 2017)) are recommended as effective only until the third/ fourth true leaf has unfurled. In the present study adult *P. chrysocephala* feed on the first four true leaves equally is in line with the later end of the recommended effective period, suggesting Lumiposa is active during the crucial period of plant establishment. However, in Northern Serbia *P. chrysocephala* have been reported feeding most intensely at the 16th – 18th true leaf (Sivčev *et al.*, 2016) which is after the recommended effective phase of Lumiposa, however the authors do note that the plant were large and seemed able to tolerate feeding injury.

This study suggests that it could be a potential benefit to crop production to have suitable host plants of a range of growth stages in order to distribute the feeding of adults, this could be in the form of trap crop boarders which are drilled prior to the crop. Allowing faster development and thus drawing *P. chrysocephala* from foraging on the emerging OSR. The potential of using trap crops for *P. chrysocephala* have been proposed (Barari *et al.*, 2005; Sivčev *et al.*, 2016) and shown some level of impact (Coston et al in prep). With further study of *P. chrysocephala* feeding timings and location (both within field and upon plant) the ultimate mechanism for trap cropping can be understood and utilised more efficiently in IPM strategies.

Studies which have examined the feeding or impact of leaf area injury on OSR have shown high levels of compensation (Nowatzki. T and Weiss. M, 1997; Susko and Superfisky, 2009; Ellis, 2015). Including when 90% leaf area is removed at the cotyledon stage (Coston et al in prep, Chapter 5 this thesis). Many of these studies simulate the injury mechanically which was shown to result in different responses from actual beetle feeding (Susko and Superfisky, 2009). To understand how OSR compensated to *P. chrysocephala* attack knowledge on how the feeding is distributed to various plant parts is crucial. Here we demonstrate little feeding on cotyledons when true leaves are also available.

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7.0 Assessing the circadian rhythm of *Psylliodes chrysocephala* L. in UK winter oilseed rape (*Brassica napus* L.):

7.1 Abstract:

With the ban on the use of neonicotinoid insecticides on oilseed rape (OSR, *Brassica napus* L.), The only alternate synthetic insecticide approved for use belong to the pyrethroid class and in order to reduce spread of resistance need to be applied as effectively as possible. One of the main targets for autumn insecticide applications in OSR is the cabbage stem flea beetle (*Psylliodes chrysocephala* L.). There has been anecdotal evidence that the most appropriate time to apply spray insecticides to target is *P. chrysocephala* because they are hypothesised to be primarily nocturnal. In this study we examine the diel activity of *P. chrysocephala,* in an OSR crop at Rothamsted Research, UK by using emergence traps set before and after sunset. There was not active *P. chrysocephala* seen prior to sunset, supporting the hypothesis that they are primarily nocturnal in the crop. Adult beetles were caught in all emergence traps suggesting the *P. chrysocephala* shelters below the soil surface in the day and emerged from the soil after dusk. If spray insecticide application is required, then the practice of doing so at dusk would coincide with the highest activity.

7.2 Introduction:

Cabbage stem flea beetle (*Psylliodes chrysocephala* L.) is a major pest of winter oilseed rape (OSR, *Brassica napus* L.) in the UK (Alford, 2003; Williams, 2010), causing severe damage and, in some cases, complete crop failure (Nicholls, 2015). As discussed, Chapter 1 this thesis), control of *P. chrysocephala* is now heavily reliant on pyrethroid sprays, although increasing resistance has been observed across the UK (Højland *et al.*, 2015). Thus, it is important to time the use of pyrethroids, with regard to the time of the season and even the time of day, to maximise exposure of the pest whilst reducing non-target exposure and minimising the number of applications to reduce resistance build up. To determine the most appropriate time to apply any insecticide sprays, a full understanding of the target pest's behaviour both in space and time is needed. Since the removal of neonicotinoid seed dressings in OSR from 2014 (EU, 2018) there has been anecdotal evidence that spraying pyrethroid at night has more of an effect than spraying during the day (Guide, 2016; Hill, 2016). To understand this principle a better understanding of *P. chrysocephala* is needed. The life cycle of *P. chrysocephala* has been studied in many countries across Europe (Thioulouse, 1987; Sivčev *et al.*, 2017; Vig, 2003) and is relatively well understood (Williams, Buechi and Ulber, 2003): adults migrate into the crop during the autumn (Thioulouse, 1987) where they feed on leaves, producing characteristic shot holing and necrosis (Alford, 2003) before reproducing within the crop and laying eggs in the soil and at the base of plants (Alford, 1979; Bonnemaison and Jourdheuil, 1954). The larvae then emerge, burrow into plant stems and feed throughout the winter within the plant petioles and stem before exiting the plant to pupate within the soil. The timings of each stage are dependent on environmental conditions and can vary from year to year (Williams, 2010). This level of detail on the yearly cycle of *P. chrysocephala* is important in predicting risk from the pest and timing of crop migration which can aid in decision making for insecticide application (Walters *et al.*, 2003). However, the daily activity cycle of *P. chrysocephala* is much less understood, and it is these daily cycles which may hold the key to improved pest control through greater refinement of the timing of application of insecticides and more reliable monitoring to improve risk and threshold assessments.

Preliminary evidence has suggested that *P. chrysocephala* are mostly active at night and are observed in far greater numbers after sun set (Diehl, 2017; Hill, 2016). This raises two questions: 1) Is *P. chrysocephala* a nocturnal species; and 2) if so, where do they shelter during the day? To answer test the timing of *P. chrysocephala* activity, emergence traps (pyramidal emergence trap with collection chamber at the upper apex to capture phototactic insects, which have been used previously to sample *P. chrysocephala* adults emerging after pupation (Conrad *et al.*, 2018; Sivčev *et al.*, 2017), to enclose area of crop and trap adult beetles during both daylight and night-time hours and thus determine timing of adult activity. A better understanding of diel activity could help improve Integrated Pest Management (IPM) by informing the best time to assess crops to selectively target *P. chrysocephala*, when determining action thresholds and ensuring that any insecticide used is applied to coincide with the peak in beetle activity and maximise pest exposure.

7.2.1 Aims of study:

This study was designed to record the activity of *P. chrysocephala* over time and to determine if the beetles are more active at day or night. Current evidence suggests they are nocturnal, but no empirical study has been performed in the UK to support this. From preliminary quadrat observations it is hypothesised that the *P. chrysocephala* is indeed nocturnal and shelters under the soil surface during the day. This study will record *P. chrysocephala* activity through direct observations and trap any emerging from below the soil surface using emergence traps.

7.3 Methods:

7.3.1 Experimental site:

Observations were made in a crop of winter OSR at Rothamsted Farm, Harpenden, Hertfordshire, UK.

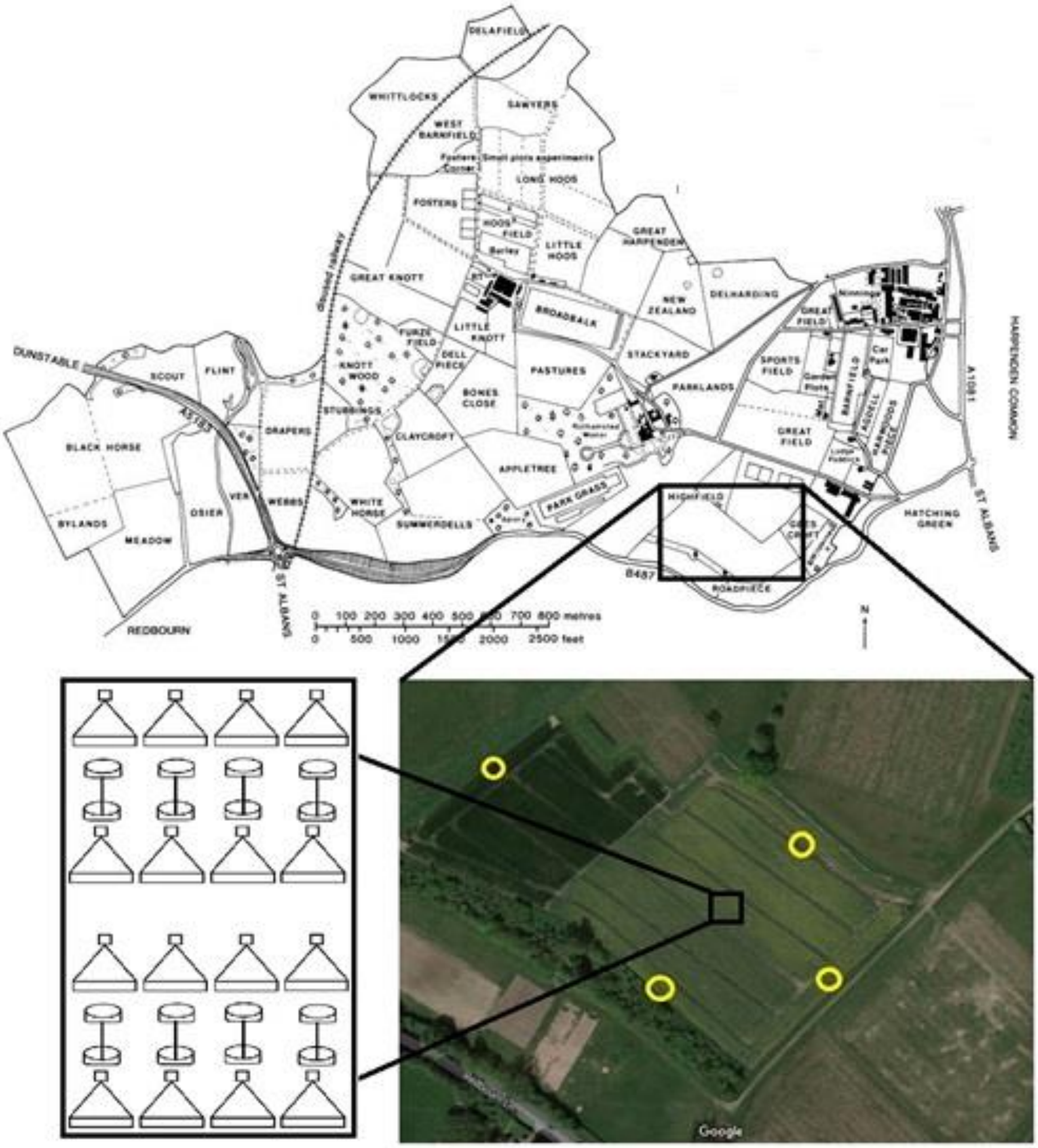

Figure 1. Map of Rothamsted Research showing the location of the experimental oilseed rape crop with locations of yellow water traps (yellow circles) and a schematic diagram of the layout of emergence traps and associated yellow water traps.

7.3.2 Sampling of *Psylliodes chrysocephala* adults at the crop edge:

Prior to any assessment of *P. chrysocephala* activity period it was first confirmed that they had migrated into the test crop. To measure this Ringot flora (Nickerson Bro, Lincolnshire, UK) yellow water traps (diameter 265mm) were set up two meters into the headlands on the 13th of September 2018 at each side of the crop, North, East, South and West (yellow circles in Figure 1). Each had two traps on a yellow stake; one at ground level and one suspended at a height of 1.5m and each trap was 1/3 filled with water and a drop of detergent (Teepol). The traps were designed to measure beetle migration into the crop (phenology and abundance) and to decipher whether this was by walking/ jumping (ground level trap) or by flying (above-ground trap). Traps were emptied and reset daily from 13th – 21st September 2018, with the numbers of beetles in traps recorded between 10:00 and 11:00 each day and traps re-set, to ensure a 24-hour exposure.

