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

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11 PhD Studentship

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13 Department of Agriculture, Policy and Development, University of Reading.

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17 February 2021

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27 **Declaration:**

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

31

A handwritten signature in blue ink that reads "D. Coston". The signature is written in a cursive style with a large, stylized 'D' and a long, sweeping underline.

32

33

34 Duncan James Coston

35

36

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278 **0. Abstract:**

279

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

295

296 **1.0 Introduction:**

297

298 **1.1 Oilseed rape:**

299 Oilseed rape (OSR, *Brassica napus* L.) is a cultivated member of the Brassica family and is
300 primarily grown on an agricultural scale as an oil crop (Redman, 2019), with the meal from
301 oil processing being sold as animal fodder (Cotrill *et al.*, 2007). Oilseed rape contributes
302 around 80% of EU biofuel production (Ouvrard and Jacquemart, 2019) and 16% of
303 worldwide oil production from crops (FAOSTAT, 2021). It is the third most widely grown
304 crop in the UK, after winter wheat and barley (DEFRA, 2015) with an average yearly value of
305 £804M (Nicholls, 2016). The primary harvest is of the seed grain for oil extraction
306 (Diepenbrock, 2000), but it is also an important break crop in arable rotations, reducing
307 weed pressure (Haramoto and Gallandt, 2004; Rick and Ann, 1995) and fungal pathogens
308 (Muehlchen, Rand and Parke, 1990). Increased rotational diversity has been shown to
309 reduce pest and weed pressure over reduced crop rotations (Brust and King, 1994).

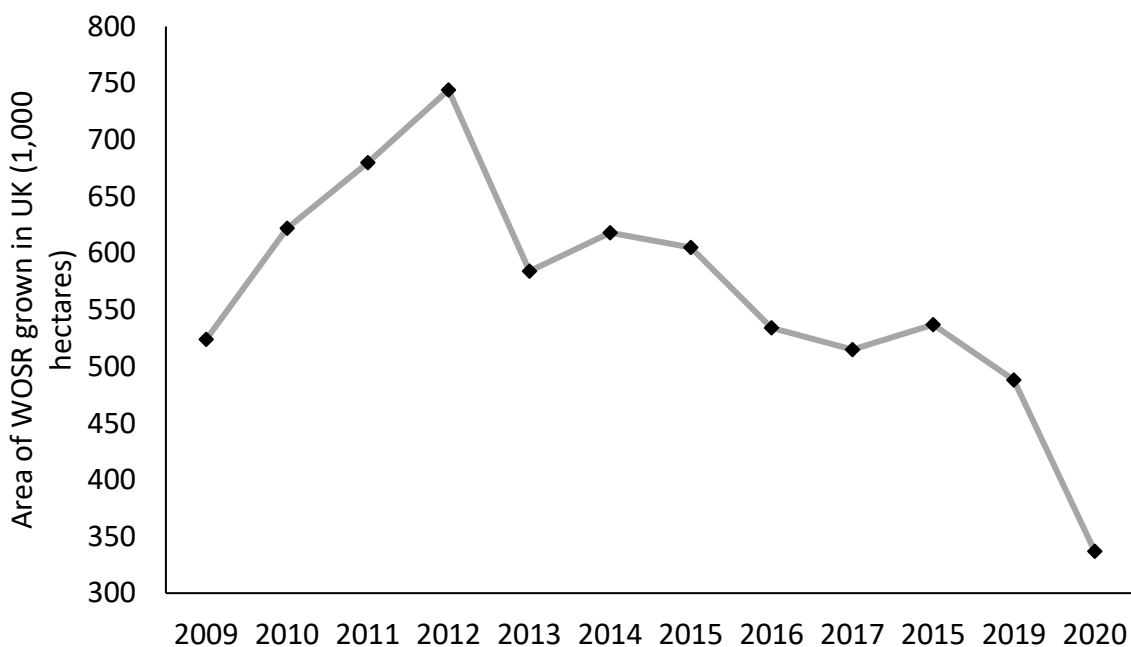
310

311 **1.2 Oilseed rape in the UK:**

312 In the UK OSR is either autumn sown or spring sown OSR, with the primary form being is
313 autumn sown (winter oilseed rape: WOSR) which requires a period of vernalisation in order
314 to form flowers (Tommey and Evans, 1992). Spring sown OSR (spring oilseed rape: SOSR)
315 does not require vernalisation and is planted in the spring and develops much faster. The
316 UK's mild winters allow WOSR to be effectively grown allowing for the increased yields, over
317 SOSR, attributed to the longer growing season (Rameeh, 2011). The area grown in UK

318 peaked in 2011/12 at just under 750,000 Ha dropping to below 550,000 Ha in 2015/16
 319 (Scott and Bilsborrow, 2019). This has continued to drop to a current level of 337,000 Ha in
 320 2020 (DEFRA statistics, Figure 1). Variations between years are to be expected as farms will
 321 not be on the same rotation stage, so some caution is needed when comparing yearly
 322 production/ planting of OSR. However, the fluctuation in market value is a major factor in
 323 the planting or OSR with crop values changing rapidly (Davies, 2019). The increase in OSR
 324 area from 2005 was in part due to EU policy to produce 20% of energy use from renewable
 325 sources of which OSR biodiesel is a component part (EREC, 2011). Then the downward
 326 trend of area grown has occurred since the restrictions (Figure 1) on using neonicotinoid
 327 seed dressings on crops that flower, came into force in 2013 and it has continued since the
 328 wider ban came into effect in 2019 (DEFRA, 2019).

329



330

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

333

334 **1.3 Pest issues and their control in oilseed rape:**

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

346

347 **1.4 Neonicotinoids:**

348 Neonicotinoids, including seed treatments, were first registered in the UK in 1994 and by
349 2008 they represented 24% of insecticides used world-wide (Jeschke *et al.*, 2011). They act
350 by binding to the insect's nicotinic acetylcholine receptor, causing excess synaptic firing
351 (Tomizawa and Casida, 2005). By contrast binding to mammalian receptors is much reduced
352 making neonicotinoids much less toxic to mammals. The seed dressings are systemically
353 taken up by the plant as it grows, conferring protection to all parts of the plant, an
354 advantage over repeated spraying which may lead to off target effects (Tomizawa and
355 Casida, 2005). This systemic nature made neonicotinoids seed dressings a very attractive
356 option to growers who could apply an insurance level of protection against pests during

357 crop establishment. At the time of interdiction neonicotinoids also allowed the diversity of
358 insecticide mode of action to aid in resistance management (Zimmer, 2015). All of these
359 positive properties led to their rapid adoption (Jeschke *et al.*, 2011). However, the systemic
360 nature, hailed by farmers as being more targeted (UK, 2020) and easier to use had the
361 downside of resulting in their presence in nectar and pollen and therefore to potential
362 exposure of non-target insects such as bees (Goulson, 2013; Gross, 2014).. Research has
363 indeed shown detrimental effects on n bees (Gill and Raine, 2014; Raine and Gill, 2015;
364 Henry *et al.*, 2012; Whitehorn *et al.*, 2012) and also on birds (Gibbons, Morrissey and
365 Mineau, 2015; Hallmann *et al.*, 2014). The level of environmental toxicity has been brought
366 into question with studies showing cumulative effects (Gill and Raine, 2014). These concerns
367 led to increased political pressure and public concern over their use.

368

369 **1.5 Neonicotinoid legislation:**

370 On the 1st of December 2013, the European commission restricted the use of three
371 neonicotinoid pesticides, Imidacloprid (registered for use in 2000, Chinook, Bayer),
372 Clothianidin (registered for use in 2008, Modesto, Bayer) and Thiamethoxam (registered for
373 use in 2007, Cruiser, Syngenta) regulation 485/2013 prohibited the use as seed treatments,
374 soil treatments and foliar application to bee-attractive crops and seed and soil treatments
375 in cereals sown between Jan and July (EU, 2013). This was expanded in 2018 under
376 regulation 2018/783-785, which prohibited the outside use of neonicotinoids on all crops
377 and only allowing use on for plant production which is 100% inside glasshouse (EU, 2018).
378 Other countries have also banned the use of some neonicotinoids, for example Fiji banned
379 the use of imidacloprid as of January 2020 (Government, 2020), the US, Canada, Australia

380 and New Zealand governments have all announced a review of neonicotinoid use
381 (Government, 2019; Zealand, 2018) and a recent report covering 17 African nations has called
382 for a review of neonicotinoid use across Africa (NASAC, 2019). Again, the main reason for
383 restrictions was in response to concerns over the potential effects of these chemicals on
384 non-target species, especially bees (Blacquiere *et al.*, 2012).

385

386 **1.6 Post neonicotinoid restrictions:**

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

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

403 (Impey, 2020). They also estimated prevention/ mitigation of *P. chrysocephala* including
404 costs of other insecticide, application costs, and re-drilling costs , totalling £18,369,369 in
405 2015/16, with a reduction of WOSR in the UK by 13% in 2016 compared with 2015 (White
406 *et al.*, 2020).

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

414

415 **1.7 *Psylliodes chrysocephala* life cycle:**

416 With the importance of OSR to pollinators, rotations, and the UK economy it is important to
417 explore alternatives to neonicotinoid-based insecticides. This requires knowledge of the
418 pests to be controlled and for OSR this is *P. chrysocephala*, which is distributed across the
419 UK and northern Europe. The life cycle of has been studied in many countries across Europe
420 (Graham and Alford, 1981; Winfield, 1992; Oakley, 2003; Holland and Oakley, 2007; Cox,
421 1998) and is relatively well understood (Williams, 2010). Adults migrate into the crop during
422 the autumn (Thioulouse, Debouzie and Ballanger, 1984; Thioulouse, 1987) where they feed
423 on leaves, producing characteristic shot holing and necrosis (Alford, 2003) before
424 reproducing within the crop and laying eggs in the soil and at the base of plants (Alford,
425 1979; Bonnemaïson and Jourdeuil, 1954). The larvae then emerge, burrow into plant stems

426 and feed throughout the winter within the plant petioles and stem before exiting the plant
427 to pupate within the soil. The two forms of feeding make *P. chrysocephala* an economically
428 important pest of OSR (Nicholls, 2016). The timings of each stage are dependent on
429 environmental conditions and can vary from year to year (Williams, 2010). The natural
430 enemy community is little understood, but it is known to be parasitized by *Tersilochus*
431 *tripartitus*, *T. microgaster*, and *Aneuclis melanarius* at the larval stage and *Microctonus*
432 *melanopus* at the adult stage (Williams and Ferguson, 2010). More recently the parasitoid
433 *Microctonus brassicae* has been examined as a potential biocontrol agent for *P.*
434 *chrysocephala* showing 44% of populations being infected in controlled environments
435 (Jordan *et al.*, 2020).

436

437 **1.8 *Psylliodes chrysocephala* impact on OSR establishment and** 438 **yield:**

439 Feeding by adult *P. chrysocephala* on the early growth of OSR can reduce stands and In
440 years of high abundance, can decimate whole fields ((2015), personal observations). making
441 establishment a lottery. There is some evidence that OSR can tolerate very high levels of leaf
442 area loss before the plant exhibits yield reductions (Freyman, Charnetski and Crookston,
443 1973; McCormick, Virgona and Kirkegaard, 2013; Ulas *et al.*, 2015) and the impact of injury
444 akin to adult feeding (shot hole injury) on early growth has been assessed for plant biomass
445 (Nowatzki. T and Weiss. M, 1997; Ellis, 2015; DEFRA, 2014) or response in plant tissue
446 concentrations of glucosinolate (Koritsas, Lewis and Fenwick, 1989; Koritsas, Lewis and
447 Fenwick, 1991; Bartlet *et al.*, 1999; Döring and Ulber, 2020). These studies examined the
448 initial response of the plant to injury and did not measure the chronic effects on later seed

449 production. The larvae are considered to be of greater concern than the adults (Williams,
450 2010) and once inside the plant stem they are difficult to target with insecticide sprays, but
451 were controlled previously through the systemic nature of neonicotinoid seed dressings, as
452 shown with the reduction in spray applications at sites under the 2015 DEFRA derogation
453 (Case, 2015; Scott and Bilsborrow, 2019). The wounds caused by *P. chrysocephala* larvae can
454 also leave plants at a higher risk of secondary infections such as stem canker (*Leptosphaeria*
455 *maculans*. K.) which has been reported to be “associated with insect wounds” (Newman,
456 1984).

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

461

462 **1.9 Resistance to pyrethroid in *Psylliodes chrysocephala*:**

463 The removal of neonicotinoid seed treatments has led to an increase in pyrethroid
464 application, up to four times the levels used before the ban (White, 2016). Scott and
465 Bilsborrow (2019) report an estimated cost of £22.2million for agrochemical (primarily
466 pyrethroids) purchase and application in 2014/15, derived from Farm Business Survey of
467 >200 farms. This represents an increase in the number of applications per crop from 1.4 to
468 2.0 (Scott and Bilsborrow, 2019) and this has increased the pressure on the insects to
469 develop insecticide resistance (Mallet, 1989). The repeated use of a single mode of action
470 can allow the percentage of pest population which exhibit resistance traits to proliferate
471 and become a major problem (Helps *et al.*, 2020). Resistance to pyrethroids has been

472 reported in *P. chrysocephala* , first in Germany in 2014 (Zimmer *et al.*, 2014) and now across
473 continental Europe (Højland *et al.*, 2015). In September 2014 resistance was found in 73%
474 of the adults in UK populations (Foster and Williamson, 2015).

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

480

481 **1.10 Protecting crops from *Psylliodes chrysocephala*:**

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

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

490

491 **1.11 Increased floral diversity:**

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

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

507 Barari *et al* (2005) reported higher numbers of *P. chrysocephala* larvae (and percentage
508 parasitism) in Turnip rape grown in association with OSR

509 Intercropping has often shown reductions in pest damage compared to crop monoculture
510 (Baux and Schumacher, 2019; Brandsæter, Netland and Meadow, 1998; Gombac and Trdan,
511 2014; Hooks and Johnson, 2004; Prasifka *et al.*, 2006) and in turn, biological control of pests
512 has been shown to be influenced by intercropping, through the increase of natural enemies
513 active within intercropping systems (Hooks and Johnson, 2003).