7.3.3 Recording of *Psylliodes chrysocephala* adult activity within the crop:

In order to collect *Psylliodes chrysocephala* from enclosed areas emergence traps (Figure) were used. Emergence traps are designed to capture phototactic-reacting animals, whereby a metal frame is buried into the ground with a pyramid frame this is then covered with a fine gage net, at the apical point of the pyramid a collection tub is placed (Figure). The traps work by enclosing an area of ground with no exit points, thereby, any insects emerging from the soil will fly to the top of the trap and get trapped in the collection tub. The traps used in this study were 1m in diameter, 1m height and covered with a 1.5mm gage net, the collection tub was 1/3 filled with 50% Ethanol to kill and preserve specimens caught. A total of 16 traps were set up in areas of uniform crop over two days (19th -20th September 2018) at four times (16:00, 18:00, 20:00 and 22:00 hours). Timing of trap establishment was based on beetle catches from the crop border yellow water trap. Two emergence traps were set at each time in two rows, perpendicular to the crop lines, resulting in two blocks of four traps (eight in total, Figure). Traps were classified as pre-sunset (16:00 and 18:00) and post-sunset (20:00 and 22:00), as sunset on the days of trap establishment was 19:31 and 19:28, respectively. These times were selected because of preliminary quadrat assessments (personal observations) showed no beetle activity prior to sunset thus to confirm nocturnal activity traps were set before and after sunset. The metal frame of the trap was placed over the crop and the internal area was carefully inspected for adult *P. chrysocephala.* Any adults observed were removed after being counted and location recorded as: i) on a plant, ii) on the substrate surface, or iii) below the substrate surface. Substrate is defined here as any non-compacted material on the soil surface (e.g., loose stones and soil clumps), which was lifted to inspect underneath. The number of plants within the frames was also recorded and the frame was pushed into the soil substrate so that there was no gap under the metal frame at ground level, through which insects might move into or out of the trap. The trap was then set by putting the net over the frame and taping the bottom of the net onto the metal frame to ensure a tight seal. A collection pot 1/3 filled with 70% ethanol to kill and preserve catches was fitted to the top of the trap and the lid tightened; at this point the trap was considered active. Traps were left for seven days after which the number of beetles in each collection pot was recorded.

For time of emergence traps establishment, yellow water traps (as described above, beetle count taken once at the end of trapping) were set equidistant between the two traps, to confirm the presence of beetles in the trapping area (Figure). The crop growth stage was recorded when the traps were dismantled by inspecting 10 randomly selected plants within the area of each block of traps.

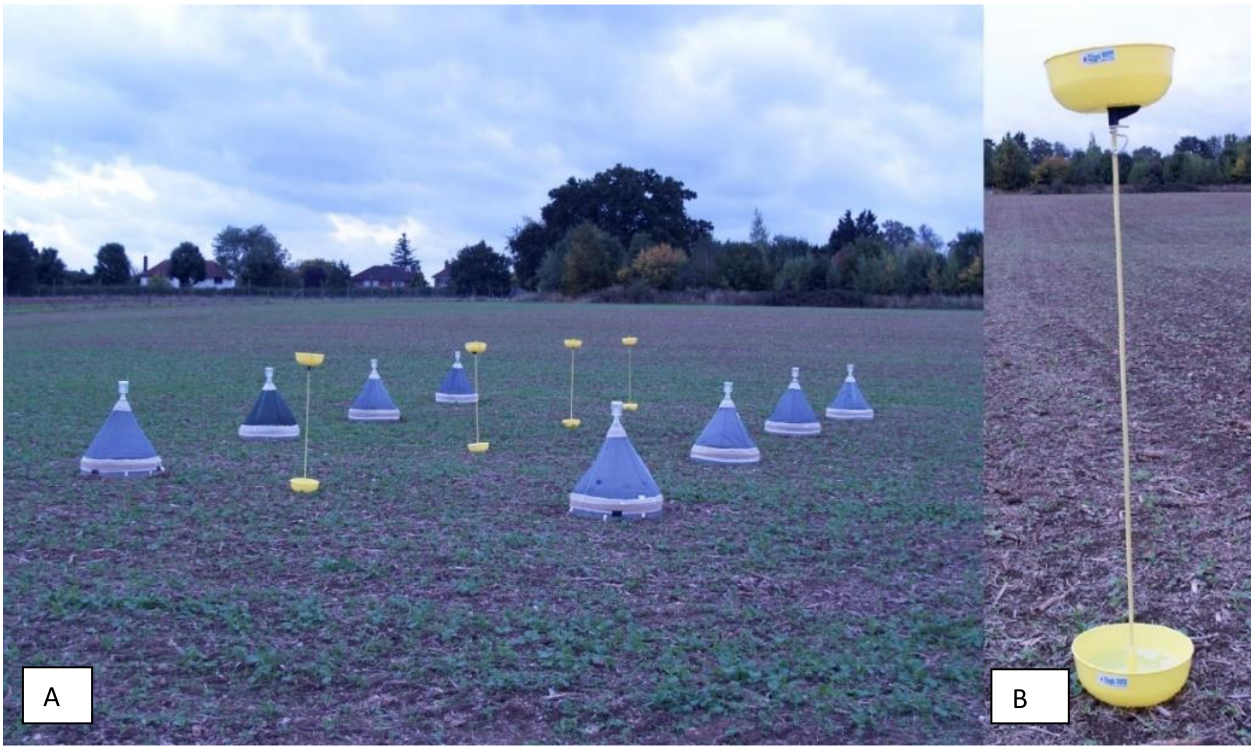

Figure 2. A, In-situ block of emergence traps with accompanying yellow water traps to assess diel activity of cabbage stem flea beetle (*Psylliodes chrysocephala*) in a crop of winter oilseed rape (*Brassica napus*). B, Yellow water trap station comprising ground-level and above-ground traps to measure the proportion of CSFB moving at ground level of aerially.

7.4 Results:

7.4.1 Yellow water trap catches:

Catches in the ground-level yellow water traps set at the crop edge confirmed the presence of *P. chrysocephala* from the beginning of sampling (Figure 3). Cumulative numbers then increased over time and there were clear trends for higher numbers in the North and East edges of the field with relatively few individuals being caught on the South or West sides (Figure 3). Only two individual *P. chrysocephala* adults were caught in the above-ground traps at different edges and different dates, so data shown are for ground-level traps only.

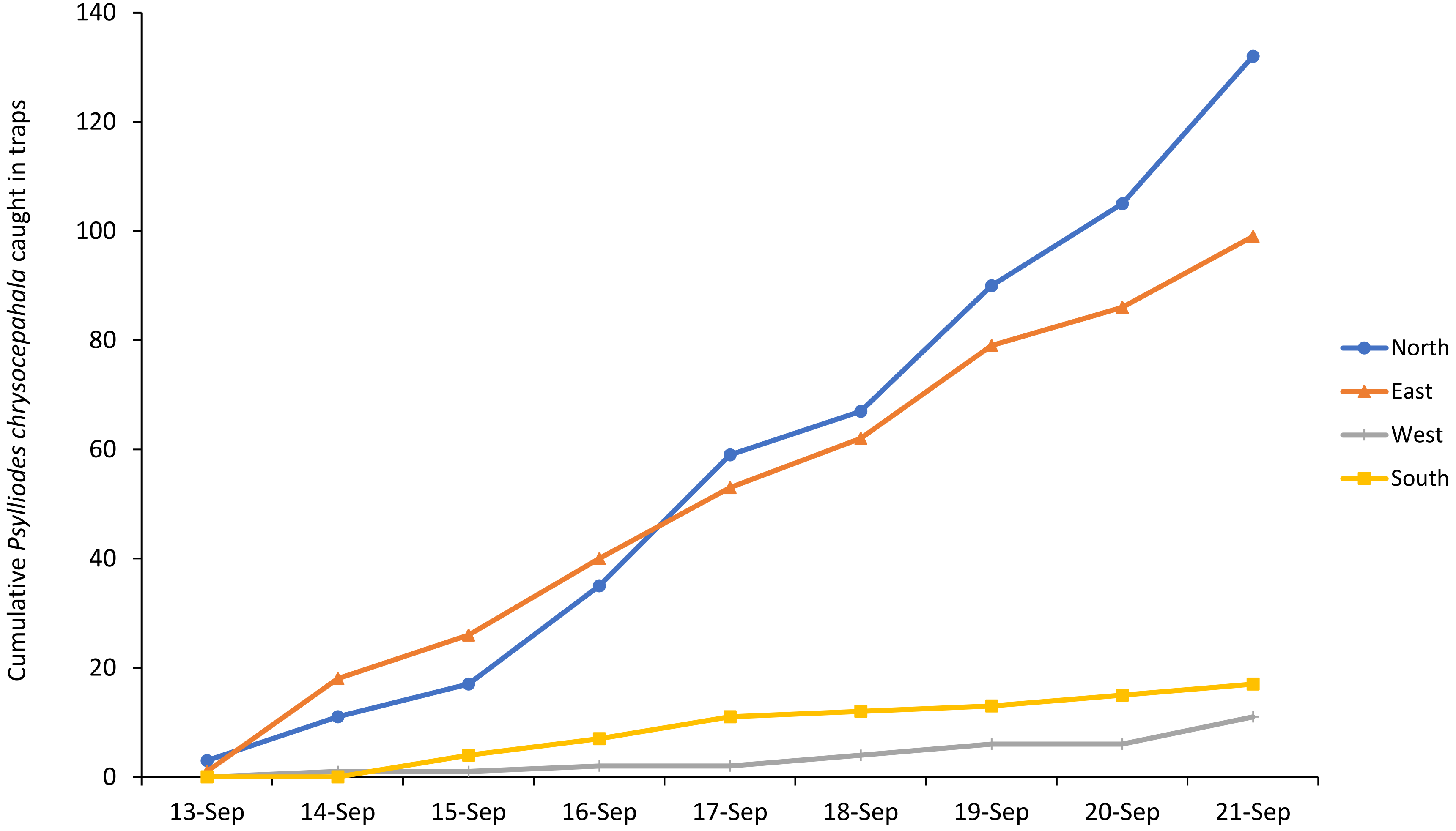

Figure 3. Cumulative numbers of *Psylliodes chrysocephala* in ground level yellow water traps placed at the four edges of a crop of oilseed rape (*Brassica napus*) in September 2018.

7.4.2 Recording of *Psylliodes chrysocephala* adult emergence within the crop:

When the traps were set up there were no beetles present before sunset (16:00 and 18:00) but after sunset active beetles were present on the substrate surface and on the plants (20:00 and 22:00, Figure 4). After seven days beetles were found in all the traps and the numbers were comparable regardless of when they were set up. The total numbers of beetles caught were variable (Figure 5), which could be attributed to an uneven pest distribution within the crop.

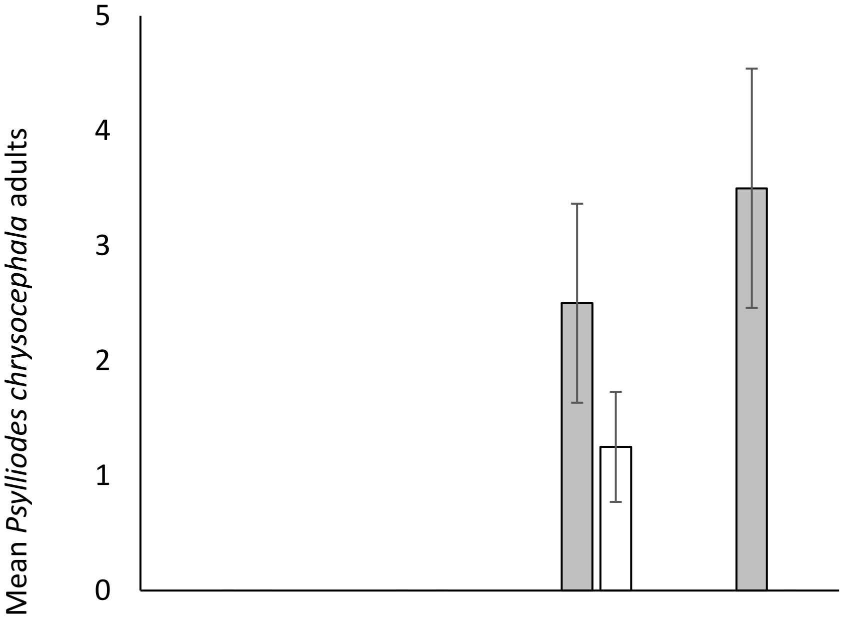

Figure 4. Mean number of *Psylliodes chrysocephala* observed when setting out emergence traps at four times of day. Grey represents adults on substrate surface and clear is adults on oilseed rape (*Brassica napus*) leaves. Numbers are from direct observations. Standard error shown.

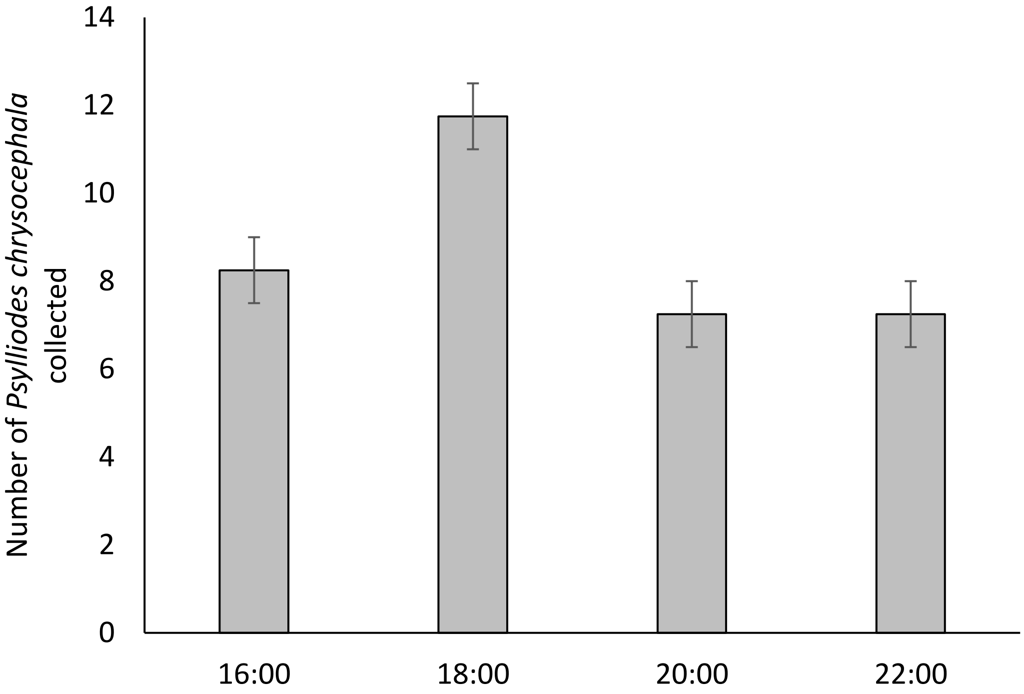

Figure 5. Mean *Psylliodes chrysocephala* adults obtained from emergence traps set out in a field of oilseed rape (*Brassica napus*) between 19th and 26th of September 2018. Standard error shown.

Data from the yellow water traps confirmed that *P. chrysocephala* were present in the crop centre adjacent to the emergence traps, mostly at ground level (Figure 6). At the end of emergence traps exposure, the growth stage of the OSR was uniform over the field with 4-5 True leaves, BBCH Growth Stage 14-15 (Lancashire *et al.*, 1991).

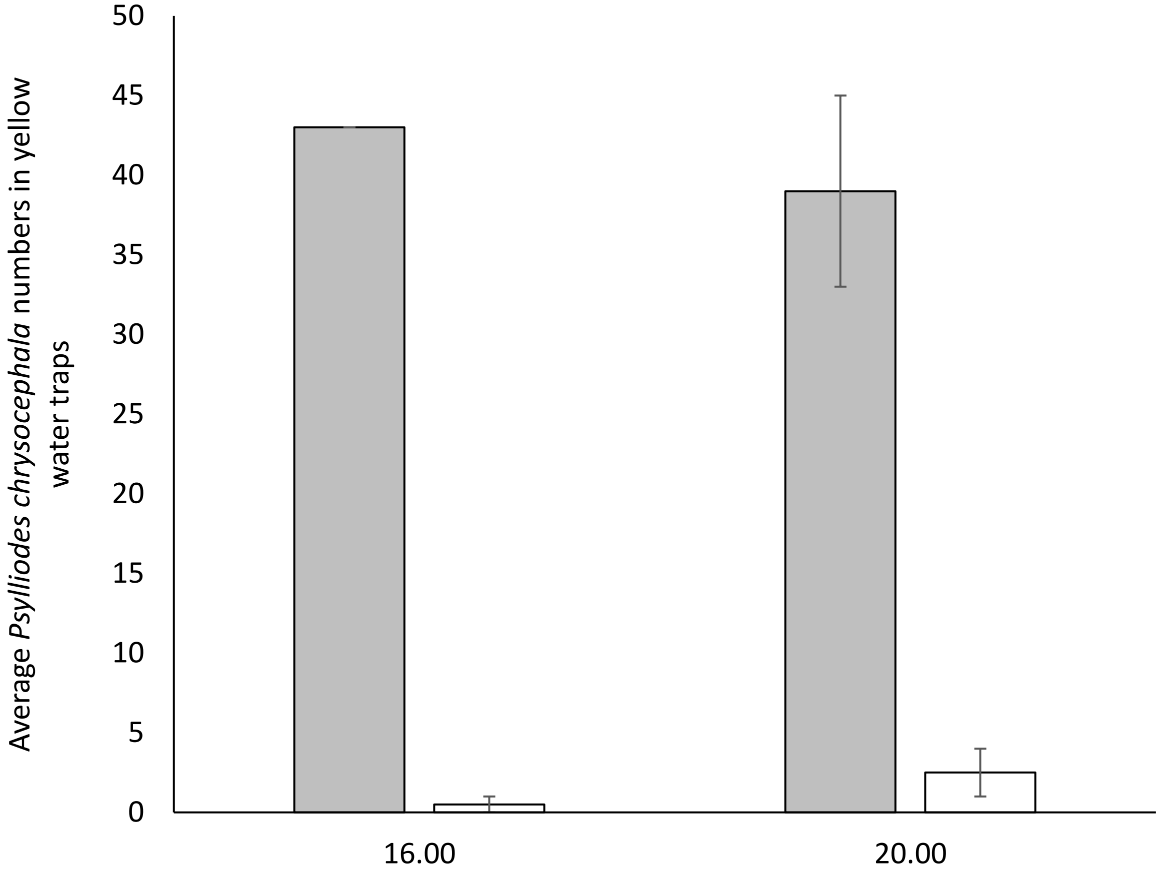

Figure 6. Average number of *Psylliodes chrysocephala* caught in yellow water traps set adjacent to emergence traps at 16.00 and 20.00 between. Grey bars represent ground-level traps and clear represents 1m above ground level traps. Standard error shown.

7.5 Discussion:

No active *Phyllodes chrysocephala* were observed before sunset during emergence trap set-up, supporting the hypothesis that they are predominantly nocturnal when within a crop of OSR (Figure). All emergence traps caught adult beetles suggesting that once within a crop of OSR the *P. chrysocephala* shelter under the soil surface during the day (Figure).

Observations of *P. chrysocephala* are known during the day (personal observations) however, here we demonstrate that once within the crop of OSR they are primarily nocturnal, with no observations before sun set. Using direct observations of crop area prior to and post sun set the split in activity observed was clearly associated with sunset. This is a step towards understanding the circadian rhythm of *P. chrysocephala* which will allow improved survey and be vital information when assessing action thresholds and application timings. Several studies in other insect species have demonstrated that circadian rhythms can vary with season (Meireles-Filho and Kyriacou, 2013; Saunders, 2009). It has been reported that once in the crop the *P. chrysocephala* wing mussels' atrophy and they no longer fly (Bonnemaison and Jourdheuil, 1954; Ebbe-Nyman, 1952). this may lead to nocturnal activity may become more prominent when location of suitable host plants is achieved, and locomotion is primarily through walking and jumping (personal observation).

The presence of adult beetles in all traps was an interesting observation and should be explored further to determine the source of the captured beetles. One possible source of the beetles is that they shelter under the soil surface during the day another is that they are newly emerged adults which entered aestivation within the crop. Observations from Hungary (Vig, 2003) and Serbia (Sivčev *et al.*, 2017), suggest that *P. chrysocephala* may remain within the previous year’s stubble and enter aestivation within the stubble area. The field used in the trial was on its second year of OSR so this cannot be discounted as a source of the captured beetles. It is, therefore, not possible to confirm that the *P. chrysocephala* shelters under the soil during the day from the data collected here.

The data presented here suggest that the best time for application of a contact insecticide targeting adult *P. chrysocephala*, would be after sunset, to coincide with the adult activity within the crop. It is also suggested that surveying for action thresholds can be carries out during the day as the majority of activity is at night.

Although post-sunset application would reduce the exposure risk to pollinating insects (Delaplane and Mayer, 2000), at least diurnal ones (Macgregor and Scott-Brown, 2020), it might also increase the risk to nocturnal insects including moth pollinators and ground-dwelling natural enemies such as carabids (Luff, 1978; Lövei and Sunderland, 1996).

The timing of action thresholds survey is important to understand the pressure from the pest accordingly. Wold-Burkness et al (2006) showed that the common asparagus beetle (*Crioceris asparagi*) is primarily active at specific times of the day (13:00-17:00). If appropriate estimations of action thresholds are to be made observations must consider, the full pest pressure. If the common asparagus aphid were surveyed outside its active period, the pressure could be underestimated.

To further understand the daily activity pattern of *P.* c*hrysocephala* further observations are required which cover the full 24hour period and through the autumn period. Prolonged direct observations or use of time sorting water traps would be a next step in unravelling the variations in activity for the *P.* c*hrysocephala* and implement IPM strategies accordingly. The nocturnal activity can be assessed using a combination of dry pitfall traps with direct observations at distance times. This method is very time consuming and alternatives have been trailed with Diehl (2017) using automated cat feeders to time sort water traps for *P. chrysocephala*, this proved highly problematic.

Psylliodes chrysocephala can even be located easily at night using a torch, in fact for collection of adults for experiments we now generally search at night with an electric aspirator (Cook lab group). The authors here suggest that direct observed of the beetles in the crop would be the most likely strategy, although labour and time intensive, to accurately assess *P. chrysocephala* activity cycles with a crop of OSR. Similar methods of observations have been utilised to examine the flight activity of rove beetles (Stylianos, Ashe and Rodney, 2004) and ground beetles (Sunderland *et al.*, 1995).

This combination of detailed life cycle, circadian rhythm coupled with habitat and weather data would improve the knowledge of *P. chrysocephala* interaction with OSR and inform decisions on IPM and development of alternative pest control strategies in the future. As suggested by (Ramsden *et al.*, 2017), for IPM to be an effective crop production strategy, it is important to have as detailed a knowledge of the pest life cycle and crop exposure levels as possible.

7.6 Acknowledgements:

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8.0 Are farmers really concerned about the neonicotinoid ban? Comparison of UK farmers views on crop protection strategies for *Psylliodes chrysocephala* in winter oilseed rape (*Brassica rape*): past, present, and future:

8.1 Abstract:

Oilseed rape is a major oil crop in the EU and under current regulation neonicotinoid insecticide use is banned. An online survey of 91 UK winter oilseed rape (WOSR, *Brassica napus*) on the issues surrounding the neonicotinoid ban on the control of the cabbage stem flea beetle (*Psylliodes chrysocephala*) and the Peach Potato Aphid (*Myzus persicae*) and how farmers are responding. The survey also aimed to capture data on farmers perceptions on application thresholds and novel methods of pest control. Respondents reported increased pest pressure since the restriction, reduced area of OSR grown and expressed concern over the future pest pressure from *P. chrysocephala*. The majority would use neonicotinoids again if the ban were lifted.

8.2 Introduction:

Oilseed rape (OSR, *Brassica napus*) is a major oil crop in the EU (Ouvrard and Jacquemart, 2019) and globally (FAOSTAT, 2021). It is attacked by several invertebrate pests (Williams, 2010b) which are primarily controlled through the use of synthetic insecticides (Williams, 2010a). In autumn sown OSR the primary pest problem comes from the cabbage stem flea beetle (*Psylliodes chrysocephala*) and virus vectors such as the peach potato aphid (*Myzus persocae*). Prior to 2013 these pests were controlled by the combination of neonicotinoid seed treatments and pyrethroid foliar sprays. Since the autumn of 2014, the accessibility of neonicotinoid seed treatments has been tightly regulated (EU, 2013). With the introduction of a blanket ban on neonicotinoid use outdoors (EU, 2018), UK growers have voiced concerns to the future of oilseed rape (OSR, *Brassica napus*) production in the UK (UK, 2020). This is thought to have resulted in a reduction of OSR area as grower's confidence of a good crop has fallen (Nicholls, 2015).

Surveys of farmers and other pest control experts have highlighted the perceived importance of neonicotinoids as a pest control strategy, with 72% opposing the ban (Zhang *et al.*, 2017). In a recent study, the relative efficacy of neonicotinoids and a wide range of alternatives pest control methods directed at protecting the crop from the cabbage stem flea beetle (*Psylliodes chrysocephala*) was trilled (Coston et al 2021, chapter 4 this thesis), demonstrating little impact from neonicotinoid seed dressings (AI: thiamethoxam) and that, in a field with high percentage of pyrethroid resistance in *P. chrysocephala* (84%), only minor reductions in larval numbers were reported. This study highlighted the potential of using cultural methods to reduce pest injury with limiting yield deficit when compared to control plots of untreated OSR.