514 One drawback of intercropping is the competition for resources with the crop (Verret *et al.*,
515 2017b)., including for light, water, nutrients, and growing space (Carof *et al.*, 2007; Kloen and
516 Altieri, 1990). In an autumn sown crop competition can be mitigated by use of frost-sensitive
517 companion plants, that will be destroyed over winter (Verret *et al.*, 2017a; Lorin *et al.*, 2015).
518 The use of herbicide resistant OSR cultivars (Clearfield) can also be utilized with susceptible
519 companion species (Cadoux *et al.*, 2015). Clearfield cultivars exhibit resistance to the
520 herbicide used alongside to control within field weeds (BASF, 2018). Thereby mitigating the
521 competition during the reproductive stage and grain filling periods of the crop.

522

523 **1.12 OSR response to *Psylliodes chrysocephala*:**

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

537 shot hole injury (akin to *P. chrysocephala*) with patch defoliation giving full recovery but
538 injury reducing seed grain yield.

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

544

545 **1.13 *Psylliodes chrysocephala* adult feeding preference:**

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

552

553 **1.14 Insecticide application thresholds:**

554 Thresholds to determine when insecticides should be applied are derived from the economic injury
555 levels and are defined as “the lowest population density that will cause economic damage” (Stern,
556 1973). Such economic thresholds are a valuable tool to reduce the cost of application by aiming at
557 keeping them to the lowest possible level needed (Ramsden *et al.*, 2017). They are often defined in
558 terms of the abundance of a pest per unit area, per plant, or per part of plant, above which the

559 economic losses are equal or greater than the cost of control (Ramsden *et al.*, 2017). However, the
560 thresholds are only as efficient as the data they incorporate on the costs of applications, finance and
561 labour, against the efficiency of the application to reduce pest pressure and avoid resistance, usually
562 without detailed data on the risks the application poses to the wider environment and any
563 confirmation of effectiveness.

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

575

576

577 **1.15 Aims of Thesis:**

578

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

586 See Chapters:

587 2.0 Nurse crops can reduce cabbage stem flea beetle (*Psylliodes chrysocephala* L.) damage
588 to oilseed rape (*Brassica napus* L.) without neonicotinoids.

589 3.0 Potential of Brassica trap crops for protecting winter oilseed rape (*Brassica napus* L.)
590 from *Psylliodes chrysocephala* damage.

591 4.0 Comparison of crop protection strategies for *Psylliodes chrysocephala* in winter oilseed
592 rape (*Brassica rapa*): past, present, and future.

593

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

596 See Chapter:

597 5.0 The impact of injury caused by *Psylliodes chrysocephala* L. adults and larvae on flower
598 and yield production in *Brassica rapa* L.

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

603 See Chapters:

604 6.0 Feeding preference of *Psylliodes chrysocephala* L. on early true leaves of Oilseed Rape
605 (*Brassica napus* L.)

606 7.0 Assessing the circadian rhythm of *Psylliodes chrysocephala* L. in UK winter oilseed rape
607 (*Brassica napus* L.).

608

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

612 See Chapter:

613 8.0 Are farmers really concerned about the neonicotinoid ban? Comparison of UK farmers
614 views on crop protection strategies for *Psylliodes chrysocephala* in winter oilseed rape
615 (*Brassica rape*): past, present, and future.

616

617

618

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

625

626 **1.16 References:**

627

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685 began in Europe in 1758, with the description of a few genera and species by the Scandinavian
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687 entomologists took up the study of chrysomelid beetles, together with other groups of beetles, and
688 many new species and genera were described from all parts of the world. This trend has, of course,
689 continued down to the present time. However, researches on the Chrysomelidae did not remain
690 restricted to systematics, and many new lines of study have been followed, especially in the present
691 century, by workers who have benefitted from the advances made in related fields of pure and
692 applied entomology. Much has been achieved in the study of the Chrysomelidae, as elsewhere, and
693 it is the aim of the present book to provide a summary and guide to these achievements. It is also to
694 be expected that this book will provide a stimulus for further studies on the Chrysomelidae, so that
695 we can anticipate continuing progress in our knowledge and understanding of this group through
696 the endeavours of an ever-increasing number of scientists. I offer my congratulations to all
697 concerned in the preparation of this book and my best wishes for its success.

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923 **2.0 Nurse crops can reduce cabbage stem flea beetle (*Psylliodes***
924 ***chrysocephala* L.) damage to oilseed rape (*Brassica napus* L.)**
925 **without neonicotinoids:**

926

927 **2.1 Abstract:**

928

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

942

943 **2.2 Introduction:**

944

945 Winter oilseed rape (OSR: *Brassica napus* L.) is an important break crop in northern
946 European agricultural systems (Redman, 2019) and the most produced vegetable oil crop
947 (Commission, 2020). Many factors influence decisions to grow or not, such as market value
948 fluctuations, crop rotation and pest /disease pressure (AHDB). In recent years pest pressure
949 from the cabbage stem flea beetle (*Psylliodes chrysocephala*, L.) has become a greater
950 concern to UK OSR growers (Coston et al in prep, Chapter 9 this thesis). This is largely
951 thought to be due to the EU ban on the use of neonicotinoid seed treatments (EU, 2018)
952 and the increase of resistance to pyrethroid insecticides, the only other registered
953 alternative chemical control currently approved for use against *P. chrysocephala* in OSR.
954 Target site resistance has been reported from Denmark (Castberg and Kristensen, 2018;
955 Højland and Kristensen, 2018), France (Bothorel *et al.*, 2018), Germany (Brandes and
956 Heimbach, 2018; Heimbach and Brandes, 2016; Zimmer *et al.*, 2014) and the UK (Foster and
957 Williamson, 2015) and a form of metabolic resistance also reported in Denmark (Højland
958 and Kristensen, 2018; Castberg and Kristensen, 2018). Metabolic resistance has been
959 highlighted in the UK with increases in resistance seen (Willis *et al.*, 2020). This is thought to
960 have contributed to a reduction in the area of OSR grown, in the UK in particular (Nicholls,
961 2015; Zhang *et al.*, 2017; Scott and Bilsborrow, 2019; DEFRA, 2019), and has subsequent
962 implications for crop rotations, as OSR is an important break crop from cereals in the arable
963 rotation (Redman, 2019).

964 With growing concern on the environmental impacts of farming (Chagnon *et al.*, 2015;

965 Goulson, 2013), the need for alternative and sustainable crop production methods is

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

979 Intercropping has been extensively trialled with brassicas (Cadoux *et al.*, 2015; Hooks and
980 Johnson, 2003; Lorin *et al.*, 2015; Lorin *et al.*, 2016; Theunissen, 1994; Verret *et al.*, 2017a),
981 often with reductions in pest damage achieved compared to crop monoculture (Prasifka *et*
982 *al.*, 2006; Baux and Schumacher, 2019; Brandsæter, Netland and Meadow, 1998; Gombac
983 and Trdan, 2014; Hooks and Johnson, 2004). For example, reductions in the numbers of
984 cabbage aphid, *Brevicoryne brassicae*, and the green peach aphid, *Myzus persicae*, have
985 been reported in broccoli (*Brassica oleracea* var. *botrytis* L.) intercropped with legumes
986 (Costello, 1994; Costello and Altieri, 1995; Cadoux *et al.*, 2015). For OSR, reductions in
987 damage from the stem weevil, *Ceutorhynchus pycitarsis*, have been recorded for
988 intercropping with: (i) faba bean (*Vicia faba*) + lentil (*Lens culinaris*), (ii) grass pea (*Lathyrus*
989 *sativus*) + fenugreek (*Trigonella foenum-graecum*) + lentil, and (iii) purple vetch (*Vicia*

990 *benghalensis*) + common vetch (*Vicia sativa*) + berseem clover (*Trifolium alexandrinum*),
991 when compared to a monoculture of OSR (Cadoux *et al.*, 2015). Although the levels of
992 reduction were not significant, this study highlights the potential of using intercropping to
993 protect *Brassica* crops from stem boring pests like *P. chrysocephala*.

994 One major drawback of intercropping systems is the potential impact of interspecific
995 competition between the companion crop and the main economic crop (Carof *et al.*, 2007;
996 Verret *et al.*, 2017b). This can be mitigated by using frost-sensitive companion plants, that
997 will be destroyed over winter by frost damage or, if necessary, by the application of a
998 specific herbicide which only kills the companion species so that it is no longer present
999 during the main crop's most important growing period (Cadoux *et al.*, 2015). Such
1000 companion planting of OSR with frost-sensitive legumes, cultivated through the autumn
1001 only and either killed off by frost or from spraying with a specific herbicide, has been shown
1002 to reduce the levels of damage from *C. pycitarsis*, although not to significant levels (Ruck,
1003 Cadoux and Robert, 2018; Cadoux *et al.*, 2015). In a follow up report, (Ruck, Cadoux and
1004 Robert, 2018) showed that the number of *P. chrysocephala* larvae in OSR stems was reduced
1005 as the biomass of legume nurse plants increased. The process of winter die-off or by
1006 herbicide use means that the companion species cannot be considered as an intercrop as
1007 the companion species are only present during the period of high pest pressure and are
1008 then removed and not harvested. The term 'nurse' crop is used, as the hypothesis is to
1009 protect the crop specifically from autumn pest damage therefore acting as a 'nurse' to the
1010 main economic crop.

1011 Another agronomic option to mitigate damage from *P. chrysocephala* is to increase the seed
1012 rate, whereby the extra seed act to dilute the activity of *P. chrysocephala* over a greater

1013 total number of plants (AHDB, 2019a). The planting rate of the OSR crop can have a
1014 significant impact on the crop productivity (Momoh *et al.*, 2004), with recommended seed
1015 application of 30seeds/m² suggested to improve yield by £29/Ha (Roques and Berry, 2016).
1016 Although reductions in yield as seed rate increases have been reported (Roques and Berry,
1017 2016; Shah *et al.*, 2014; Momoh and Zhou, 2001), current advice is to increase the seed rate
1018 to compensate for expected losses due to *P. chrysocephala* (AHDB, 2019b; BASF, 2018),
1019 however, there is no qualitative data or published evidence to support this advice.

1020

1021 **2.2.1 Aims of study:**

1022

1023 I aimed to assess the impact of OSR seed rate and the efficacy of using frost-sensitive nurse
1024 plants as part of a modified companion planting system to mitigate *P. chrysocephala*
1025 damage in a replicated field experiment conducted in collaboration with NIAB, Cambridge,
1026 UK at a field site near Duxford. I hypothesised that:

1027 (i) Levels of damage will be lower on OSR associated with a nurse crop due either to reduced
1028 host plant location, reduced acceptance (Finch and Kienegger, 1997b) or by “trap crop”
1029 effects (Finch and Collier, 2012; Barari *et al.*, 2005).

1030 (ii) Levels of damage will be reduced as OSR seed rate increases due to dilution effect

1031 Three distinct types of nurse crop mixes were tested, based on commercially available cover
1032 crop mixtures and the plant species commonly found in them (see Methods, Table 1). Each
1033 was grown with the OSR, which was itself sown at four seed rates, two ‘standard’ rates (60,
1034 and 80, seeds/m² and two increased rates (100 and 120 seeds/m²). Levels of *P.*

1035 *chrysocephala* injury to the OSR and nurse species were assessed twice in the autumn, along

1036 with a measure of the percentage survivorship of the nurse plants and crop plants. Using
1037 these data, I build on knowledge of nurse cropping systems for OSR and highlight avenues
1038 for further research into cultural control methods for protecting OSR from *P. chrysocephala*.

1039

1040 **2.3 Methods:**

1041

1042 **2.3.1 Treatments and experimental layout:**

1043 Data for this study was collected from an already established NIAB trial at Duxford (Lordship
1044 Farm, Hinxton, Cambridgeshire), which was on light, sandy soil. Field trials were designed
1045 and implemented by NIAB in autumn 2015. The experiment was designed in a randomized
1046 block, with four treatment mixtures (Figure 1). Each treatment was sown in a plot (nurse
1047 type) split into four sub-plots of OSR cv Charger, sown at four seed rates: 60, 80, 100 and
1048 120seeds/m² representing two relatively standard seed rates and two higher rates (Figure
1049 1), i.e., plot level is nurse crop mix and sub-plot the OSR seed application rate. Seeds were
1050 supplied by Kings seeds (<https://www.kingsseeds.com/Home>). Plots were 12 x 8m and
1051 subplots were 12m x 2m and were separated by a tractor wheeling lines along all sides, 1m
1052 (Figure 1).

1053 Plots were drilled on 08th September 2015. The nurse crop mix was applied using the same
1054 drill but with coulters raised to allow seed to land on the soil surface. The plots were then
1055 rolled, to distribute and compact the seed mix; this also helps to conserve moisture. The aim
1056 was to apply the OSR and nurse mixtures using standard farm machinery to simulate a

1057 drilled crop with a broadcast sown nurse mixture. The resulting plots of OSR alone (control)
 1058 and OSR with the nurse crops are shown in Figure 42 and Figure 33.

1059

1060 Table 1. Nurse crop mix composition. Grown in companion with oilseed rape (*Brassica*
 1061 *napus*) at potential pest control measures for the cabbage stem flea beetle (*Psylliodes*
 1062 *chrysocephala*). Details given of species and cultivar where available and the seed
 1063 application rate in seeds/m².

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

1064

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

1065

1066 Figure 1. Schematic of the field experiment used to test the effects of different nurse crop
1067 mixtures and crop seed rate on *Psylliodes chrysocephala* damage to oilseed rape. Positions
1068 of each treatment in the randomized block structure. Colour denotes plot: Blue – Mix A
1069 (Fenugreek: *Trigonella foenum-graecum*), Orange – Mix B (Pak Choi: *Brassica rapa* var.
1070 *chinensis* cv. Joi Choi, Chinese Cabbage: *Brassica rapa* subsp. *Pekinensis* Lour, Salad rocket:
1071 *Eruca sativa*, and Linseed: *Linum usitatissimum* cv. Abacus), Grey – Mix C (Common vetch:
1072 *Vicia sativa*, Crimson clover: *Trifolium incarnatum*, Berseem clover: *Trifolium alexandrinum*,
1073 and Persian clover: *Trifolium resupinatum* cv. lightning) and Yellow – Mix D (OSR: *Brassica*
1074 *napus* cv. charger monoculture) and numbers show the OSR seed rate in sub-plots. Each
1075 sub-plot was 2m x 12m with a tractor wheeling line on all sides.