Despite this and other evidence to support their effectiveness, given the reported strength of support for neonicotinoid use, it is important to understand farmer willingness and barriers to adopting these alternative, non-chemical methods. Linking the responses of farmers with field trials at Rothamsted (Coston et al 2021 chapter 4 this thesis) on the efficacy of alternative control methods, a survey an online survey, built in Qualtrics (Provo, 2015), was used to explore UK OSR farmers willingness to adopt alternative control methods for *P. chrysocephala* and *Myzus persacae* in autumn OSR following the ban on neonicotinoids, what the barriers towards their use were and the perceived effectiveness of both neonicotinoid and non-neonicotinoid control methods. The survey also aimed to collect data on farmers opinions on the neonicotinoid restriction, likelihood of reverting to them if legislation changes and characteristics of the farm (Location, size) and the farmer (experience, education).

8.2.1 Aims of study:

This survey of UK farmers was aimed to determine which methods of autumn pest control trailed at Rothamsted in 2016-17 (chapter 4 this thesis) would be acceptable to farmers. The primary aims were to understand what the current understanding of pest control practices and how effective they are. The secondary aim was to find out what concerns or barriers to use they have for alternative practices being trilled in research.

8.3 Methods:

8.3.1 Survey structure:

The survey was developed on the online survey platform Qualtrics (Provo, 2015). In February 2019, a pilot of the survey using 19 agriculture students from the University of Reading was performed. No changes were made from the pilot; therefore, the responses were retained in the final analysis.

The survey comprised 35 questions (see appendices) divided into five sections,

(i) farm (location and area of OSR between 2012 and 2019) and growers' characteristics,
(ii) Pest pressure from *P. chrysocephala* and *M. persacae* and view of control efficacy from neonicotinoids and pyrethroids,
(iii) use of current insecticide application thresholds and view of reliability,
(iv) effectiveness of natural enemies in controlling *P. chrysocephala* and *M. persacae*.
(v) views on alternative control methods for *P. chrysocephala* and *M. persacae* and willingness to use these methods.

The survey was approved by the ethical committee of the School of Agriculture, Policy and Development, University of Reading. In line with ethical approval, the collected data can only be shared in summary and all participants are kept anonymous.

8.3.2 Survey dissemination:

The survey was live from March to October 2019. The survey was distributed using social media, leafleting at Cereals 2019 (see appendices) and by direct contact through. The survey was also disseminated by the NFU and Farmers Weekly.

8.3.3 Statistical analysis:

Analysis of relationships between the probability of a respondent's willingness to use an option relative was conducted using standard logistic regression modelling in R (R core team, 2020). Separate analyses were conducted for each option relative to:

i) Farmer demographics (size of holdings, AES participation, Organic participation, years of farming experience, education level and income level),
ii) Perceptions of risk from each of the two pests (importance as a pest, effectiveness of natural enemies as pest control, concern over neonicotinoid and pyrethroid resistance and change in damage following neonicotinoid restrictions),
iii) Their choice of pest control prior to neonicotinoid restrictions (neonicotinoid seed treatment, pyrethroid sprays, trap crop boarders, intercropping/cover crops, altered sowing density, topping and other),
iv) Their choice of pest control prior to neonicotinoid restrictions (pyrethroid sprays, other pesticides, trap crop boarders, intercropping/cover crops, altered sowing density, topping and other).

v) farmers unwillingness to use each option relative to the perceived disadvantages (too expensive, too unreliable, ineffective, increase non-insect pests, increase weeds, unsightly, labour intensive, lower crop yield/quality).

Due to the low sample variation, the majority of these analyses did not return any significant effects and are not commented upon.

8.4 Results:

8.4.1 Overview of respondents:

In total 91 useable answers were returned which covered 17,000 to 23,000h over the 2012-2019 time in the survey (Figure 1 and Figure2). Nationally, OSR area was recorded as 488,000 to 744,000ha in the same period ((DEFRA, 2019, Figure 1). The area under observation in the study comprises less than 0.05% of the UK OSR production. The low response rate is typical of online surveys of farmers (e.g., Breeze et al., 2019).

Respondents tended to have large farms (76% had >200 ha farms), be very experienced (67% had >20 years farm business experience) and many had higher education (only 7% had O-levels/GCSEs as their highest qualifications, 49% were graduates or postgraduates). Farmer participation in Agri-Environment schemes (AES) was high at 60% in just AES and a further 14% in both organic and AES while only 22% were in neither Organic nor AES.

8.4.2 Regional distribution of respondents:

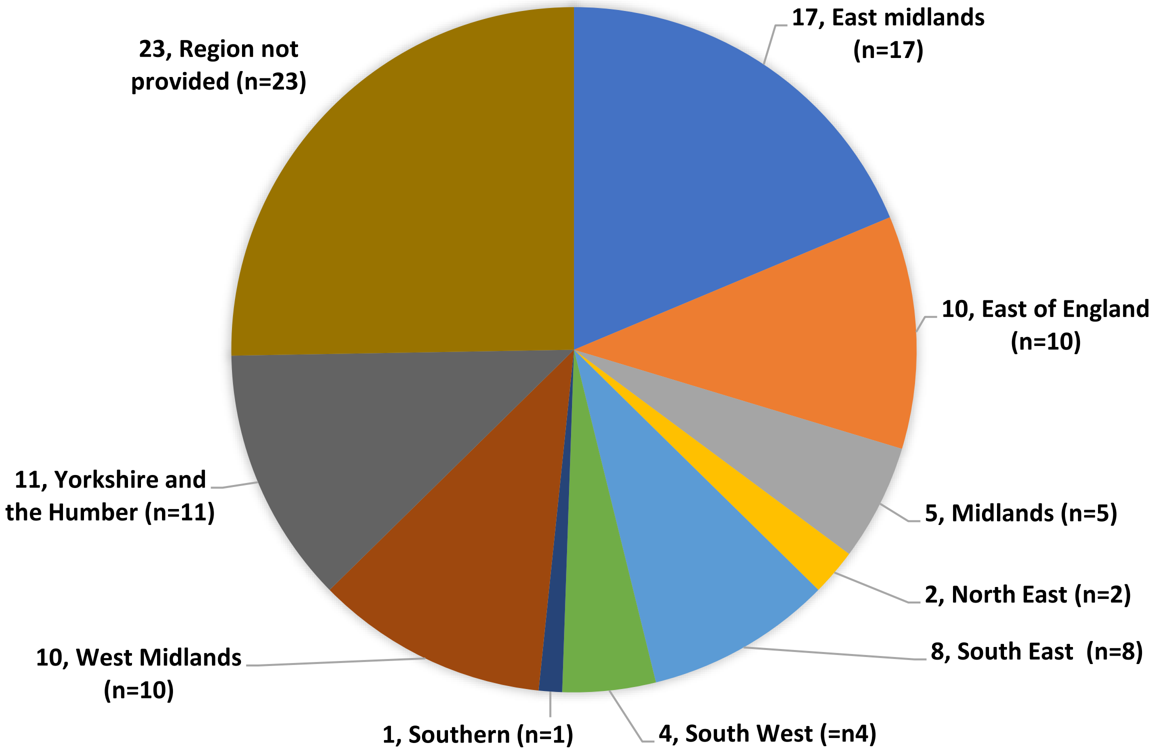

Figure 1. Regional distribution of respondents to survey (n=91). Refined to the nearest level of accuracy available based on respondents' answers.

8.4.3 Area of winter oilseed grown:

Average area of OSR planted by respondents has declined by 25% between 2012 and 2019 with sharp drops in the last year (Figure 2 and Figure3), mirroring a similar decline in UK OSR as a whole (Figure 2).

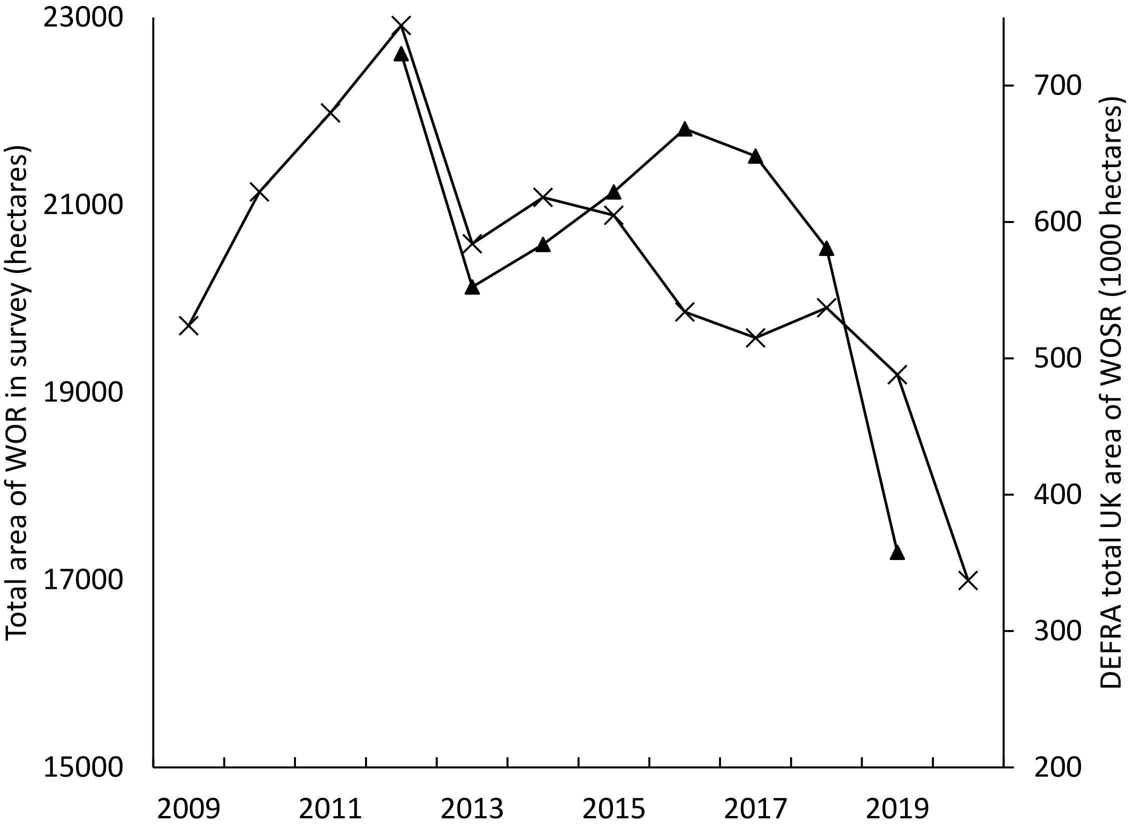

Figure 2 Combined total area, in hectares, of winter oilseed rape (WOSR, *Brassica napus*) grown between 2012 and 2019 by survey respondents (closed triangle, left axis). Data presented in grand total of all 91 respondents with standard error of means shown. For trend comparison the UK winter OSR area is also given (data from DEFRA statistics 2009-2020, in thousand hectares, cross, right axis).

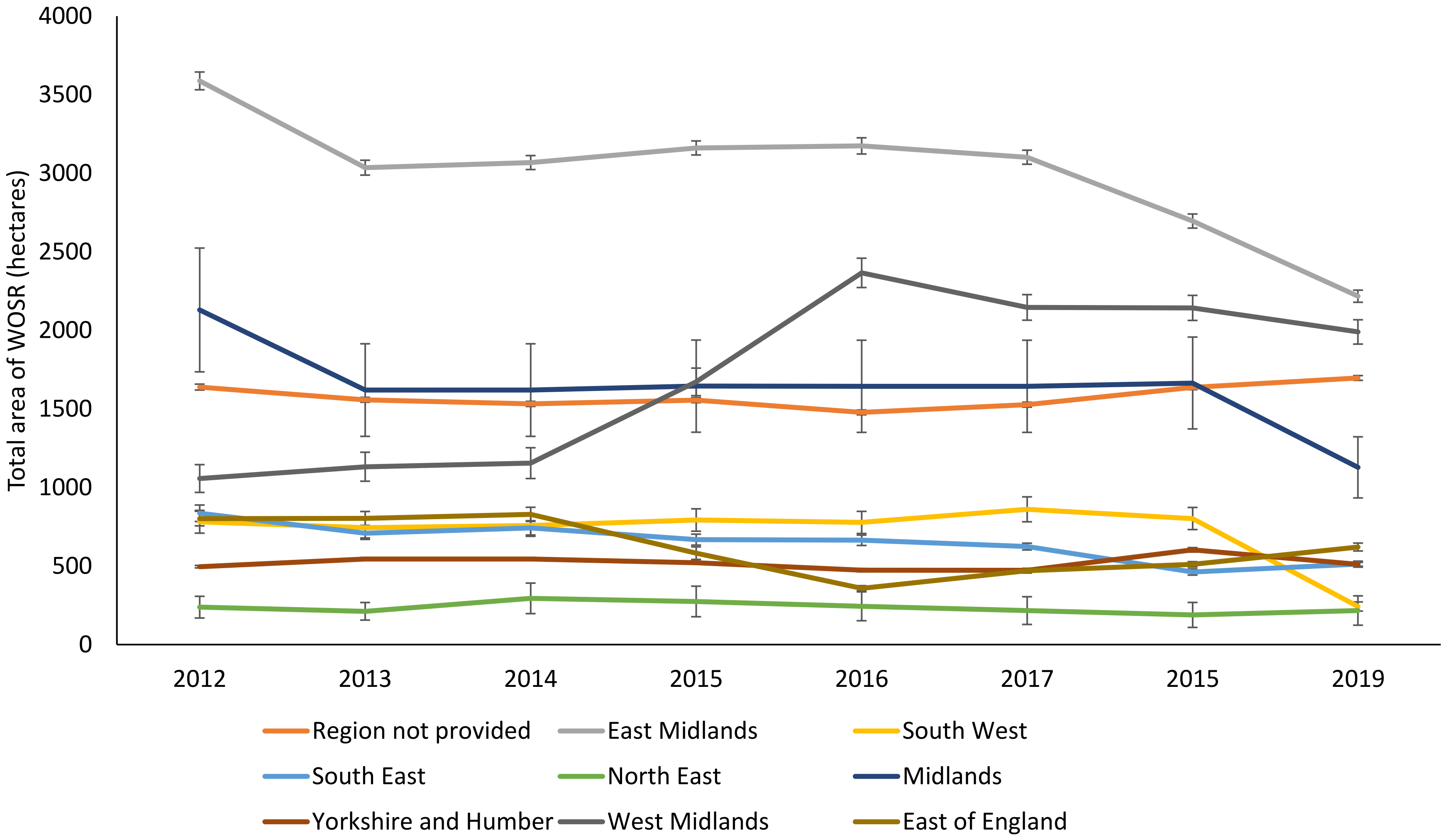

Figure 3. Regional break down of winter oilseed rape (OSR, *Brassica napus*) of survey respondents with standard error of means given.

8.4.4 Perceptions of pest damage:

Psylliodes chrysocephala was considered “very important” or “extremely important” by 74% of respondents (Figure4) while *M. persacae* was only considered very important by 3% of respondents and 65% considered them “not at all important” and “slightly important”. Few respondents (34%) named other pests, mostly slugs and pigeons, and only 16% rated these as very or extremely important.

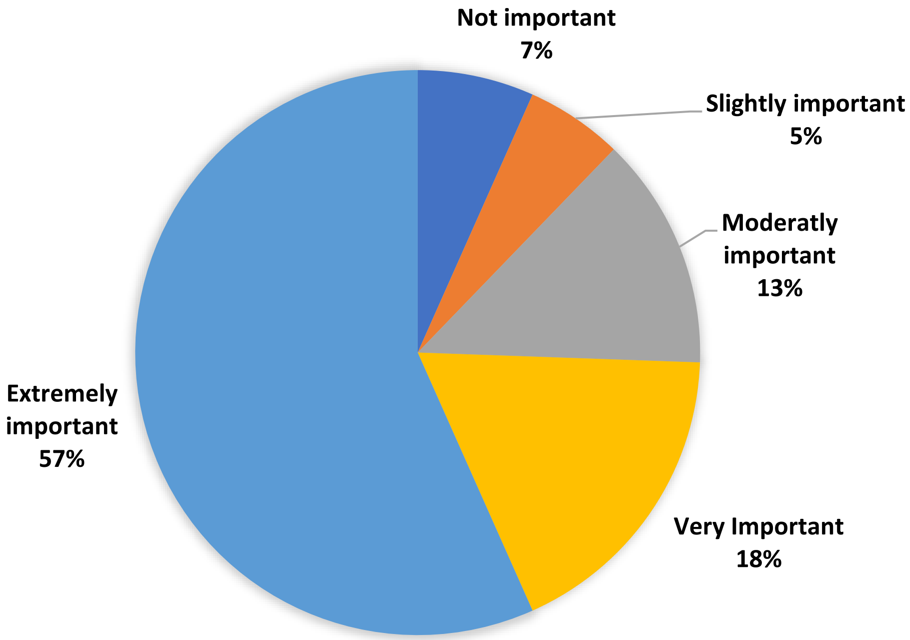

Figure 4. Importance of *Psylliodes chrysocephala* to decision making for growing oilseed rape in the UK.

8.4.5 Synthetic insecticide use:

Prior to the neonicotinoid restrictions 95% of respondents used neonicotinoid seed dressings to control *P. chrysocephala* while 43% used pyrethroid sprays (41% and 29% respectively for peach potato aphid). On average, prior to restrictions, farmers used 1.78 measures to control *P. chrysocephala* and 0.85 to control for peach potato aphid. By contrast, after the restrictions, respondents used an average of 2.08 measures to control for *P. chrysocephala* and 0.57 measures to control for *M. persacae*, indicating that they have not increased efforts to control the latter but have had to expand efforts to control the former.

8.4.6 Post Restriction:

Since the restrictions, to control *P. chrysocephala*, 70% have increased their use of pyrethroids, 43% altered their sowing density (up from 4% pre-restriction), 35% practice intercropping (up from 7% pre restrictions) and 31% have adopted new pesticides. For *M. persacae*, 21% have increased pyrethroids and 13% have adopted new pesticides.

8.4.7 Efficiency of insecticide applications:

In terms of effectiveness, 90% of respondents felt neonicotinoids were >50% effective at controlling *P. chrysocephala* (Figure5) while only 38% felt that they were as effective in controlling *M. persacae*.

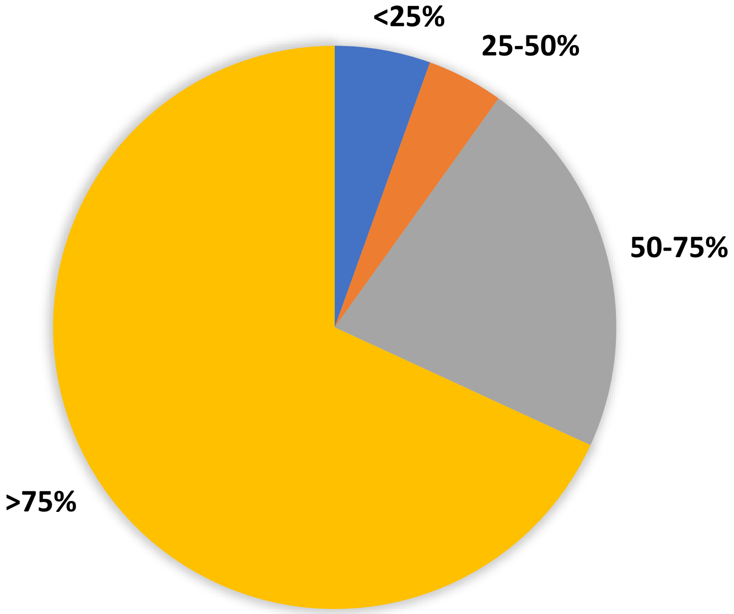

Figure 5. Perceived effectiveness of neonicotinoid seed dressings in protection Oilseed rape (*Brassica napus*) from *Psylliodes chrysocephala* pest damage.

8.4.8 Resistance:

Concerns of pyrethroid resistance in *P. chrysocephala* were expressed by 83% of respondents expressed some degree of concern (Figure6) and 63% to neonicotinoid sprays but only 42% were concerned about resistance to neonicotinoid seed dressings. Concern over each *M. persacae* was more mixed with no definite trends emerging apart from 54% of respondents expressing concern that they could become resistant to neonicotinoid seed dressings.

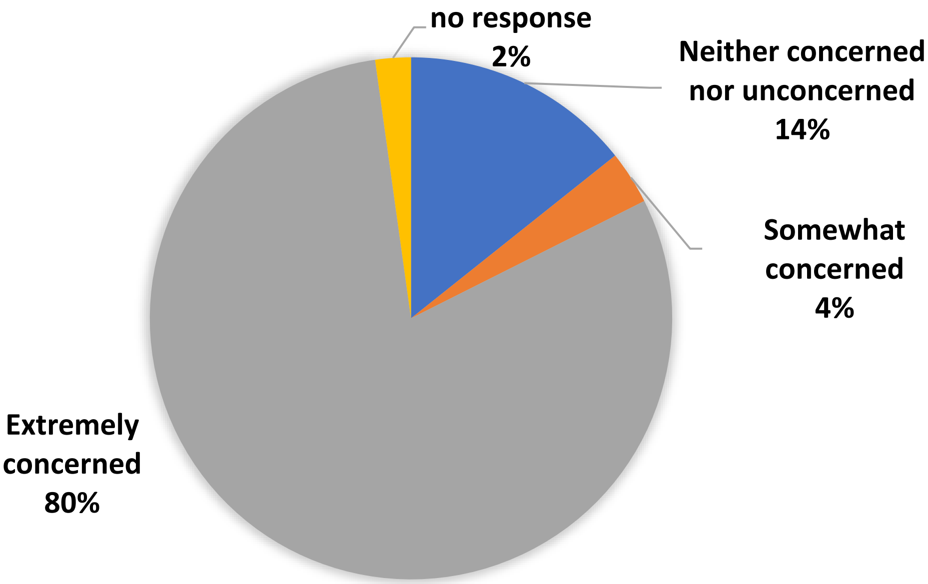

Figure 6. Level of concern of resistance to pyrethroids in *Psylliodes chrysocephala*.

8.4.9 If the ban were rescinded:

If they were made available again, 75% of respondents were somewhat likely or extremely likely to resume the use of neonicotinoids (Figure7). When asked to explain their response, many respondents expressed a preference for neonicotinoids as they were more targeted and, they believed, effective at controlling pests without harming beneficial insects. However, several farmers expressed that they would not readopt to protect wildlife and beneficial insects such as bees and natural enemies.

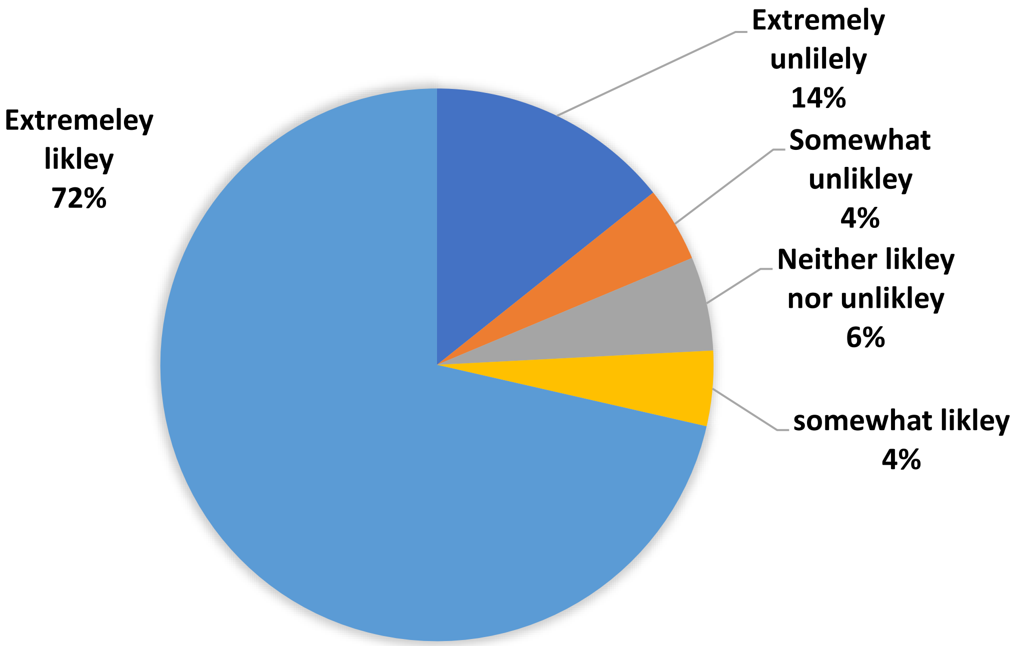

Figure 7. Likelihood respondents would use neonicotinoids if the current ban were rescinded. Based on 91 respondents.

8.4.10 Application thresholds and natural enemies:

A high proportion of respondents used spray thresholds, 91%, with 82% indicating that they carried out such spraying at threshold levels of pests observed either by themselves or at the advice of an agronomist. However only 11% of respondents felt that this strategy was very or extremely effective while 53% felt it had little or no effect.