1076

1077 **2.2.2 Assessment of plant density:**

1078 The establishment success of both the OSR crop and the nurse crop species was assessed
1079 once (26-27th October 2015) using 0.25 m² quadrats. The total number of crop plants and
1080 each of the nurse crop species was recorded from four randomly placed quadrats per plot
1081 (Figure 2). These values were combined to give a measure for plant establishment per 1m².
1082 Due to the difficulty of discriminating between the three species of clover (mix C, Table 1) at
1083 early growth stages they were recorded collectively ('clover').

1084 Values for plant establishment for each species were calculated using the following formula:

1085

1086
$$(species\ total\ plants\ in\ the\ 4\ quadrats / seed\ rate\ per\ m^2\ applied) \times 100.$$

1087

1088 **2.2.3 Assessment of leaf area damage by adult *Psylliodes***

1089 ***chrysocephala*:**

1090 Plant damage resulting from adult *P. chrysocephala* feeding was assessed twice on 26-27th
1091 October (average GS= 12-15) and 24th November 2015 (average GS= 13-19). Within each
1092 plot, 10 plants of OSR and five plants for each nurse crop species (clover scored collectively)
1093 were scored to the nearest 5% of leaf area lost (Figure 8). Injury was measured as a
1094 percentage leaf loss and estimated by eye and calibrated by consensus of field survey team
1095 (Sam. Cook, Martin. Torrence, and Trish. Wells), with 10 plants being assessed as a group to
1096 form a relative measure of injury. Adult feeding of *P. chrysocephala* can be identified as they
1097 produce circular holes in the leaf, which form characteristic 'shot holing' patterns (Figure 8).

1098 However, damage levels were high and could not always be attributed unequivocally to *P.*
1099 *chrysocephala* because slug and pigeon damage are likely contributing factors. The growth
1100 stage of each OSR plant assessed was also recorded according to the BBCH scale (Lancashire
1101 *et al.*, 1991).

1102

1103 **2.2.4 *Psylliodes chrysocephala* larval infestation:**

1104 Comparisons between *P. chrysocephala* larval infestation of OSR in each treatment was
1105 planned. However, poor crop survival meant that this was not possible.

1106

1107 **2.2.5 Crop yield:**

1108 Comparisons of crop yield attributes between treatments were planned but assessments
1109 were not possible due to the total loss of the OSR crop in the experimental area. This led to
1110 the trial being terminated in January 2016.

1111

1112 **2.2.6 Statistical analysis:**

1113 Crop density was analysed using a generalized linear mixed model due to the treatment
1114 layout and assuming a binomial distribution from the proportion of seed survival to plant at
1115 time of assessments. A mixed model was used due to the split-plot design of the
1116 experiment.

1117 Differences between treatments in crop growth stage and leaf area damage at the two
1118 assessment dates were analysed using analysis of variance (ANOVA) as a split plot treatment
1119 design.

1120

1121 **2.3 Results:**

1122

1123 **2.3.1 Plant density:**

1124 Total plant density (companion and OSR plants combined) did increase in the mixtures over
1125 OSR monoculture ($F_{273.35,9}=91.21$; $P < 0.001$) with higher plant density seen in treatment B >
1126 C > A and lowest in D, confirming germinations of the nurse species (Figure 2). The density
1127 of OSR crop plants was not affected by nurse crop mix type ($F_{1.94,9}=0.65$; $p=0.605$, Table 2
1128 and Figure 3). However, there was an effect at sub-plot level with increasing OSR plant
1129 density as OSR seed rate increased ($F_{65.32,35.9}=21.77$; $p < 0.001$, Table 2 and Figure 4). There
1130 was no interaction between mix type and seed rate ($F_{8,9,35.9}=0.99$; $P=0.466$, Table 2).

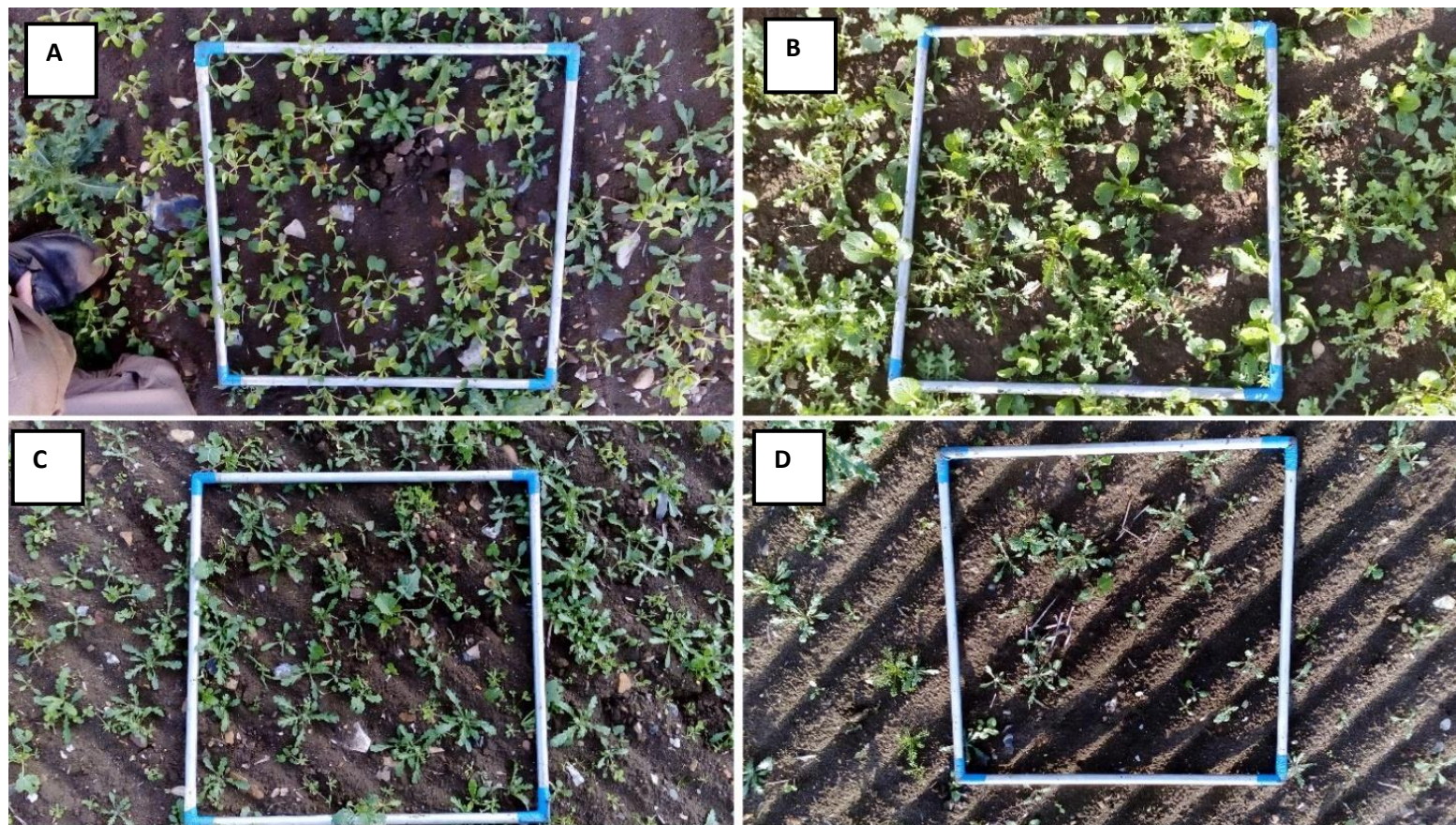
1131

1132 Table 2. Oilseed rape (*Brassica napus*, OSR) mean plant density 48 days after drilling when
 1133 grown with nurse crop mix at four seed rates (60,80,100 and120seeds/m²). Treatment
 1134 codes; A = Fenugreek (*Trigonella foenum-graecum*); B = Brassica mix (*Chinese cabbage*
 1135 (*Brassica rapa subsp. Pekinensis (Lour.) Rupr*), Pak Choi (*Brassica rapa var. chinensis*), Salad
 1136 rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum*); C = Legume mix (Common vetch
 1137 (*Vicia sativa*), Crimson clover (*Trifolium incarnatum*), Berseem clover (*Trifolium*
 1138 *alexandrinum*) and Persian clover (*Trifolium resupinatum*); D = Oilseed rape crop
 1139 monoculture control. Standard error of differences given in brackets, approximate average
 1140 standard error of differences: 1.546 (calculated on variance scale). No statistically significant
 1141 difference observed between nurse mix (rows), increasing OSR seed rate significantly
 1142 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)

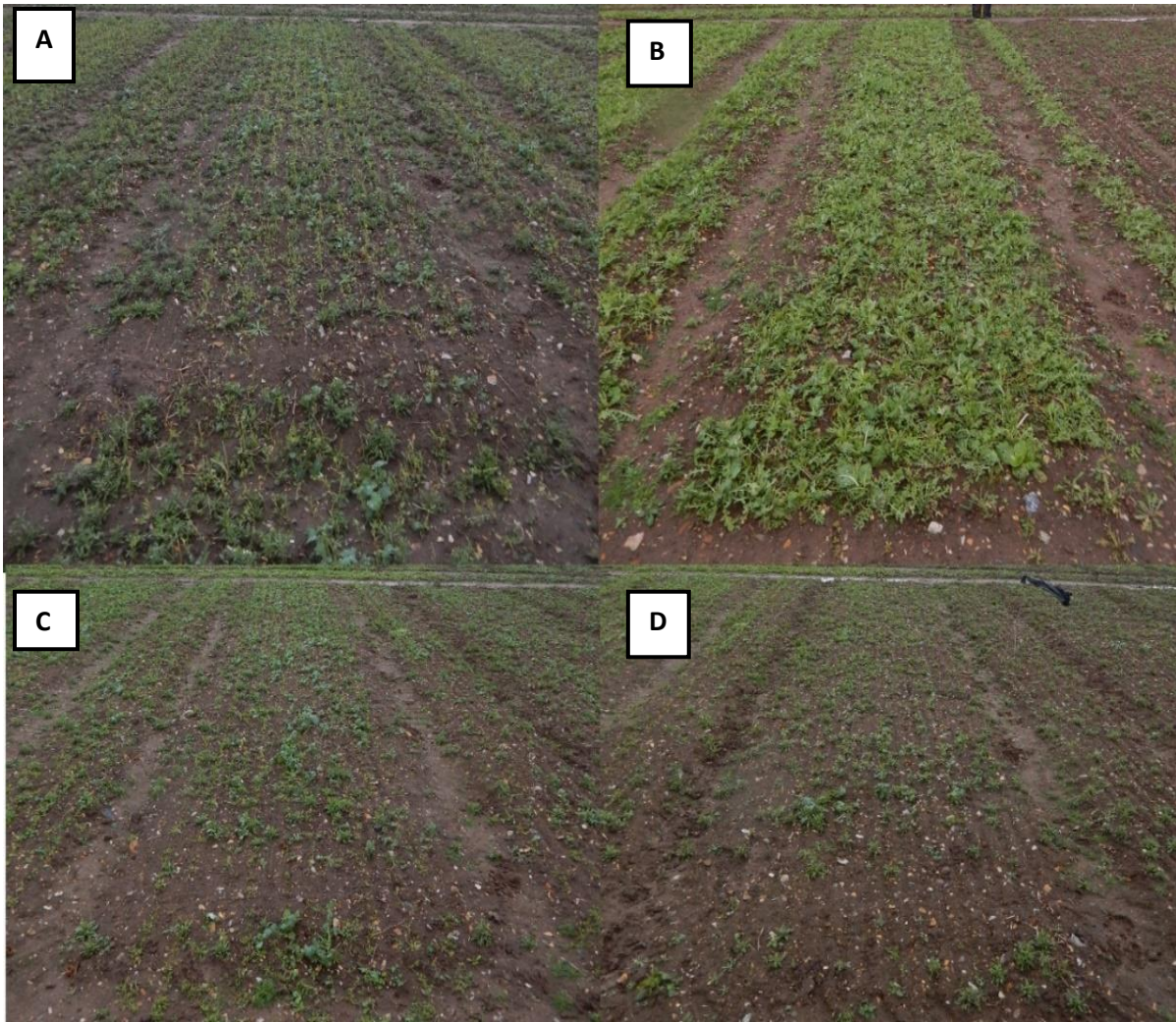
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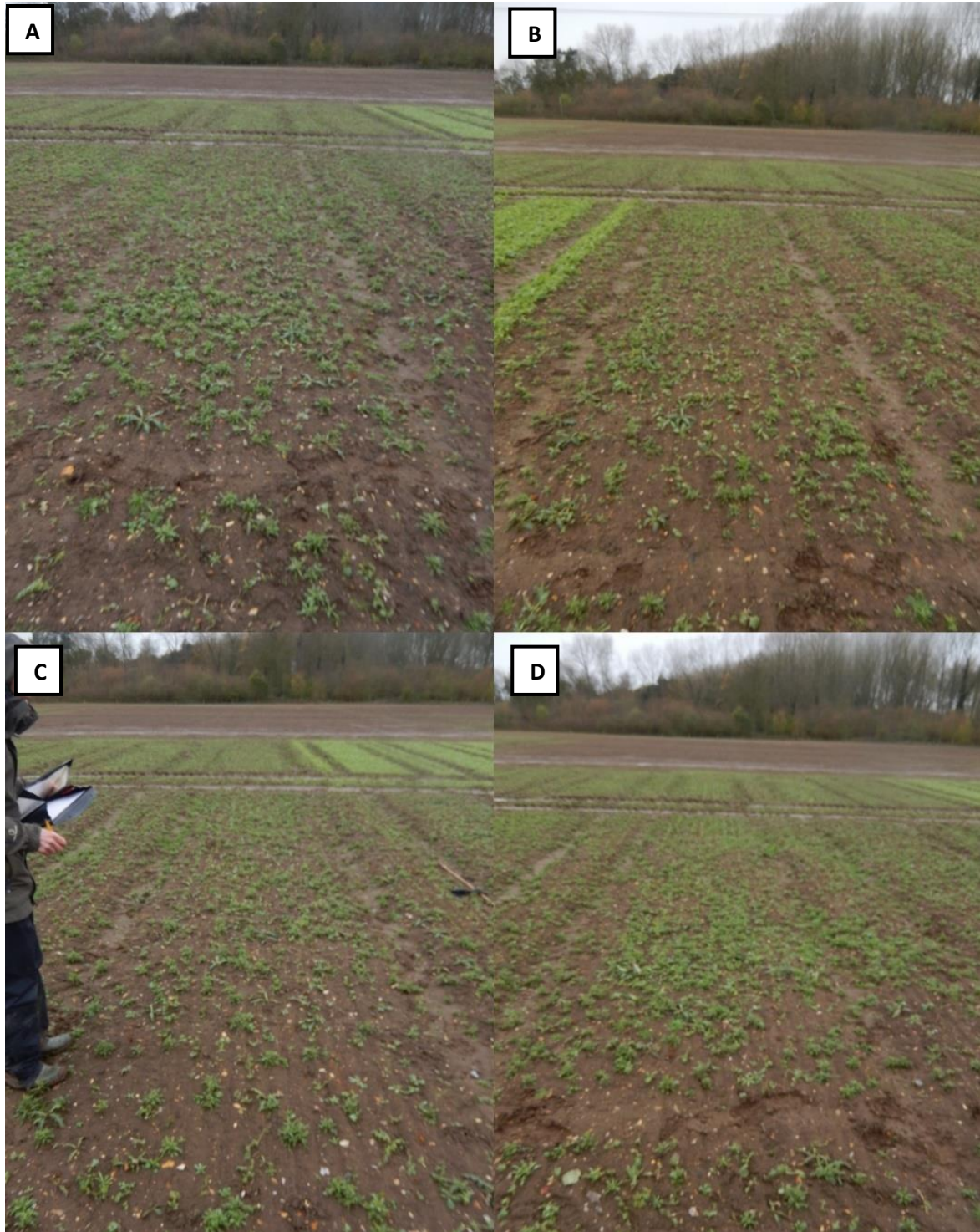
1146 Figure 2. Representation of plant establishment in plots of oilseed rape (*Brassica napus*) with different ‘nurse’ companion crops, showing one
 1147 randomly selected quadrat per treatment (plant density in four quadrats were assessed per plot). A = Fenugreek (*Trigonella foenum-*
 1148 *graecum*); B = Brassica mix (Chinese cabbage (*Brassica rapa subsp. Pekinensis* (Lour.) Rupr), Pak Choi (*Brassica rapa var. chinensis*), Salad rocket
 1149 (*Eruca sativa*) and Linseed (*Linum usitatissimum*); C = Legume mix (Common vetch (*Vicia sativa*), Crimson clover (*Trifolium incarnatum*),
 1150 Berseem clover (*Trifolium alexandrinum*) and Persian clover (*Trifolium resupinatum*); D = oilseed rape crop monoculture control. All images of
 1151 plots with oilseed rape at 100 seeds/ m². Photographs taken 26/10/2015.