8.4.11 Pest control by natural enemies:

Similarly, 76% of respondents felt that natural enemies alone had <50% effectiveness in controlling *P. chrysocephala* and 56% that they had <50% effectiveness in controlling *M. persacae*.

8.4.12 Post restriction changes in pest pressure:

When asked to consider how the damage inflicted by pests has changed since the ban on neonicotinoids, 80% of respondents felt that damage by *P. chrysocephala* had risen sharply since the ban (Figure8) while only 20% felt damage from *M. persacae* had risen at all (Figure8).

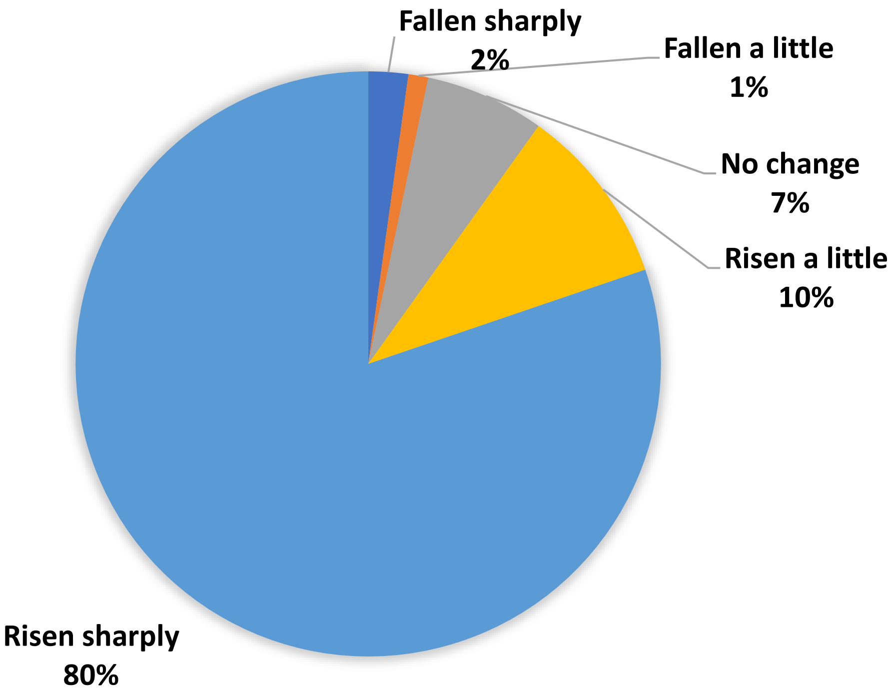

Figure 8. Perceived change in crop damage from *Psylliodes chrysocephala* since the restriction on the use of neonicotinoid seed dressings came into effect in in the UK in 2014.

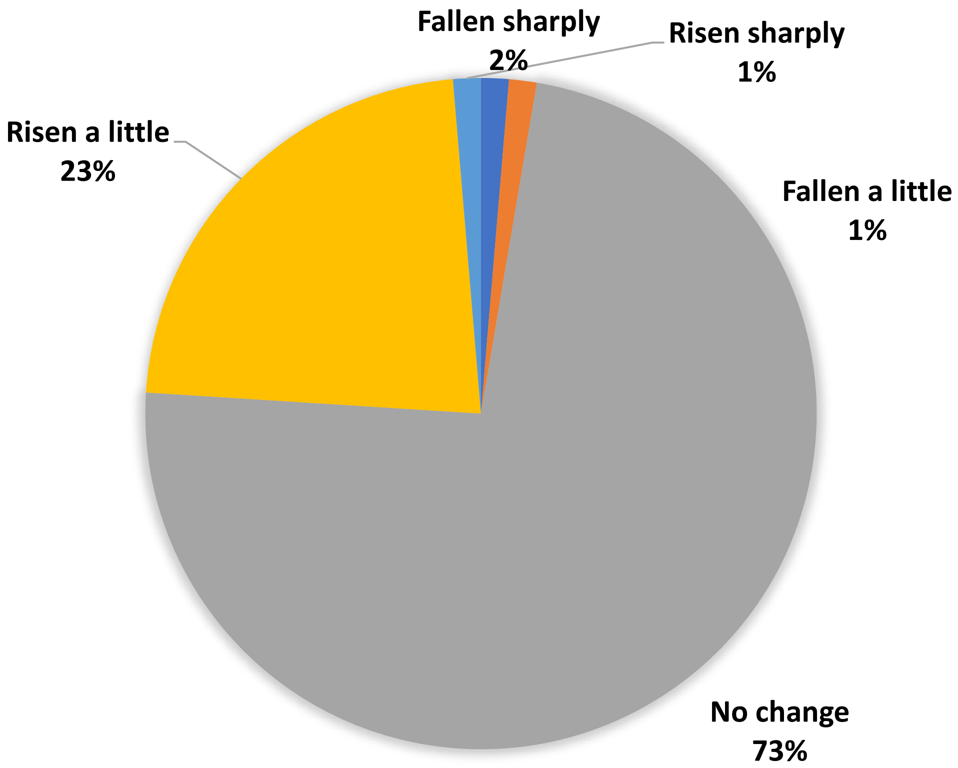

Figure 9. Perceived change in *Myzus persacae* damage to oilseed rape crop since the restriction on neonicotinoid seed dressing came into force in the UK.

8.4.13 Adopting new methods:

Respondents were asked if they would adopt any of a number of alternatives to chemical control if sufficient evidence was supplied. Of the options presented for *P. chrysocephala*, 88% would adopt insect resistant varieties 81% would adopt intercropping or cover crops, 71% would alter sowing density and 63% would use trap crop boarders. For *M. persacae*, there was substantially less interest in adopting new measures, only 42% would adopt resistant cultivars, 34% would adopt intercropping, 21% would adopt trap cropping and 20% would alter sowing density. When asked why they would not adopt any of the measures given, no measure was a common concern (all <30% of responses). However, topping the crop before stem elongation was always the measure that most respondents indicated they would be unwilling to use, notably for lowering yield and quality (26% of respondents) being ineffective (23% of respondents) and unreliable (21% of respondents).

8.4.14 Open questions:

Of the questions where open answers boxes were available there was relatively little responses for Q 8, 13, 17, 21, 24 and 25 (Appendix) with each being utilised in 3, 5, 3, 5, 2 and 1 times, respectively. Questions 5, 11 and 12 were filled out more frequently with 19, 47 and 21 responses, respectively. For question 15 on other pests causing a problem, respondents stated slugs (n=15), pigeons (n=3) and cabbage root fly (n=2) out of 19 total responses. Question 11 elicited the most engagement with 47/91 respondents leaving a comment, highlighting the importance of the topic to the participants. The main concern raised was the inability to grow OSR without the use of neonicotinoids (n=12) with the increase in *P. chrysocephala* as a problem since the restriction in 2014 and ban in 2018 being stated in 9 responses. Neonicotinoids were stated to not work by 6 participants with an equal amount saying they are more targeted and better for the environment than a broad spray application. The impact on non-target species was highlighted by 8 responses with impact on beneficials mentioned 6 times and impact on soil biodiversity thrice. The two main comments for question 28 were related to needing robust evidence alternative techniques work (n=5) and a lack of knowledge of potential techniques (4). Barriers to botanical diversification (trap crop or cover crop) were stated as the increase in labour would not be attainable during the busy time of year when OSR seed bed is prepared and seed drilled (n=3). In other words, alternative methods are too labour intensive to establish and won’t be used. In specific relation to the topping of crop prior to stem elongation 3 respondents made the comment that topping the field at that time of year (February-April), when the soil is wet and cannot easily support heavy machinery, would be too damaging to the crop and leave rut ways in the field, this was countermarked by 2 respondents that using sheep to graze rather than topping would be preferred with one saying the sheep would be preferred to prevent the damage caused by heavy machinery on wet soil.

8.4 Discussion:

8.4.1 Overview:

The key research interest of the survey was to explore trends in farmer preferences towards different alternatives and barriers towards the adoption of options they would like to adopt. This will be conducted using logistic regression analysis (due to the large number of binary response variables assessed) or similar statistical methods. However, due to the bias towards agri-environmental farmers, there is some caution to be exercised in interpreting these results, however as agri-environmental farmers are more likely to be early adopters of alternative methods this is not expected to hider the strength of the outputs. Otherwise, much of the remaining analysis will be descriptive, looking into the unique insights that this survey provides regarding farmers rationales for using different methods of pest control and their answers to open questions where they could state their opinions freely.

8.4.2 Area change:

The participants of this survey have reported significant reductions of the area of OSR they grow since the neonicotinoid restriction came into effect. The reduction in area's grown in in line with other reports on reductions in OSR post the neonicotinoid restriction (Nicholls, 2015; DEFRA, 2019). This is evidence that the farmers are highly risk adverse and were heavily reliant on the insurance of seed treatments in OSR. This was born out by the proportion of respondents who would use neonicotinoids is the ban were rescinded.

8.4.3 Crop damage levels:

The levels of damage from *P. chrysocephala* to crop plants is perceived to have risen sharply since the neonicotinoid ban highlighting, the importance farmers put on neonicotinoid seed treatments in reducing *P. chrysocephala* feeding damage. In a recent study the effect of neonicotinoid seed dressings was brought into doubt with little to no reduction in leaf area loss or larvae numbers from *P. chrysocephala* when compared to untreated OSR (Coston et al 2021 – chapter 4 this thesis). The findings in plot-based experiments are in contrast to the level of effectiveness perceived from neonicotinoid seed dressings expressed by respondents.

8.4.4 Pest resistance to insecticide:

Farmers are fairly confident that *P. chrysocephala* won't become resistant to neonicotinoid seed dressing but will become resistant to sprays – however despite some concern from nearly half the respondents regarding *P. chrysocephala* resistance to neonicotinoid sprays they are very likely to resume using them. The high levels of concern raised by respondents to pyrethroid resistance developing in their region is in line with a recent survey of *P. chrysocephala* resistance in the UK reporting widespread resistance (Willis *et al.*, 2020), see Figure 10. The levels of resistance reported by Will *et al*., (2020) were from adult beetles and in a recent study (Coston et al 2020 chapter 4 this thesis) showed up to 84.9% resistance. However, in this field trial the application of pyrethroids significantly reduced the number of *P. chrysocephala* larvae in plants. Suggesting that even when adult populations exhibit high levels of resistance there is still a potential protection against eggs and larvae of *P. chrysocephala*. Further investigation into the susceptibility of *P. chrysocephala* to pyrethroids at different life stages should be carried out soon.

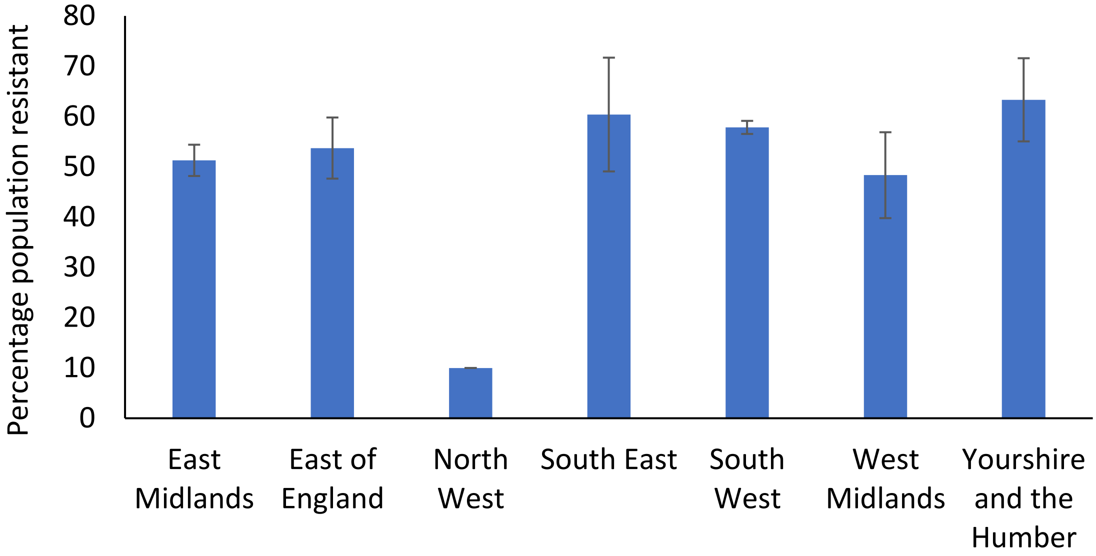

Figure 10. Percentage population of *Psylliodes chrysocephala* exhibiting resistance to pyrethroid insecticide (data from Willis *et al.,* (2020)).