1152

1153 Figure 3. Full plot photographs of oilseed rape (*Brassica napus*) with different 'nurse'
 1154 companion crops mixtures: Treatment A = Fenugreek (*Trigonella foenum-graecum*); B =
 1155 Brassica mix (*Pak Choi (Brassica rapa var. chinensis)*, Chinese cabbage (*Brassica rapa subsp.*
 1156 *Pekinensis (Lour.) Rupr.*), Salad rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum*); C =
 1157 Legume mix (*Common vetch (Vicia sativa)*, *Crimson clover (Trifolium incarnatum)*, *Berseem*
 1158 *clover (Trifolium alexandrinum)* and *Persian clover (Trifolium resupinatum)*); D = Oilseed rape
 1159 crop monoculture. All are plots sown at 120 oilseed rape seeds/m². Images taken on
 1160 24/11/2015, 7 weeks post drilling.

1161



1162

1163 Figure 4. Oilseed rape (*Brassica napus*) monoculture plots (Mix D) showing the four seed
1164 rates applied: A. 60, B. 80, C. 100, and D. 120 seed/m². Images taken on 24/11/2015, 7
1165 weeks post drilling.

1166 **2.3.2 Crop growth stage:**

1167 When crop growth was assessed in October all plants were at GS 13 to GS 14; there was no
1168 statistically significant difference between nurse crop mix type ($F_{3,9} 1.06$; $p=0.412$) or OSR
1169 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
1170 more evidence of a treatment effect in the November assessment, with OSR plants in
1171 treatment B (the Brassica species nurse mix) being at less developed growth stage than
1172 those in other treatments, although this was not statistically significant ($F_{3,9} =3.44$, $p=0.065$,
1173 Table 3).

1174

1175

1176 Table 3 Oilseed rape (*Brassica napus*) mean growth stage (BBCH scale (Lancashire *et al.*,
 1177 1991)) for October (26/27/10/2015) and November (24/11/2015) in plots with different OSR
 1178 seed rates and nurse crop mixtures: **A** = Fenugreek (*Trigonella foenum-graecum*); **B** =
 1179 Brassica mix (Chinese cabbage (*Brassica rapa subsp. Pekinensis* (Lour.) Rupr), Pak Choi
 1180 (*Brassica rapa var. chinensis*), Salad rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum*);
 1181 **C** = Legume mix (Common vetch (*Vicia sativa*), Crimson clover (*Trifolium incarnatum*),
 1182 Berseem clover (*Trifolium alexandrinum*) and Persian clover (*Trifolium resupinatum*); **D** =
 1183 Monoculture oilseed rape crop control. No statistically significant difference observed.

OSR seed rate	A - Fenugreek		B - Brassica		C – Legume		D - Monoculture	
	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

1184

1185 **2.3.3 Crop injury:**

1186 A high level of damage was observed on the OSR plants for all nurse crop treatments and all
1187 OSR seed rates (Figure 5). There was a significant effect of nurse crop mix B in which a
1188 reduction of the percent leaf area loss was observed for OSR plants assessed in October ($F_{3,9}$
1189 =11.48, $p=0.002$) and November ($F_{3,9} =13.28$, $p=0.001$). Crop damage with mixtures A and C
1190 was comparable to controls (Figure 6 and Figure 7).

1191 There was also a significant effect of OSR seed rate on damage observed in October ($F_{3,36} =$
1192 3.07, $p= 0.040$) with reduced damage as seed rate increased (Figure 6), however, this was
1193 not apparent in the November assessment ($F_{3,36} = 0.42$, $p= 0.737$; Figure 7). There was no
1194 significant interaction between nurse crop mix type and OSR seed rate for either month
1195 (October= $F_{9,36} = 1.59$, $p=0.155$, November= $F_{9,36} = 0.76$, $p=0.657$).

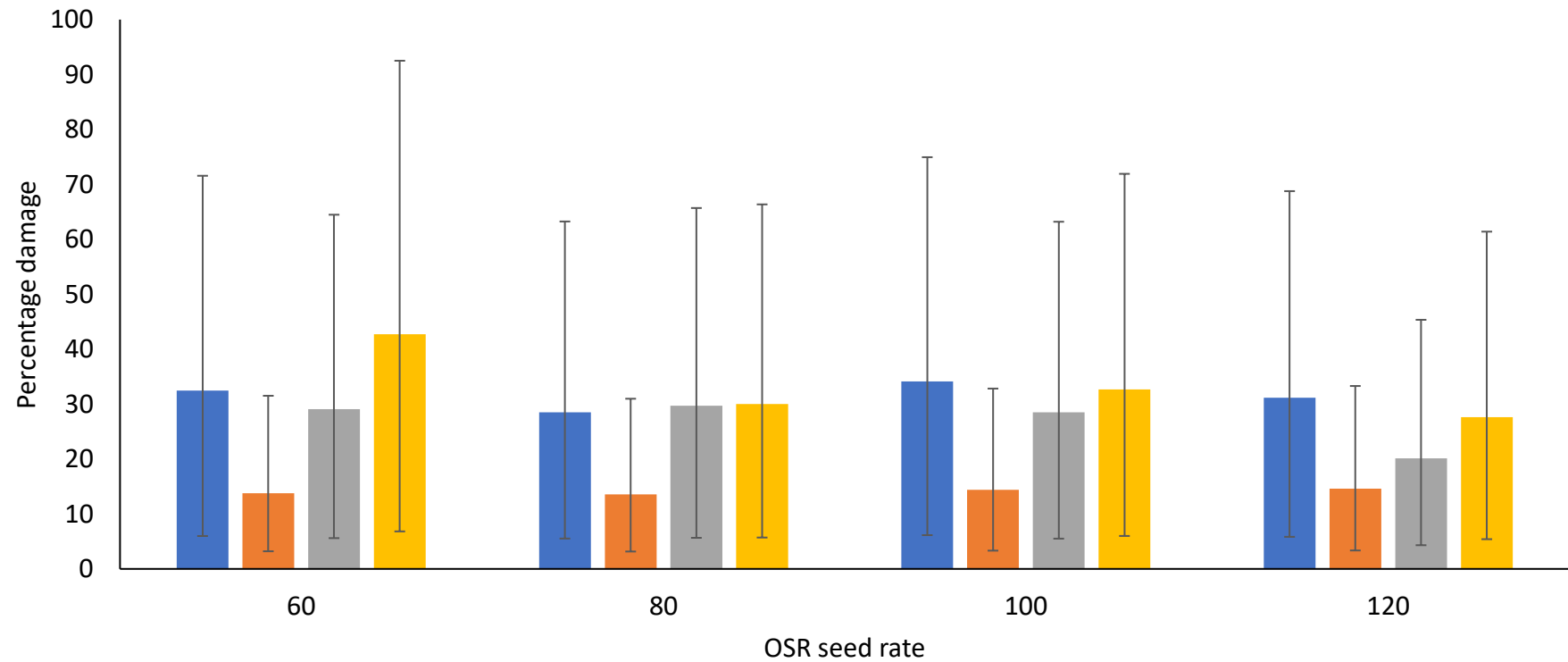


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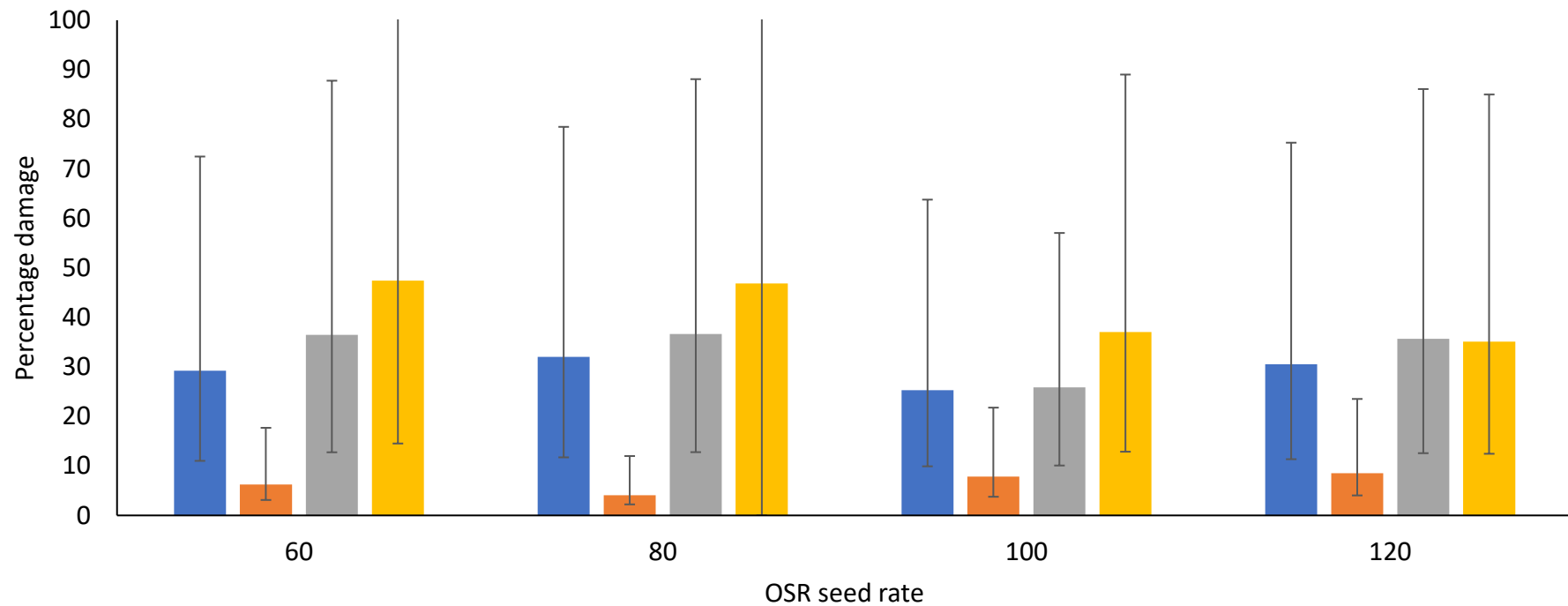
1197

1198 Figure 5 Representative photographs of leaf area injury seen on oilseed rape (*Brassica*
1199 *napus*) showing signs of shot-hole feeding injury, indicative of *Psylliodes chrysocephala* (A)
1200 and high levels of leaf area loss (B). Photographs taken: 24/11/2015.



1201

1202 Figure 6. October leaf area injury recorded on oilseed rape (OSR, *Brassica napus*) as percentage leaf area lost. Grown with four 'nurse crop'
 1203 mixtures and using four OSR seed rates. Treatment; Blue = Fenugreek (*Trigonella foenum-graecum*); Orange = Brassica mix (Chinese cabbage
 1204 (*Brassica rapa subsp. Pekinensis* (Lour.) Rupr), Pak Choi (*Brassica rapa var. chinensis*), Salad rocket (*Eruca sativa*) and Linseed (*Linum*
 1205 *usitatissimum*); Grey = Legume mix (Common vetch (*Vicia sativa*), Crimson clover (*Trifolium incarnatum*), Berseem clover (*Trifolium*
 1206 *alexandrinum*) and Persian clover (*Trifolium resupinatum*); Yellow = Oilseed rape crop monoculture control. Bars show 95% CI.



1207

1208 Figure 7. November leaf area injury recorded on oilseed rape (OSR, *Brassica napus*) as percentage leaf area lost. Grown with four 'nurse crop'
 1209 mixtures and using four OSR seed rates. Treatment; Blue = Fenugreek (*Trigonella foenum-graecum*); Orange = Brassica mix (Chinese cabbage
 1210 (*Brassica rapa* subsp. *Pekinensis* (Lour.) Rupr), Pak Choi (*Brassica rapa* var. *chinensis*), Salad rocket (*Eruca sativa*) and Linseed (*Linum*
 1211 *usitatissimum*); Grey = Legume mix (Common vetch (*Vicia sativa*), Crimson clover (*Trifolium incarnatum*), Berseem clover (*Trifolium*
 1212 *alexandrinum*) and Persian clover (*Trifolium resupinatum*); Yellow = Oilseed rape crop monoculture control. Bars show 95% CI.

1213 **2.3.4 Nurse crop injury:**

1214 Levels of *P. chrysocephala* feeding injury on the non-brassica nurse crop species was either
1215 not observed or was negligible. Injury was observed on just three fenugreek plants (n=160)
1216 and only at 5% area injury. Leaf injury was only observed rarely on clover (22/160 plants)
1217 and Vetch (16/160 plants). The only nurse crop plants to be injured frequently by *P.*
1218 *chrysocephala* were the Pac Choi and Chinese Cabbage in mix B, which had levels of injury
1219 akin to the OSR (Figure 88 and Figure 99). Both the Pac Choi and Chinese Cabbage had lower
1220 injury levels than the OSR crop plants in October ($F_{2,295}=11.95$, $P < 0.001$) but higher levels in
1221 the November sample ($F_{2,296}=52.32$, $P < 0.001$). There was no effect of seed rate in either
1222 month (October: $F_{3,295}=0.14$, $P = 0.931$, November: $F_{3,296}=0.91$, $P = 0.474$). The linseed in this
1223 mix was not observed to be injured at either month. Salad rocket was observed to be
1224 injured by *P. chrysocephala* but only to low levels with only four times the injury covered
1225 more than 15% of leaf area.



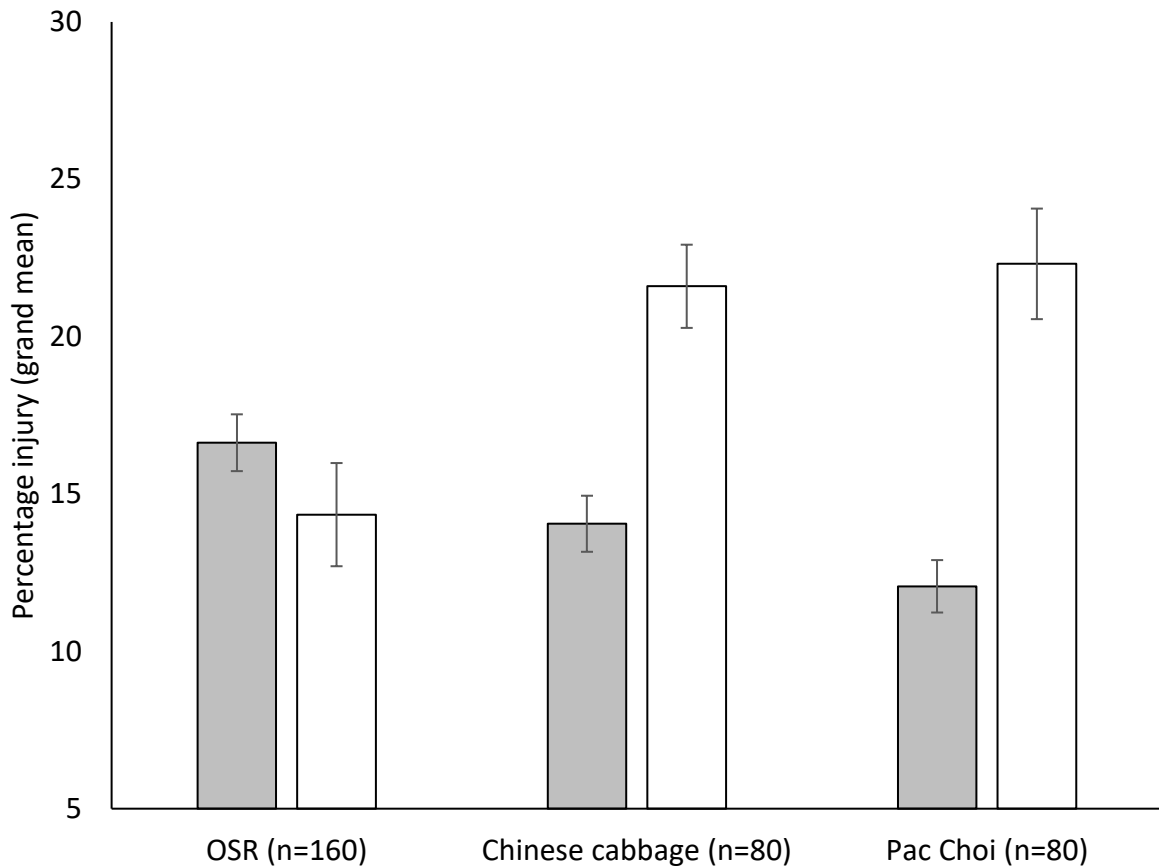
1226



1227

1228 Figure 8. Representative photographs of leaf area injury from *Psylliodes chrysocephala*
1229 present on A, Chinese cabbage (*Brassica rapa subsp. Pekinensis (Lour.) Rupr*) and B, Pac Choi
1230 (*Brassica rapa var. chinensis*). Photographs taken: 24/11/2015.

1231



1232

1233 Figure 9. Percentage leaf area injury observed in on Oilseed rape (OSR, *Brassica napus*),
 1234 Chinese cabbage and Pac Choi from Mix B (Brassica mix: (Chinese cabbage (*Brassica rapa*
 1235 *subsp. Pekinensis* (Lour.) Rupr), Pak Choi (*Brassica rapa* var. *chinensis*), Salad rocket (*Eruca*
 1236 *sativa*) and Linseed (*Linum usitatissimum*) showing injury attributed to *Psylliodes*

1237 *chrysocephala*. Data shown is grand mean of all plants measured in October (**grey**) and
 1238 November (**clear**). Sample number and standard error of difference of mean shown.

1239

1240

1241 **2.4 Discussion:**

1242

1243 In the autumn/ winter of 2015-16 the abundance of *Psylliodes chrysocephala* was very high
1244 resulting in extremely high feeding injury (Figure 6 and Figure 7). This led to the
1245 abandonment of this trial in January 2016 as all the OSR within the field was destroyed.
1246 However, data collected during crop establishment and early crop growth did show that
1247 increasing in-field plant diversity reduced feeding injury from *P. chrysocephala* (Figure 6),
1248 supporting hypothesis (i) – but the composition of the nurse crop mixtures was found to be
1249 important in realising this effect. Increasing seed rate did show reductions in the levels of
1250 injury to OSR plants supporting hypothesis (ii).

1251 Levels of injury to the OSR was only significantly reduced compared to the control in Mix B
1252 (Pack choi, Chinese cabbage, salad rocket and linseed). This effect was persistent and was
1253 observed in both the October and November assessments (Figure 6 and Figure 7). This
1254 shows that there is potential for feeding injury on the crop to be diluted in the early growth
1255 phase when grown in combination with other brassicas. The reduction of injury to OSR was
1256 apparent during the early growth stages, although the level per plant was higher in the OSR
1257 than the brassica companion plants (October assessment, Figure 9). The distribution of
1258 injury was reversed later in the season with the Chinese Cabbage and Pac Chois both
1259 receiving more feeding injury than the OSR suggesting they may be a preferred forage
1260 source at later growth stages and the potential to act as trap crop (Shelton and Nault, 2004).
1261 High levels of feeding observed on the Chinese Cabbage and Pac Choi (Figure 9). Showing
1262 potential of both Chinese Cabbage and Pac Choi to act as a suitable trap crop under the
1263 definitions of Finch and Collier (2012) as a suitable host plant.

1264 No injury was recorded on the linseed and little on the salad rocket suggesting they are not
1265 palatable to *P. chrysocephala*. The reductions on OSR plant leaf injury observed here may
1266 partly be due to dilution of the crop amongst other suitable host plants as was seen when
1267 the OSR seed rate is increased the level of injury per plant reduces. The level of injury per
1268 plant when in combination of a diverse brassica mix is greater than the increase in seed
1269 number along. This apparent extra effect from diverse brassica mix however, may be
1270 conveying secondary benefits from increased green surface area. As the other mixtures did
1271 not show any reduction in the injury on OSR the dilution of feeding is more likely than
1272 inherent benefits from increased green surface area. White *et al.*, (2020) reports of similar
1273 reductions of *P. chrysocephala* injury to the OSR crop when grown with mustard (*Sinapis sp*)
1274 and berseem clover (*Trifolium alexandrinum*).

1275 Fenugreek was tested as a potential nurse crop as it has a very pungent odor (Leela and
1276 Shafeekh, 2008); it was postulated that this could mask host plant location and/or repel or
1277 deter *P. chrysocephala* infestation. There was no evidence for this, with no reductions in
1278 feeding observed in this treatment compared to the control (Figure 6 and Figure 7). The
1279 presence of host masking volatile chemicals is unlikely to influence host location in a
1280 specialist forager (Finch and Collier, 2012). *Psylliodes chrysocephala* is a crucifer specialist
1281 and has thus evolved specific chemo receptors to detect volatile chemicals from host plants
1282 (Bartlet *et al.*, 1999) and receptors for non-host volatiles would be limited (Visser, 1983).

1283 There is no reason for them to have evolved receptors for fenugreek volatiles and it would
1284 be unlikely for the fenugreek to alter the chemical composition or volume of other plant
1285 species (Finch and Collier, 2012; Bruce and Pickett, 2011). This does not preclude secondary
1286 factors from fenugreek influencing *P. chrysocephala* host location, such as increased green
1287 surface area reducing the visual cues for landing when entering a crop. The increase in the

1288 green surface index has been suggested as a mechanism behind the success of some
1289 intercrop mixtures, and not through chemical repellent or masking effects (Finch and Collier,
1290 2012).

1291 Legumes were present in two of the treatments in this study (Mix A and C) and showed no
1292 direct effect on the levels of *P. chrysocephala* feeding; the levels of feeding injury on the
1293 OSR in these treatments was comparable to controls (Figure 6 and Figure 7). Clover has
1294 been shown to reduce injury from *P. chrysocephala* when cover reached 200g/m² (Ruck,
1295 Cadoux and Robert, 2018; Cadoux *et al.*, 2015). The biomass of the nurse mixtures were not
1296 recorded in this study and it is possible that it was insufficient to reduce injury as seen by
1297 (Finch and Kienegger, 1997a). Who showed reductions in multiple insect pests in OSR when
1298 clover covered >50 of the “vertical profile of the crop plant”. Postulating the lack of contrast
1299 between plants and soil affecting the insects host location ability.

1300 Recommendations from AHDB to combat *P. chrysocephala* pressure include increasing seed
1301 rate in areas prone to high *P. chrysocephala* pressure (AHDB, 2019a). I found evidence to
1302 support this strategy; reductions in the level of *P. chrysocephala* feeding per plant was
1303 observed as seed rate increased in October (Figure 6) but the effect was transient; by the
1304 time of the November assessment the levels of damage were not influenced by plant
1305 density (Figure 7). The reductions in adult feeding observed in October is likely to be due to
1306 a dilution effect i.e., with increased host plant abundance the amount of *P. chrysocephala*
1307 feeding per plant will be diluted. Or the higher green cover at higher seed rate disrupting
1308 the host location by reducing contrast with soil (Finch and Collier, 2012). The lack of any
1309 effect by the November assessment may be due to a leveling off between seed rates of
1310 green surface cover as plants at lower density develop and cover more area. The reduced

1311 difference may also be due to high abundance of *P. chrysocephala* at the site increasing the
1312 levels of feeding observed. In a recent report by White *et al* (2020) the impact of seed rate
1313 was shown to be variable but did suggest a trend towards reductions in *P. chrysocephala*
1314 injury at higher plant density.

1315 Increases in green cover have been shown to reduce pest landing by disrupting landing
1316 stimuli (Crawley, 1983). If this were the case for *P. chrysocephala* here, then reductions in
1317 feeding would be expected with increased green cover. Green cover was increased in the
1318 mixture plots over monoculture (Figure 3) and as OSR seed rate increased (Figure 4), but no
1319 reduction of injury was seen in any other than the Brassica mix (Figure 6 and Figure 7). This
1320 suggests that host location was not influenced by plant density alone in terms of green
1321 surface cover but by brassica density.

1322 Cultivation of monoculture crops has become the norm for almost all arable crops grown
1323 worldwide, however, there is increasing evidence to support the hypothesis that increased
1324 botanical diversity within the crop is not only financially viable for the farmer but is also of
1325 great importance to improving within field biodiversity (Ratnadass *et al.*, 2012). In a survey
1326 of farmer opinions to increased floral diversity in Switzerland, the uptake of methods such
1327 as intercropping was higher where knowledge of their benefits was present (Baux and
1328 Schumacher, 2019). In a similar survey of UK OSR growers, the use of nurse cropping was
1329 considered to have potential, but growers lack proof of effectiveness (Coston *et al* 2021,
1330 Chapter 8 this thesis). Here the potential of nurse cropping in OSR with Brassicas to reduce
1331 early growth injury and promote crop establishment shows promise but is in need of further
1332 investigation to understand the mechanisms of success.

1333

1334 **2.5 Acknowledgments:**

1335

1336 I'm grateful to Simon Kightley, NIAB for the opportunity to collaborate on this experiment
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1338 from the field site. Thanks to Martin, Sam Cook and Trish Wells for assistance with data
1339 collection; special thanks to Trish for keeping up morale. Suzanne Clark helped with data
1340 analysis. Finally, thanks to funding for my PhD under the LAWES agricultural scholarship.

1341

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1481 **3.0 Potential of Brassica trap crops for protecting winter oilseed**
1482 **rape (*Brassica napus* L.) from *Psylliodes chrysocephala* feeding:**

1483

1484 **3.1 Abstract:**

1485

1486 In recent years pressure on oilseed rape (OSR, *Brassica napus* L.) production has increased
1487 as the result of a key pest, the cabbage stem flea beetle (*Psylliodes chrysocephala* L.)
1488 developing resistance to pyrethroid insecticides, and the current EU ban on the use of
1489 neonicotinoid outdoor use. There is therefore an urgent need for further research into
1490 alternative pest protection methods. This study assesses the potential of two Brassica
1491 species as trap crops, and their efficacy as a pest protection method for reducing the
1492 pressure from *P. chrysocephala* on OSR. The relative effect of neonicotinoid seed dressing
1493 was also tested. In the year of the experiment *P. chrysocephala* pressure was so high it
1494 completely eradicated the trial, however, both trap crops tested survived (with and without
1495 neonicotinoid seed treatment) and data presented here suggests that both show potential
1496 as a trap crop for *P. chrysocephala*. The neonicotinoids seed treatment did not improve the
1497 survival of OSR. Implications for autumn pest control are discussed.