8.4.5 Adopting new methods:

The majority of respondents were open to novel methods of OSR cultivation in terms of new methods for *P. chrysocephala* protection. They were less inclined to adopt methods for protecting the crop from *M. persacae,* which was perceived as of little importance by the majority of respondents. One method which shows promise in reducing *P. chrysocephala* larvae is the topping or grazing of the crop. This was trilled in the linked field experiment and showed significant reductions in larvae numbers (Coston et al 2021 – chapter 4 this thesis). However, the subsequent delay in flowering brought on by topping left these plants at greater risk to pollen beetle (*Brassicogethes aeneus*). The respondents were concerned about such factors but were more concerned on the practicalities of using machinery on the crop before OSR stem elongation. An issue which can be removed by grazing the crop instead of topping, which was noted by respondents. Recent work at AHDB is examining the potential of sheep grazing as a means of reducing *P. chrysocephala* in plants (White *et al.*, 2020). There is also evidence in spring OSR from Canada and Australia that if grazing is timed correctly little reduction in the grain yield is seen (Kirkegaard *et al.*, 2008; Seymour *et al.*, 2015). This survey shows that farmers are willing to use these methods and are awaiting evidence of effectiveness. The primary issue respondents had to alternative methods was a lack of evidence of effectiveness. Some of this lack of evidence may be brought on by the limited access to scientific journals in the farming community.

8.4.6 Conclusion:

The levels of concern about loss of sufficient crop protection in OSR with the removal of neonicotinoid seed dressings highlights, the level of importance they were regarded by growers. This being said one respondent made special note on the issues of ease of use and I quote “Neonics have allowed poor establishment techniques to remain viable. Planted correctly, there is no risk of insect attack-Insects only attack stressed plants.” This and the willingness to adopt new methods by growers such as intercropping or elevated seed rate shows there is a willingness to adapt to a new non neonicotinoid cropping system. It is highlighted the uptake of novel sustainable crop protection practices are in need of furthers robust evidence to convince farmers of their effectiveness.

8.5 Acknowledgments:

I would like to thank farmers weekly, the NFU and Rothamsted research for publicising the survey and aiding in its distribution to appropriate growers. The university of Reading agricultural students who participated in the testing of the survey.

8.6 Appendices:

Q00 Thank you for participating in this study on British Farmer’s perceptions of pest issues and management in winter oilseed rape. We hope the findings of this survey will help identify how British farmers feel about pest management strategies to common oilseed rape pests. This study is part of a PhD project conducted by The University of Reading and Rothamsted Research and should take no more than 20mins to complete. This study is funded by Lawes Agricultural Trust, and has been designed, administered and all data collected will be analyzed by The University of Reading (UK). As part of this survey, we will ask a few basic demographic questions (e.g. your age, location of your farm etc.) in order to identify trends in perceptions between farmers. Under data protection law, we are required to inform you that the use of the personal data we may hold about you is on the lawful basis of being a public task in the public interest and where it is necessary for scientific or historical research purposes. All of the information collected will be held by the University of Reading (Data controller) and by Qualtrics, the online platform that this survey uses. Both the University of Reading and Qualtrics are fully complicit with EU and UK data protection laws. The data collected in this survey is only intended for use as part of the PhD research project "Quantifying the impacts of the neonicotinoid restriction on oilseed rape pest control, pollination and productivity", although summarised versions of this data may be made available for later analysis. Should you wish to withdraw your answers from this survey at any time prior to the publication of results please call me on 0118 378 6419 or e-mail t.d.breeze@reading.ac.uk and quote the questionnaire ID below – this number is linked to your responses. If you withdraw from a research study, which processes your personal data, dependent on the stage of withdrawal, we may still rely on this lawful basis to continue using your data if your withdrawal would be of significant detriment to the research study aims. The survey does not ask for your name, or the address of either you or your farm operations. Your individual responses will be held by the University of Reading for the duration of the project and then destroyed. Anonymised summaries of responses from all participants will be retained by the University of Reading indefinitely for use in future work. The data will be stored on a secure drive only accessible to the University of Reading (Dr Tom Breeze, Duncan Coston) and will not be passed on to any third parties. By participating you are consenting to these terms of data storage and use which have been approved by the University of Reading's Ethics.

Q1: Please enter a unique identifier text (e.g. the present date and time) so we can retrieve your questionnaire upon request.

Q2: Which region are your farming activities based in?

Q3: Approximately how large is your farming operation currently?

Less than 20 Hectares

20-50 Hectares

51-100 Hectares

101-200 Hectares

More than 200 Hectares

Q4: Approximately how many hectares of winter oilseed rape did you/ do you plan to plant in each of the following years? This does not have to be exact. Please put NA if you were not farming or 0 if you did not grow winter oilseed rape that year.

2012 2013 2014 2015 2016 2017 2018 2019

Q5: How important are levels of autumn pests in determining the amount of winter oilseed rape you grow?

	Extremely important	Very important	Moderately important	Slightly important	Not at all important
Cabbage stem flea beetle					
Peach potato aphid					
Other (please state)					

Q6: Following the restrictions on neonicotinoid insecticide use, how much do you believe that pest damage from autumn pests in your winter oilseed rape crops has changed?

	Damage has fallen sharply	Damage has fallen a little	No change in pest damage	Damage has risen a little	Damage has risen sharply
Cabbage stem flea beetle					
Peach potato aphid					

Q7: How effective do you believe neonicotinoid seed treatments were/ are at controlling autumn pests in your winter oilseed rape crops?

	Less than 25% effective	25-50% effective	50-75% effective	More than 75% effective
Cabbage stem flea beetle				
Peach potato aphid				

Q8: How concerned are you about the cabbage stem flea beetle becoming resistant to common synthetic insecticides?

	Extremely concerned	Somewhat concerned	Neither concerned nor unconcerned
Neonicotinoid seed dressings			
Neonicotinoid sprays			
Pyrethroid sprays			
Other (please state)			

Q9: How concerned are you about the peach potato aphid becoming resistant to common synthetic insecticides?

	Extremely concerned	Somewhat concerned	Neither concerned nor unconcerned
Neonicotinoid seed dressings			
Neonicotinoid sprays			
Pyretheroid sprays			
Other (please state)			

Q10: If restrictions on neonicotinoid seed treatment use in winter oilseed rape were lifted, but you had the option of using untreated seed, how likely are you to use neonicotinoid seed treatment again?

Extremely likely, Somewhat likely, Neither likely nor unlikely, Somewhat unlikely, Extremely unlikely

Q11: Please use this space if you wish to explain your answer further

Q12: At any point since 2012, have you used any pest spray threshold strategies (only spraying when a certain number of pests are detected)?

Yes or No

Q13: If you do not use pest spray thresholds which of the following most accurately describes your spraying strategy?

Spray by date, Spray by crop growth stage, Other (please state)

Q14: Which of the following most accurately describes your inspection and spraying strategy?

Inspect one field, spray all fields if any are found to be above the threshold.

Only spray fields where threshold levels of pests have been observed.

A mix of the above.

I don't know – this was carried out by/under advice from agronomists.

Q15: How reliable do you think current pest threshold strategies are at protecting your winter oilseed rape crops?

Extremely effective, Very effective, Moderately effective, Slightly effective, or Not effective at all.

Q16: Without insecticides, how much do you think arthropod natural enemies (e.g. ground beetles, wasps, ladybirds etc) alone could control cabbage stem flea beetle and peach potato aphid in winter oilseed rape?

	Less than 25% effective	25-50% effective	51-75% effective	More than 75% effective	None at all
Cabbage stem flea beetle					
Peach potato aphid					

Q17: Before the restrictions on neonicotinoid seed treatments, did you use any of the following measures to control autumn pests in your winter oilseed rape crop? (please tick all that apply)

	Neonicotinoid seed treatment	Pyretheroid spray applications	Trap crop border	Intercrop or cover crops	Altered sowing density	Topping the crop before stem elongation	Other
Cabbage stem flea beetle							
Peach potato aphid							
Other (please state)							

Q18: Which crops did you use as trap crops or intercrops before the restrictions on neonicotinoid seed treatments?

Q19: How did you alter your sowing density of winter oilseed rape before the restrictions on neonicotinoid seed treatments?

Q20: Which other methods did you use to control these pests before the restrictions on neonicotinoid seed treatments?

Q21: Following the restrictions on neonicotinoid seed treatments what measures have you taken to control autumn pests in your winter oilseed rape fields?

	Extra applications of pyrethroid insecticide	Using other insecticides not normally applied	Trap crop border	Intercrop or cover crops	Altered sowing density	Topping the crop before stem elongation	Other

Cabbage stem flea beetle
Peach potato aphid
Other (please state)

Q22: Which crops did you use as trap crops or intercrops following the restrictions on neonicotinoid seed treatments?

Q23: How did you alter your sowing density of winter oilseed rape following the restrictions on neonicotinoid seed treatments?

Q24: Which other methods do you use to control these pests following the restrictions on neonicotinoid seed treatments?

Q25: If there were further evidence that they could potentially control autumn pests, would you be willing to use the following in your winter oilseed rape crops?

	Trap crop border	Intercrop or cover crops	Altered sowing density	Topping the crop before stem elongation	Insect resistant cultivars	Other
Cabbage stem flea beetle						
Peach potato aphid						
Would not use						

Other (please state)	

Q26: Which other measures would you want to use to control autumn pests in your winter oilseed rape crops? Please use this space to tell us about them and why you would like to use them.

Q27: If you are not willing to use any of the following management options, please indicate why not? (please tick all that apply)

	Trap crop border	Intercrop or cover crops	Altered sowing density	Topping the crop before stem elongation	Insect resistant cultivars
I believe it is too expensive					
I believe it is too unreliable					
I believe it is ineffective					
I believe it will increase risks from non-insect pests (e.g. slugs, pigeons etc.)					
I believe it will increase weed burden					
I believe it is unsightly					
I believe it is too labour intensive					
I believe there is potential to affect the yield or quality of the harvest crop					
Other					

Q28: Please use this space to tell us about other reasons why you would not want to use these methods on your winter oilseed rape crops

Q29: How long have you been involved in farming at a business level?

Under 5 years, 5-10 years, 11-15 years, 16-20 years, or more than 20 years.

Q30: What is the highest level of formal education in subjects relevant to farming you possess?

GCSE/O-Level, A-level, Undergraduate degree, Postgraduate degree, or Other (including BASIS).

Q31: Do you participate in the Countryside Stewardship or any other agri-environment scheme (government or private)?

Yes or No

Q32: Which agri-environment scheme do you participate in?

Q33: Do you participate in any organic or low input farming schemes (government or private)?

Yes or No

Q34: Which organic or low input farming scheme do you participate in?

Q35: Finally, please indicate your average net farm income in your last two financial years using the categories below.

Less than zero (loss), 0 to less than £5,000, £5,000 to less than £10,000, £10,000 to less than £20,000, £20,000 to less than £30,000, £30,000 to less than £50,000, More than £50,000 or I would rather not say.

Figure 11. Promotional flyer distributed at events and to farmers as a means of distributing the survey.

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9.0 Concluding discussion:

The restriction of neonicotinoids across the EU in 2013 (EU, 2013) and its subsequent re-enforcement and expansion to a total ban in 2018 (EU, 2018). This has manifested in reductions in cropping area in the UK (DEFRA, 2019; Scott and Bilsborrow, 2019) and wide publication of farmers concerns of growing OSR without neonicotinoids (Guardian, 2020). The development of alternatives is vital to maintain OSR in the UK cropping system (Impey, 2020). The continued reduction in OSR production may be reducing the complexity of field rotations removing the benefits of complex systems in limiting disease and weed prevalence (Hilton *et al.*, 2018; Malik, 2010; Weiser *et al.*, 2018).

9.1. Methods of pest control targeted at *Psylliodes chrysocephala:*

9.1.1 Seed treatments:

In the work presented here the effectiveness of novel cropping systems to protect OSR from *P. chrysocephala* were directly compared to OSR treated with a neonicotinoid seed treatment (Cruiser®, AI: thiamethoxam). In no assessments in any of the field trails undertaken in this thesis did neonicotinoid seed dressings reduce pest injury, mortality or affect yield in comparison to un-treated seed. The lack of any detectable difference between treated and un-treated seed puts doubt into the level of protection provided. The data presented here would support the ban on neonicotinoid use in the fact no appreciable benefit to the crop was detected.