1498 **3.2 Introduction:**

1499

1500 Since 2014 the use of neonicotinoid seed dressings has been restricted in OSR with a
1501 complete ban of outside use imposed in 2018 (EU, 2018). Pest management of *P.*
1502 *chrysocephala* is becoming particularly problematic for OSR growers, (EU 2018) and that
1503 which efficacy is under questioning with increasing resistance in field populations of the beetles
1504 (i.e., the pyrethroids Brandes and Heimbach 2018, Foster and Williamson 2015, Castberg
1505 and Kristensen 2018). The reduction in control options has led to a renewed research effort
1506 into alternative non-chemically based methods of pest control.

1507 One such method, which shows potential, is 'trap cropping', a system whereby a plant
1508 species which is more attractive to the pest than the crop, is grown alongside the crop in
1509 order to act as a trap for pests, reducing direct feeding and subsequent larval infestation in
1510 the main crop (Barari *et al.*, 2005; Hokkanen, 1991; Shelton and Badenes-Pérez, 2006;
1511 White, Ellis and Kendall, 2018). Trap crops may also influence the levels of pest species by
1512 providing valuable resources to natural enemies such as nectar, alternative prey, shelter and
1513 non-crop habitat (Skellern and Cook, 2018).

1514 Several studies have investigated the effectiveness of trap crops in high value vegetable
1515 brassicas. For example, it has been suggested that trap cropping could be employed to
1516 protect cabbage from the cabbage flea beetle (*Phyllotreta* spp.) and therefore reduce the
1517 need for insecticide application (Bohinc and Trdan, 2012). Several studies have shown the
1518 potential of trap crops in managing diamondback moth (*Plutella xylostella*) in cabbage and
1519 cauliflower (George, Collier and Port, 2009; Shelton and Nault, 2004).

1520 Research in oilseed rape (OSR, *Brassica napus*) has shown that trap crops can act as a pest
1521 control measure for a wide range of pests as outlined in Table 1.

1522

1523 Table 1 Pest insect species which show potential for control via trap cropping in brassica
1524 systems.

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

1525

1526 Barari *et al.* (2005) examined the use of turnip rape (*Brassica rapa*) as a trap crop for *P.*
1527 *chrysocephala* in winter OSR showing reduced larval infestations in OSR plots grown in
1528 association with the trap crop compared with control plots and this was independent of
1529 whether or not insecticide was applied to the trap crop. Sivčev *et al.* (2017) also showed
1530 that the beetles were attracted to turnip rape, and more beetles emerged from soil growing
1531 turnip rape than OSR.

1532 Winter turnip rape cv Jupiter (*Brassica rapa* var. *olifera*) has shown promise as a trap crop in
1533 winter oilseed systems against pollen beetle (Skellern and Cook, 2018). Similarly, Tyfon, a
1534 hybrid between stubble turnip (*Brassica rapa* subsp. *rapifera*) and chinese cabbage (*Brassica*

1535 *raps* subsp. *Pekinensis* (Lour.), which is used as fodder for sheep (Gottstein, 2008) has also
1536 been shown to be a possible trap crop for pollen beetle winter OSR systems (Cook *et al.*,
1537 2013). The efficacy of these species as a trap crop for *P. chrysocephala* is unknown.

1538 In this study, the potential of trap cropping with turnip rape or Tyfon to reduce *P.*
1539 *chrysocephala* adult feeding damage and larval infestation in winter OSR are tested. The
1540 interactions between the trap crop (a nature-based cultural control) and a synthetic
1541 chemical-based (neonicotinoid seed treatment: Cruiser, active ingredient thiamethoxam)
1542 crop protection strategy were tested. The aim was to test whether trap cropping is a
1543 realistic alternative to neonicotinoid seed dressings, as an example of how cultural control
1544 methods might perform compared to synthetic agrochemicals. As *P. chrysocephala* are
1545 known to feed on a wide range of brassica species (Alford, Nilsson and Ulber, 2003;
1546 Williams, 2010). It is expected that they will actively feed on both trap types and reduce the
1547 amount of damage to the OSR crop. If there is an effect from using trap crop boarders, it
1548 would be expected to reduce the level of injury on the OSR in association with a trap crop. If
1549 injury is seen to be even across treatments, there will be a lack of evidence of the
1550 effectiveness of trap cropping for *P. chrysocephala*.

1551

1552

1553 **3.2.1 Aims of study:**

1554

1555 This study was designed to test the effectiveness of two trap crop species in reducing pest
1556 pressure from *P. chrysocephala* in OSR crops. The impact of treating the trap crop with a
1557 neonicotinoid seed dressing was also tested. The null hypothesis is that the OSR within plots
1558 will be unaffected by trap crop presence of seed treatment and be comparable to control
1559 plots. I hypothesized that turnip rape trap crop borders will reduce pest pressure in the
1560 OSR plots as shown by Barari et al (2005) and a similar effect will be seen for Tyfon. I
1561 hypothesized that the neonicotinoid treatment will kill the beetles feeding on the trap crop,
1562 reducing the level of injury to the trap and the oilseed rape plot associated with it, inferring
1563 increased crop protection from *P. chrysocephala* over un-treated trap crop treatments
1564 through the toxic effect of the neonicotinoid.

1565

1566

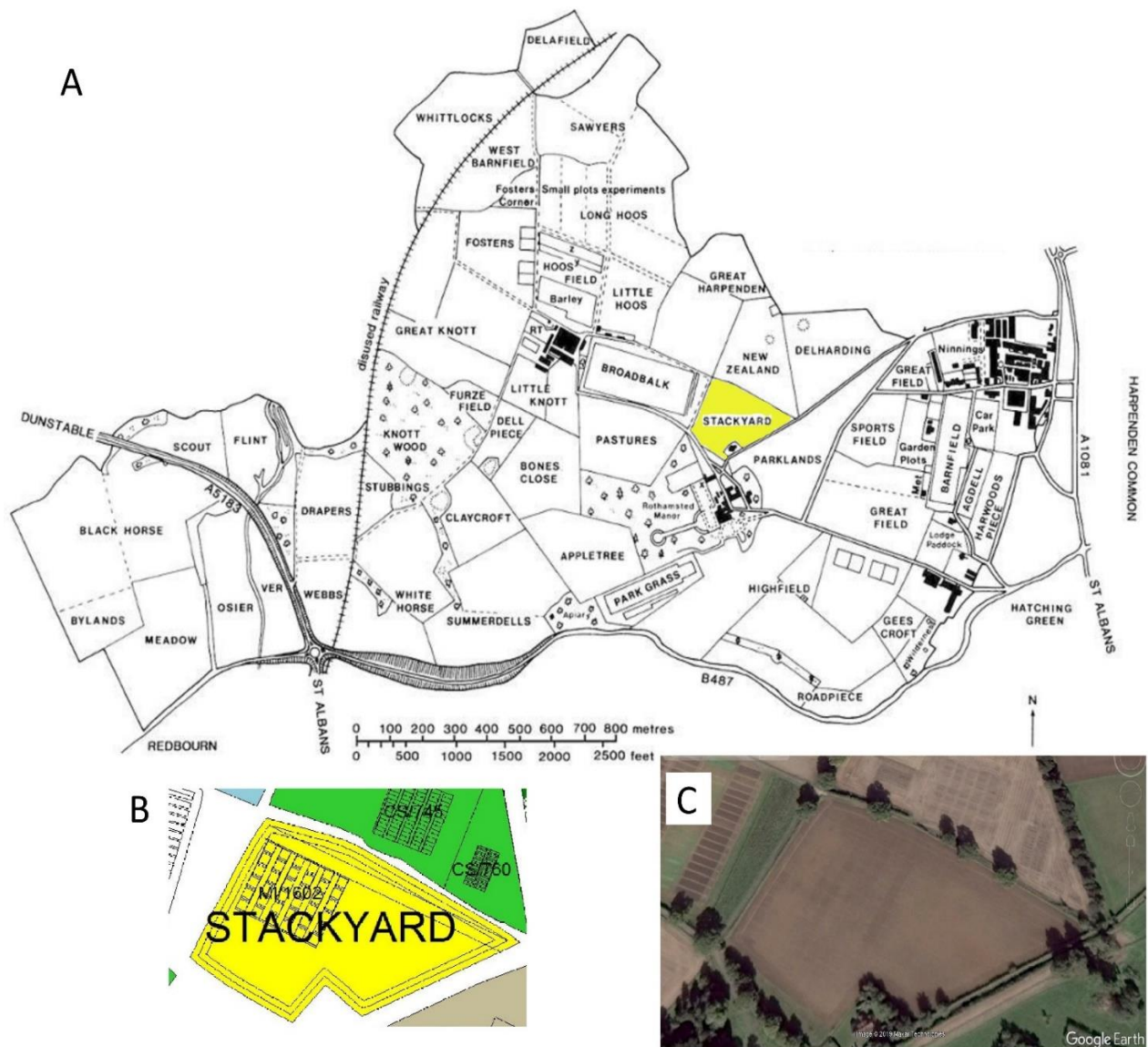
1567 **3.3 Methods:**

1568

1569 **3.3.1 Treatments layout and drilling procedure:**

1570 The experiment was established on 17th September 2015 at Rothamsted Research (Figure 1)
1571 and comprised six treatments (Figure 22 and Table therein), laid out in plots measuring 9m
1572 x9m. Each treatment was replicated six times in a quasi-complete Latin square design, with
1573 each treatment occurring once in each row and column and every treatment is a horizontal
1574 and a vertical neighbour to every other treatment twice, to exclude directional effects and
1575 to balance, as far as possible, any effects of neighboring treatments on each other. The
1576 treatments comprised: OSR (cv. DK Exalte, *Brassica napus*) main crop with or without a 1m-
1577 wide trap crop border of either turnip rape (cv. Jupiter, a restored hybrid variety, *Brassica*
1578 *rapa*) or Tyfon (a hybrid between stubble rape, *Brassica rape oleifera* and Chinese cabbage,
1579 *Brassica rape* subsp. *pekinensis*). Each trap crop was sown either using neonicotinoid-
1580 treated seed (Cruiser, at 15ml/kg, active ingredient: Thiamethoxam) or untreated seed.
1581 Oilseed rape without a trap crop acted as a control and OSR sown using neonicotinoid-
1582 treated seed was used as a treated control. Plots and trap borders were drilled using a
1583 Haldrup SB-25 plot drill (Halsrup, <https://www.haldrup.net/en/seeders/sb25.html>). OSR was
1584 sown at 50seeds/m² and the trap crop borders were sown at 150 seeds/m², which is the
1585 recommended sowing rates based on their seed weights (LG 2016). All other agronomy
1586 was per standard farm practice.

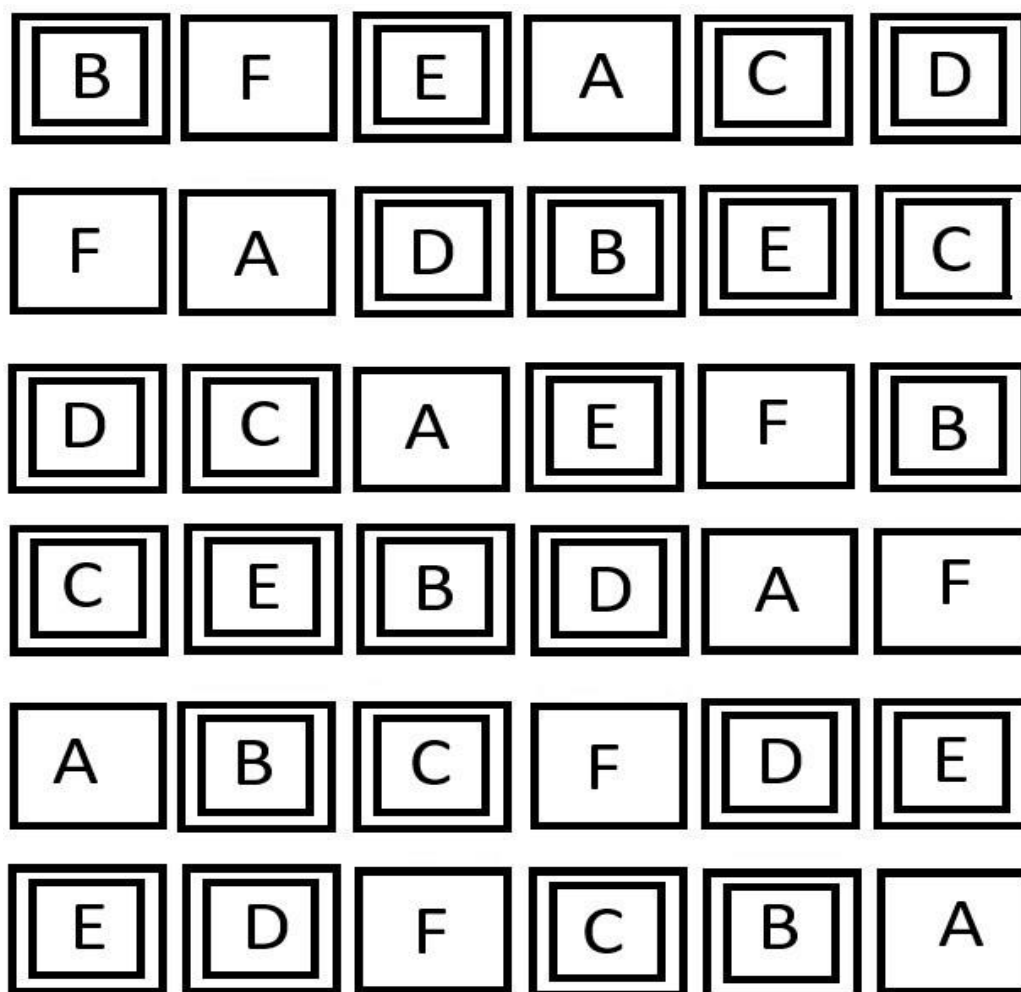
1587



1588

1589 Figure 1. A. Rothamsted Farm map showing the location of the experimental field
 1590 (Stackyard); B. the location of the experiment within the field; and C. Google Earth image
 1591 (19th September 2015) of the field.

1592



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

1593

1594 Figure 2. Treatment structure of the field experiment testing two different types of Brassica

1595 trap crop (turnip rape and Tyfon) borders on crop protection of an oilseed rape (OSR,

1596 *Brassica napus*) main crop from damage by the pest *Psylliodes chrysocephala*, and the effect

1597 of treating the trap crop with neonicotinoid seed treatment (Cruiser, at 15ml/kg).

1598 **3.3.2 Plant density:**

1599 Plant density for both the OSR and the trap crops was used as a measure of the success of
1600 establishment and was recorded on 3rd to 5th November 2015, using 0.25m quadrats. As
1601 plant density of the whole experiment could not be assessed in one day, assessment was
1602 carried out in two experimental blocks (12 plots) per day to avoid temporal bias in sampling.
1603 Four quadrats were randomly placed in the central 7m² area of all plots (OSR) and one
1604 quadrat was surveyed on each of the trap crop borders where present (see Figure1 and
1605 Figure 2). No assessments were done on the borders of the plots without a trap crop as
1606 plant density of OSR in the borders was assumed to be the same as the rest of the plot.

1607

1608 **3.3.3 Leaf area injury by adult *Psylliodes chrysocephala*:**

1609 To measure the levels of feeding injury by adult *P. chrysocephala* to the OSR and the trap
1610 crop, plants were visually inspected for characteristic shot hole damage (Figure). Each plant
1611 was scored to the nearest 5% of leaf area removed, by estimated visual assessments. This
1612 rapid assessment allowed for data to be collected by one individual and ensured consistency
1613 between measurements. Within the central 7m² of each plot, a total of 12 OSR plants were
1614 examined. Where a trap crop was present, three trap crop plants from each border side
1615 were examined for damage (totaling 12 plants per plot). Two rounds of assessments were
1616 carried out one on all plots on the 28th of October and the second split between one block
1617 on the 12th and the other two between the 16-17th November 2015, again assessing 1 or
1618 two blocks of the experiment each time to ensure consistency between treatments. The
1619 growth stage of the plants assessed for injury was also recorded according to the BBCH scale

1620 (Lancashire et al. 1991); at this stage of crop development this involved recording the
1621 number of true leaves present.

1622

1623 **3.3.4 *Psylliodes chrysocephala* larval infestation:**

1624 *Psylliodes chrysocephala* larval infestation of crop and trap crop plants was assessed using
1625 destructive sampling of whole plants. Where present, five OSR plants were collected from
1626 the central 7m² area of each plot. For treatments with a trap crop border (Treatments B, C,
1627 D and E) one plant was taken from each of the four borders. Plants were sampled carefully
1628 to ensure good representation from the whole area of the plot. This mitigated possible pest
1629 congregation as *P. chrysocephala* within a field has been shown to be patchy (Ferguson *et*
1630 *al.*, 2002). Plants were removed gently from the ground by pulling the stem and were placed
1631 in labelled plastic bags and transferred to cold storage (5°C) prior to processing. The number
1632 of larvae per plant was assessed by dissecting the stems and petioles using a scalpel under a
1633 light microscope and recording the number of larvae and the larval instar. *Psylliodes*
1634 *chrysocephala* larvae develop within Brassica stems passing through three distinct larval
1635 instars as described by Ebbe-Nyman (1952). Plants were collected for sampling on 10th
1636 February 2016 and dissections were completed by 2nd March 2016. Dissections were carried
1637 out to ensure that on each day equal number of plants were dissected from treatment plots
1638 within a block to limit any temporal effects of plant storage.

1639

1640 **3.3.5 Statistical analysis:**

1641 Plant density data were transformed to \log_{10} plant number recorded per quadrat to account
1642 for zero counts. An analysis of variance (ANOVA) with blocking to account for the rows and
1643 columns of the Latin square design was performed to analyze differences in plant density
1644 within the central areas of each plot. A mixed model (REML) was used to analyze
1645 differences in plant density in the trap crop borders due to unbalanced distribution of trap
1646 crop plots.

1647 Growth stages were analyzed separately for each of the two sample rounds (1st round: 28th
1648 of October and 2nd round: the 12th, 16-17th of November). A mixed model (REML) analysis
1649 was used to incorporate the treatment structure and allow for missing values as some
1650 plants were damaged too much to accurately determine growth stage.

1651 Leaf area damaged was logit transformed and adjusted to allow for 0% and 100% values in
1652 the data set. An analysis of variance (ANOVA) was performed on the data from plot centers.
1653 A mixed model was used to analyze differences between trap crop type in order to account
1654 for blocking structure.

1655 When plants were collected for assessment of the numbers of larvae present in February
1656 2016, a third of the plots (12/36) had no OSR plants and no treatment had the target
1657 number of 30 plants necessary for a robust analysis. A mixed model (REML) was used due to
1658 the data being unbalanced. Total number of larvae per plant were \log_{10} transformed with an
1659 adjustment for zero observations ($n+1$).

1660 All statistical analyses were performed using GenStat V18, for windows (VSN International,
1661 Hemel Hempstead).

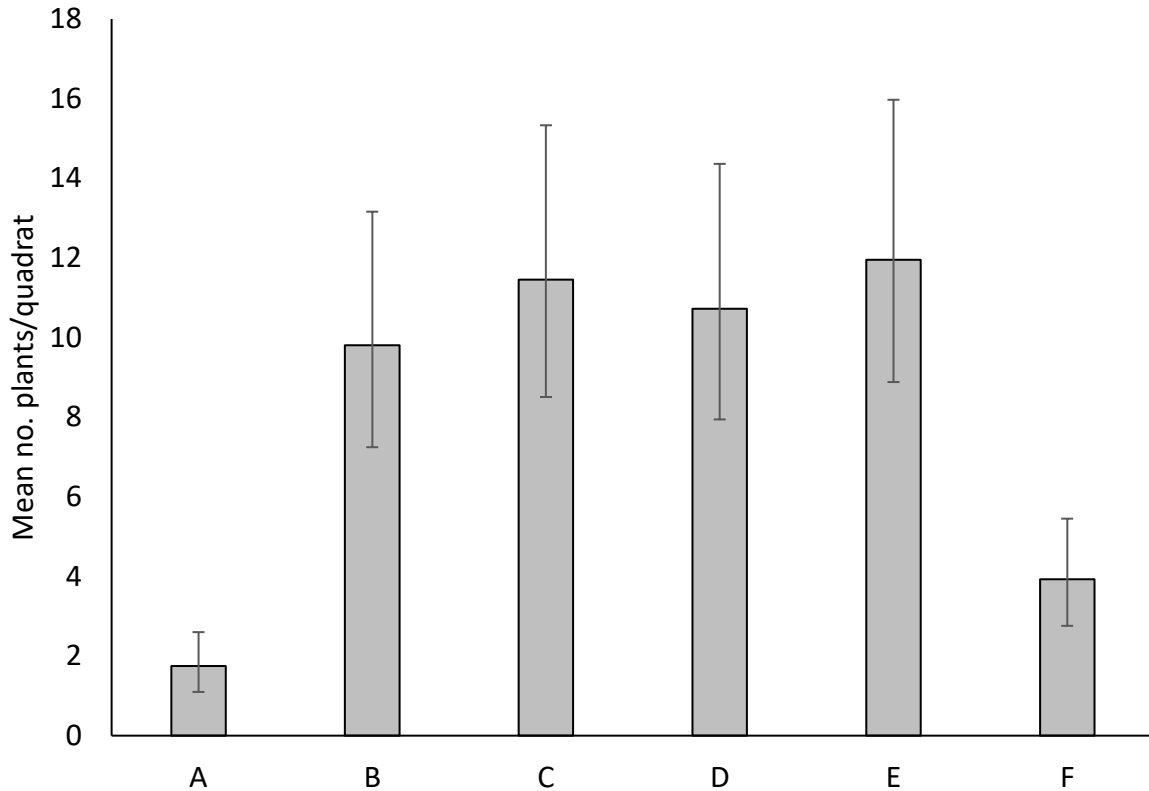
1662 **3.4 Results:**

1663

1664 **3.4.1 Plant density:**

1665 The trap crop borders successfully established (Figure 3 and Figure) with no difference in
1666 plant density between the turnip rape and Tyfon trap crop types ($F_{0.01,10.6}=0.01$, $P=0.943$)
1667 nor was there an effect of the neonicotinoid seed treatment ($F_{1.03,10.6}=1.03$, $P=0.332$,
1668 Figure).

1669 The presence of a trap crop increased the OSR density ($F_{1,20}=10.10$, $P=0.005$). The mean
1670 plant density of the OSR without a trap crop boarder were below the 20-25 plants/m²
1671 (Roques and Berry 2016), with or without neonicotinoid seed treatment, where a trap crop
1672 was present OSR density was above the 20-25 plants/m² (Figure 3 and Figure 4). There was
1673 no significant effect of the trap crop type ($F_{1,20}=0.21$, $P=0.648$) or from the use of the
1674 neonicotinoid seed treatments to the trap crop seed ($F_{1,20}=0.87$, $P=0.362$) on OSR density.



1675

1676 Figure 3. Grand mean number of oilseed rape (OSR) plants per quadrat for each treatment.

1677 A = Untreated OSR; B = Untreated OSR with a turnip rape trap crop border; C = OSR with a

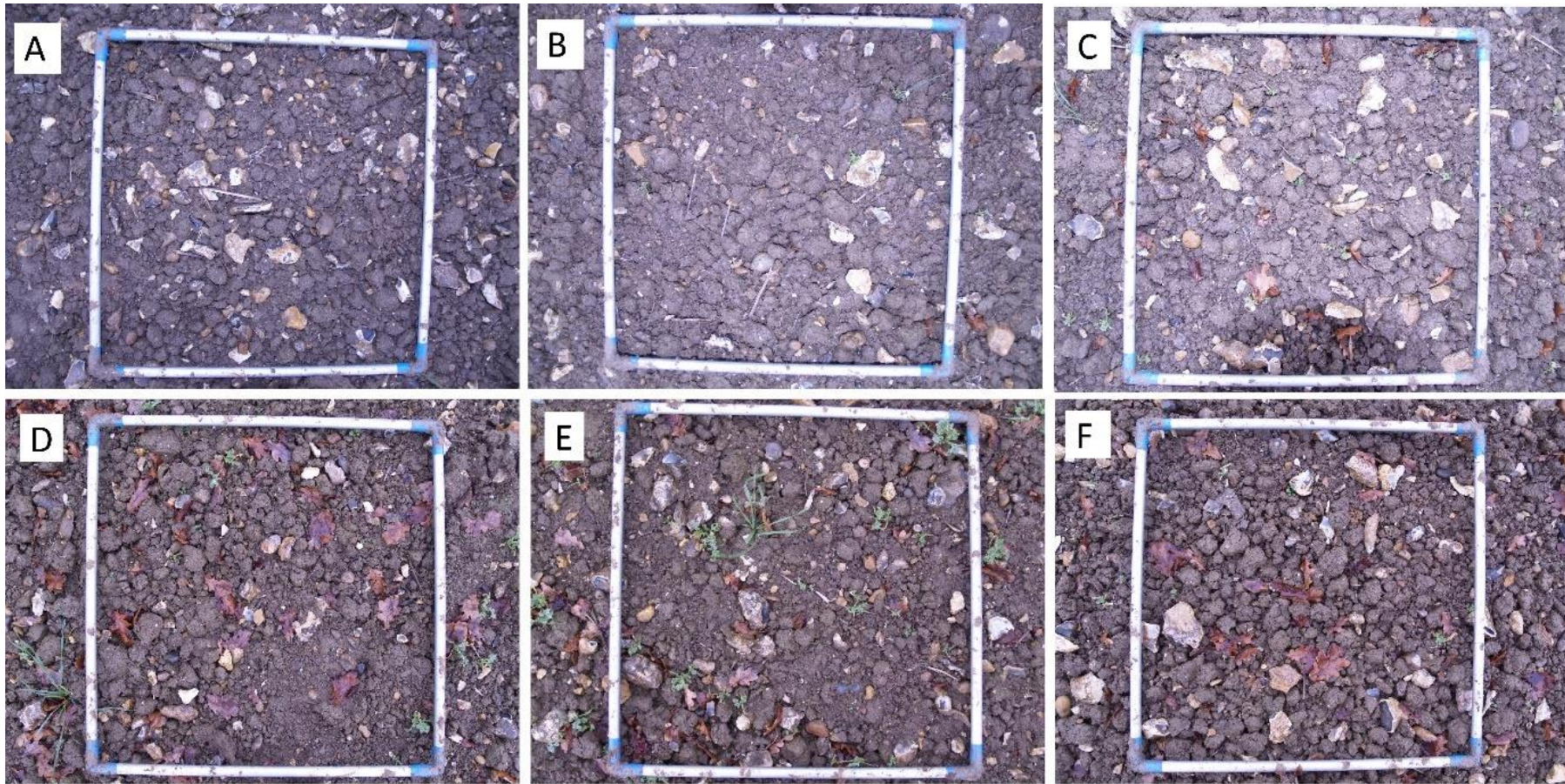
1678 turnip rape trap crop border with neonicotinoid-treated seed; D = Untreated OSR with a

1679 Tyfon trap crop border; E = OSR with a Tyfon trap crop border using neonicotinoid-treated

1680 seed; F = OSR using neonicotinoid-treated seed. Seed treatment Cruiser, at 15ml/kg.