At the time of the experiment, the seed treatment Lumiposa™ (AI: cyantraniliprole) was registered for use in Poland, Hungary and Romania to protect OSR from *P. chrysocephala* attack, but not in the UK yet (NFU, 2018). No effect on the level of *P. chrysocephala* feeding or larval infection in relation to un-treated OSR was observed in this thesis (chapter 4). Lumiposa™ is recommended to protect early growth OSR until BBCH 13-14 from *P. chrysocephala* and is marketed to “protect seedlings producing uniform and heathy stands” (DuPont, 2017). The data shown here would suggest that the time of Lumiposa recommended active period is outside the preferred feeding of adult *P. chrysocephala*, reducing the efficacy as an aid to crop establishment.

9.1.2 Spray application:

High levels of pyrethroid resistance have been reported across the UK (Willis *et al.*, 2020). The fields in this study were no different with resistance exhibited in up 84.9% resistance in field populations (chapter 4). When examining the reductions in *P. chrysocephala* larvae a significant reduction was seen when pyrethroids were applied (chapter 4), suggesting that the eggs or larvae are more susceptible. This variation in toxicity to differing life stages may be in part due to the form of resistance being expressed (Panini *et al.*, 2016). where metabolic resistance is concerned, where the toxic compounds are metabolically broken down, a cost to resistance applies (Kliot and Ghanim, 2012; ffrench-Constant and Bass, 2017). This mode of action exhibiting resistance may be limited or impossible at the egg or larval stages (Zimmer, 2015). One further hypothesis is that the cost of resistance exhibited by *P. chrysocephala* also has negative effects on the adult fecundity. The sub-lethal effects of insecticide have included a reduction in fecundity (Lee, 2000; Rivero *et al.*, 2011). Further investigation into the sub-lethal effects of pyrethroids on *P. chrysocephala* may aid in interpreting the reduction in larvae see here from its application.

The reduction in larvae reported here suggests that even at high adult resistance Pyrethroids do express a level of pest control. The timing of application to avoid adults and target eggs or larvae should be examined along with the variations in toxicity at different developmental stages.

9.1.3 Seed application rate:

Responses of pest pressure from *P. chrysocephala* were shown fluctuate depending on the time of observation (chapter 4), lower larvae numbers at lower seed rate in November but significantly higher in February (60seeds/m2 against 100 and 120seeds/m2). Slight increases in yield were apparent as the seed rate applied reduced, recording declining yield return with increasing seed rate, although not significant. The level of adult feeding was in line with the findings of Alves et al. (2015) that reported crops sown using low seed rates suffer from greater adult *P. chrysocephala* feeding activity per plant than crops sown using higher seed rate. The increase in seed rate did trend towards lower yield returns but these were not significantly different (chapter 4). The increase in plant density is a likely cause for reduced yield as a response to increased competition between plants (Roques and Berry, 2016; Berry and Spink, 2006). The limited yield penalties reported here suggest that current advice to increase seed application to reduce *P. chrysocephala* problems (AHDB, 2019) is worth further investigation.

9.1.4 Trap cropping:

The pollen beetle has been shown to migrate into the crop from the edge (Cook *et al.*, 2007). Therefore, aligning a trap crop as a boarder will aid in disrupting the pollen beetle's migration into the crop centre (Mauchline *et al.*, 2017; Juhel *et al.*, 2017). The migration pattern of *Psylliodes chrysocephala* do not appear to colonise the field edges equally (chapter 4 and 7). The raises the potential to design an area of migration-based trap crop area rather than a full field boarder. This could be set as an area along with a flower margin, shown to benefit pollination services (Haaland, Naisbit and Bersier, 2011). Thereby the area of flower margin contains brassicas as a dead-end trap crop (Veromann *et al.*, 2014). Therefore, combining attributes of both a trap crop and pollinator reserve, increasing the ecological benefit to the crop.

Future survey work into farmers willingness to adopt measures more specifically could explore whether farmers who are willing to use a flower boarder would add brassicas to this mix, as if seen in many flower mixes for birds. In addition to the potential of OSR seed to incorporate a trap crop element for both *P. chrysocephala* and pollen beetle if left to flower in the margin, a benefit for pollinators as well.

9.1.5 Nurse cropping:

The use of brassica nurse crops may be considered for further research combined with the grazing or topping of the crop prior to stem elongation as shown in Chapter 4 to reduce *P. chrysocephala* numbers. The lack of major frosts and limited action from the herbicide application led to the nurse cropping being cleared along with the OSR, so no measurement on yield could be attained. The issues involved in small plot experiment here would put more doubt into the full field scale use of the mix tested here.

9.1.6 Defoliation:

When OSR is defoliated prior to stem elongation it exhibits a remarkable ability to compensate from the injury (Syrovy, Shirtliffe and Zarnstorff, 2016; Ramachandran, Buntin and All, 2000; ADAS, 2013). The development of dual use canola in Australia (Kirkegaard *et al.*, 2008a) shows that this can be possible without significant yield penalties. The use as a pest control method for *P. chrysocephala* has been receiving attention more recently in the UK in response to the neonicotinoid ban. The use of this method a component of field trials in this thesis (chapter 4). Where the crop was mown to 5cm and shown to significantly reduce the number of *P. chrysocephala* larvae (Chapter 4), whilst the plant density at harvest was not affected by defoliation (chapter 4). This has been reported in other similar studies using different modes of defoliation e.g. Kirkegaard *et al* (2008b) have shown sheep grazed OSR can compensate from the injury with little to no yield penalty if controlled and timed correctly and this was reflected here (chapter 4). Due to the delay in flowering, induced by the defoliation, the effected plants were severely damaged by pollen beetle (*Brassicogethes aeneus*). Which are known to be more damage to less developed plants (Dosdall and Mason, 2010). This highlights the importance of timing of defoliations is not only crucial to reduce direct damage to the plant but to avoid leaving defoliated plants more open to secondary pest attack. The method of defoliation was a major concern of farmers when asked their views on alternative pest control methods (chapter 8). The concept of defoliation or sheep grazing was generally supported (chapter 8). However, concerns were raised on the use of machinery to accomplish the defoliation at the appropriate time of season (chapter 8). They did indicate a preference to using sheep to avoid soil bed damage (chapter 8). Further work into sheep grazing as a means of pest control is currently under way at AHDB (White *et al.*, 2020). There is a potential to combine the technique with nurse cropping to combat the issues around removal of non-crop plants (chapter 4). By developing a nurse crop mix which is a suitable host for *P. chrysocephala* and a forage plant of sheep this may then be an effective means of removing the cover and gaining the benefits of soil cover of the nurse over winter which in this study showed reducing *P. chrysocephala* adult feeding injury on the OSR crop and the reduction in larvae number reported here and in other studies (White *et al.*, 2020; White, Ellis and Kendall, 2018; White, 2019).

9.2. Assessment of *Psylliodes chrysocephala* in the field:

9.2.1 Feeding location on plant:

Data shown in Chapter 6 demonstrated a feeding preference for true leaves over cotyledon. If this is to be confirmed in the field future research surveying *P. chrysocephala* injury would be wise to also record the leaf location to build a bigger data set to determine is the *P. chrysocephala* does have any preferential feeding location on the plant.

9.2.2 Nocturnal feeding diurnal sheltering:

The data presented in chapter 7 suggests that *P. chrysocephala* are primarily active at night making direct observations during this time critical. Using emergence traps the hypothesis that *P. chrysocephala* are primarily nocturnal was supported and that during daylight hours they shelter under the soil surface. This information supports current advice for synthetic insecticide application being carried out at night. This idea was partly brought forward to avoid exposure to day-flying bees to pest protection products. Data collect here supports evidence that this is also a benefit for application in order to maximise likelihood of contacting the desired pest. It also shows that to accurately measure the abundance and therefore pest load to crop during night.

However, this suggests that examining the interactions with *P. chrysocephala* and below soil nematodes could be fruitful. Levels of entomopathogenic nematodes have been shown to be at low density in OSR fields in Europe but are being tested as a pest control option against *P. chrysocephala* by inundation/inoculation of fields (Hokkanen *et al.*, 2006) and the survival of these nematodes can be boosted by providing ground cover and restricting UV radiation at soil level. Reduction of *P. chrysocephala* of up to 73% was reported (Hokkanen *et al.*, 2006) although as the authors state the target pest during that trial was pollen beetle. This especially linked to the diurnal resting period of the *P. chrysocephala* being under the soil surface and therefore near potential entomopathogenic nematodes (Tangtrakulwanich *et al.*, 2014). Distributions of *P. chrysocephala* have been shown to be variable across a field (Thioulouse, Debouzie and Ballanger, 1984; Ferguson *et al.*, 2006). Although it is not known if this variability is consistent over seasons. With the strong directional bias reported here coupled with the knowledge that *P. chrysocephala* shelters below soil during the day the potential exists to target the inundation/inoculation proposed by Hokkanen *et al* (2006).

9.2.3 Migration into the field:

Migration into fields from the surrounding landscape of *P. chrysocephala* was measured in the study with water traps along crop edges in two fields in 2016 and one field in 2018. Each sowed a distinct bias in catch numbers to certain edges (in 2016 South and South-East and in 2018 North-East). Further investigation would be needed to determine if this bias is due to factors such as wind direction, known to influence aphid migration into fields (Mauchline *et al.*, 2017), or location of aestivation sites for the beetles. The data may already exists in farmers records for application thresholds.

9.3. Direct impact of *Psylliodes chrysocephala* on Oilseed rape:

When examining the direct interactions of pest injury on crop growth OSR was shown to compensate well to leaf area loss but less well to low larvae introduction (chapter 5). When larval numbers are higher (25 introduced to plant, 6 estimated taking up residency) levels of flower production are reduced, and subsequent yield matrix are equally reduced (chapter 5). This expands on work on the physiological response of OSR to flea beetle feeding, with Nowatzki and Weiss (Nowatzki. T and Weiss. M, 1997) reporting reduction in biomass but high level of survival when attacked by *Phyllotreta crucigerae,* and increased glucosinolate production when infected with *P. chrysocephala* larvae (Koritsas, Lewis and Fenwick, 1991; Koritsas, Lewis and Fenwick, 1989). The higher levels of glucosinolate reported in seeds in this study when plants were infected with high numbers of *P. chrysocephala* larvae (chapter 5) is evidence that the increase in glucosinolate reported by Döring and Ulber (2020) in early response to larval infection are still evident when measuring the end seeds (chapter 5). The effect of which was reductions in floral potential, a primary concern for both the farmer and pollinators (Westphal, Steffan-Dewenter and Tscharntke, 2009; Holzschuh *et al.*, 2012), and reductions in yield amount and quality (chapter 5). This confirms the concerns raised by farmers that *P. chrysocephala* is a major and potential increasing problem to OSR production (UK, 2020). Further work is needed to understand in detail the interactions between *P. chrysocephala* larvae and OSR and the methods outlined here (chapter 5) allow simple and quick establishment of experiments without expensive equipment or long-term husbandry of *P. chrysocephala.* These high numbers of larvae do not occur every season (personal observation). With the abundance in 2015 being particularly high resulting in complete crop failure (chapters 2 and 3), even when treated with neonicotinoids, chapter 3). There is some evidence suggesting that *P. chrysocephala* populations abundance peaks every 7 years in Germany and Sweden (Nilsson, 2002). There is potential to monitor such trends using the Rothamsted insect survey data (https://insectsurvey.com/), although this has not been explored yet (Shortall, C. personal communication). Further investigation of long running data sets may be useful to examine the cycles in the UK and predict years of high abundance. The addition of this data into any farmer support, such as proPlant (Johnen *et al.*, 2010), tools would be a great benefit.

9.4. Conclusion:

This thesis has attempted to quantify the impacts of the neonicotinoid restriction on OSR pest control, and production through field trials testing the relative effect of neonicotinoid seed treatments to alternative options available to farmers. The findings demonstrate that neonicotinoid insecticides are likely to be less effective than perceived (Chapters 4 and 8) and that alternative measures can be effective at mitigating the risks of common oilseed rape pests (Chapters 2-4). Key to the effective implementation of these measures will be deeper understanding of a) the ecology of pests (Chapters 5-7), the economic impacts of the damage caused (Chapters 5 and 6) and farmer acceptance of these methods in relation to their perceived drawbacks (Chapter 8). These showing that the effect of neonicotinoid seed treatment can be achieved with ecological based methods. The crop failure in two of the field experiments demonstrate the problems facing farmers, who cannot afford a total crop failure. No method tested was enough to protect OSR and produce a viable crop, including neonicotinoid treated seed. The data collected here suggests that control of *P. chrysocephala* can be achieved through non-chemical based methods and further research is needed to develop these.

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