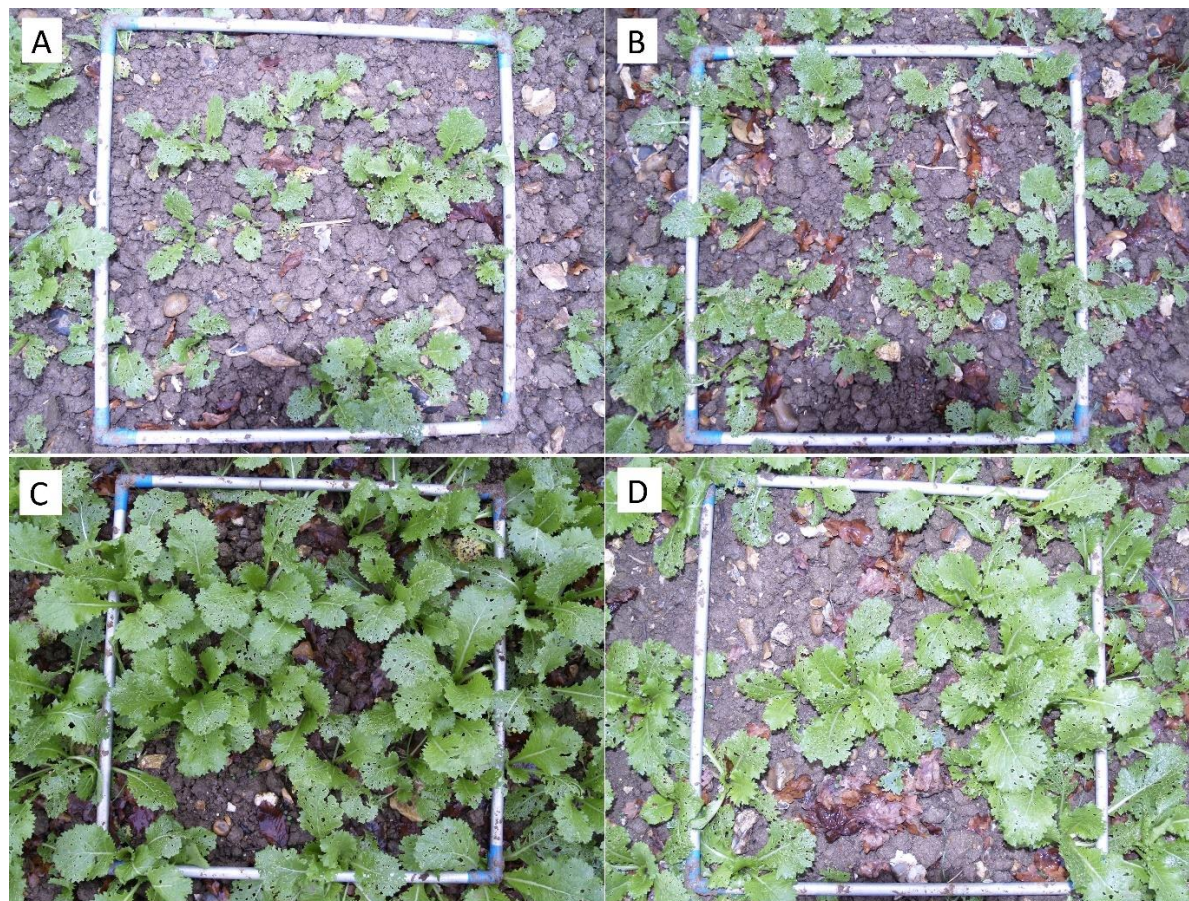
1681 Standard error of means shown.

1682



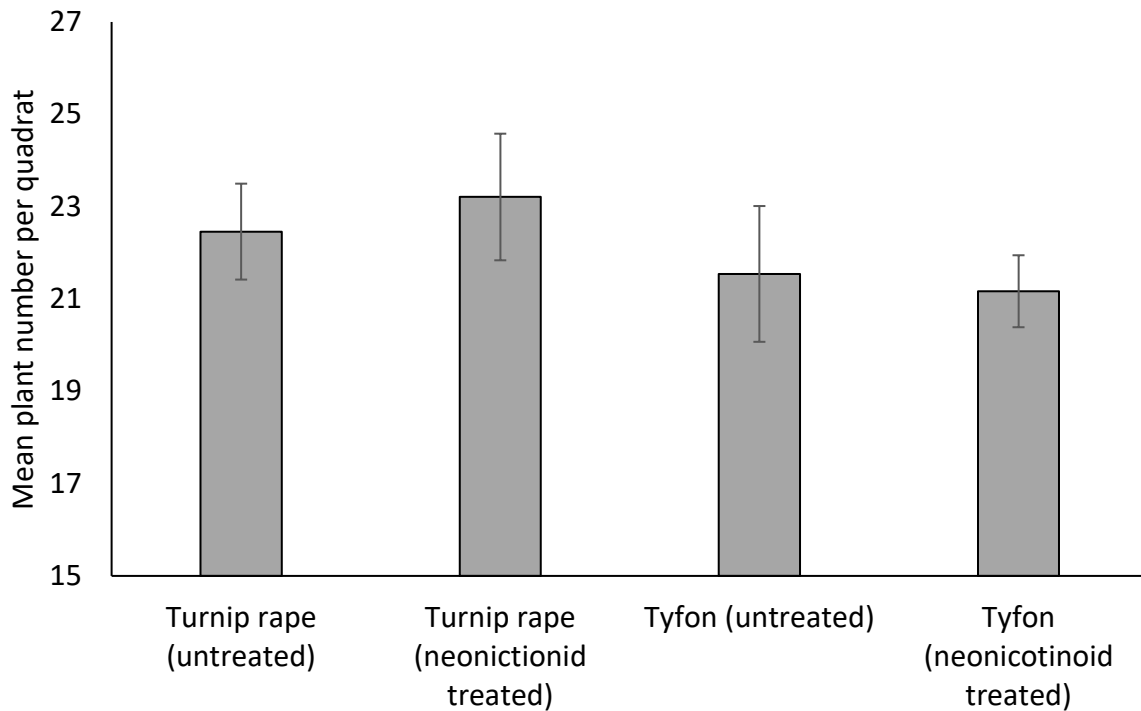
1683

1684 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
1685 with neonicotinoid seed-treated turnip rape border; (D) OSR with Tyfon border; (E) OSR with neonicotinoid-treated Tyfon border; and (F) OSR
1686 with neonicotinoid seed treatment. Seed treatment Cruiser, at 15ml/kg. Photos taken 3/11/2015, 47 days after drilling.



1687

1688 Figure 5. Representative photograph of quadrat samples within each trap crop type. (A) Turnip rape (*Brassica rapa*) sown using untreated
1689 seed; (B) Turnip rape sown with neonicotinoid treated seed; (C) Tyfon sown with untreated seed; (D) Tyfon sown with neonicotinoid-treated
1690 seed. Seed treatment Cruiser, at 15ml/kg. Photos taken 3/11/2015, 47 days after drilling.



1691

1692 Figure 6. Mean \pm SE plant density of turnip rape and Tyfon trap crop borders sown using
 1693 untreated or neonicotinoid-treated seed. Seed treatment Cruiser, at 15ml/kg.

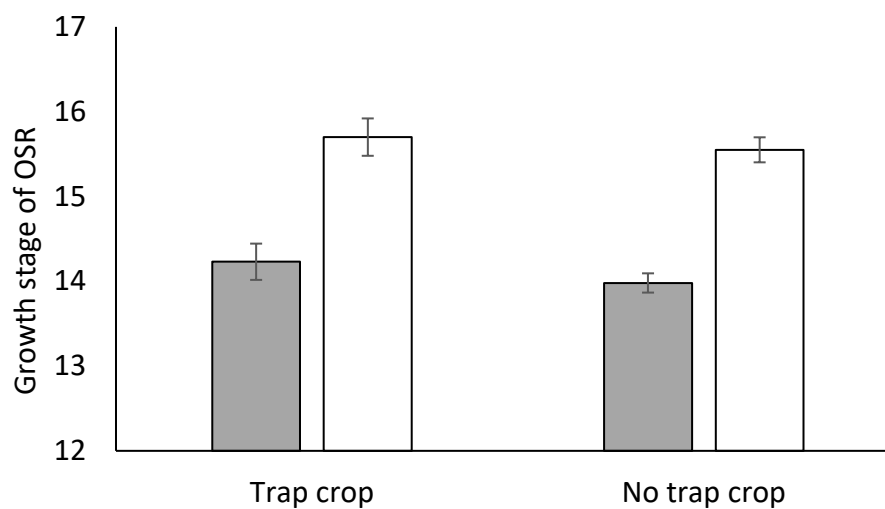
1694

1695 3.4.2 Growth Stage:

1696 At the October assessment the levels of adult *P. chrysocephala* feeding damage were
 1697 already high and one full plot from treatment A (Control) could not be accurately assessed
 1698 for growth stage due to a complete lack of surviving plants. Overall, when grown in
 1699 association with a trap crop border the OSR was at a more advanced growth stage than in
 1700 the absence of a trap crop (GS 14.23 and GS 13.98, respectively; Figure ; $F_{1,19.8}=8.43$,
 1701 $P=0.009$); there was no significant effect of the trap crop type on growth stage of OSR
 1702 ($F_{1,18.9}=0.02$, $P =0.883$) and no significant effect of the neonicotinoid seed treatment
 1703 ($F_{1,18.9}=0.2$, $P =0.659$). By November, the growth stage of the OSR in the crop centres had
 1704 advanced in the more backward treatments and there were no differences between

1705 treatments (in association with trap crop GS 15.70, monoculture GS 15.55; Figure ;
1706 $F_{1,20.5}=1.7, P=0.206$). Due to high levels of injury, a total of 60/432 OSR plants could not be
1707 measured for growth stage in the November assessment due to a complete lack of leaf
1708 material from heavy damage.

1709



1710

1711 Figure 7. Oilseed rape (OSR, *Brassica napus*) growth stage in October (grey) and November
1712 (clear) when grown in association with a Brassica trap crop or not. Growth stage based on
1713 BBCH scale (Lancashire *et al.*, 1991); mean \pm SE shown.

1714

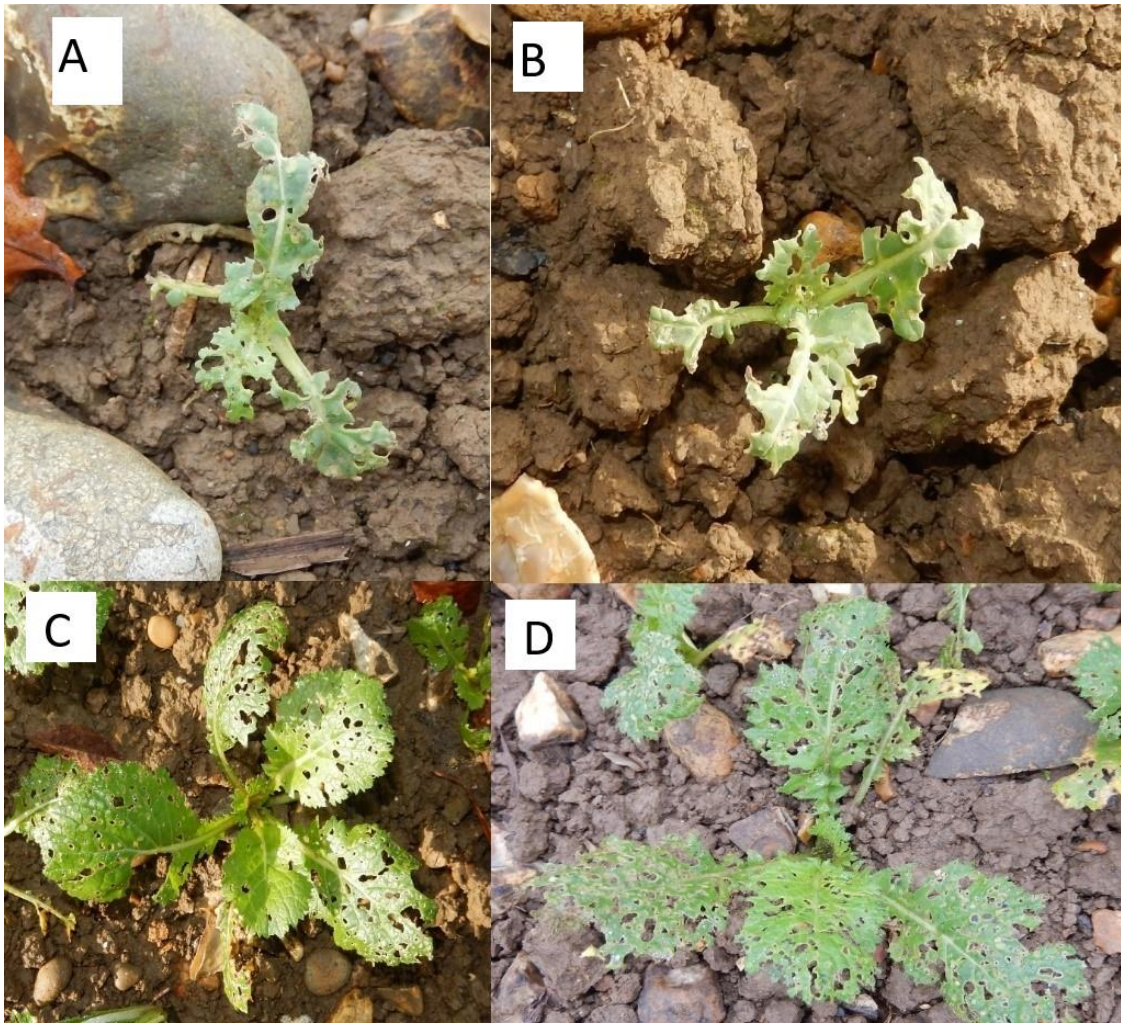
1715 **3.4.3 Leaf area injury from *Psylliodes chrysocephala*:**

1716 Levels of damage observed on the OSR crop plants were very high in all treatments (Figure
1717 and Figure). Some plots (N=12/36) did not have enough plants to assess the target number
1718 of ten plants. For example, in the first round of observations (October assessment), plot 36
1719 (treatment A, untreated OSR control) had a total of just 8 plants within the OSR main crop

1720 central area (7m²). At the October assessment there was a significant reduction in the
1721 amount of damage observed on crop plants grown in association with a trap crop border
1722 compared with treatments without a trap crop ($F_{1,20}=24.2$, $P<0.001$; Figure). There was no
1723 significant effect of the neonicotinoid seed treatments ($F_{1,20}=0.52$, $P=0.48$) nor between the
1724 trap crop type ($F_{1,20}=0.0$, $P=0.995$). The same pattern was seen for the November
1725 assessment with the only significant effect being the presence of a trap crop ($F_{1,20}=17.76$,
1726 $P<0.001$); it did not make a difference if the trap crop was sown using neonicotinoid-treated
1727 seeds or not ($F_{1,20}=1.28$, $P=0.271$), nor was there any effect of the trap crop type ($F_{1,20}=0.19$,
1728 $P=0.665$).

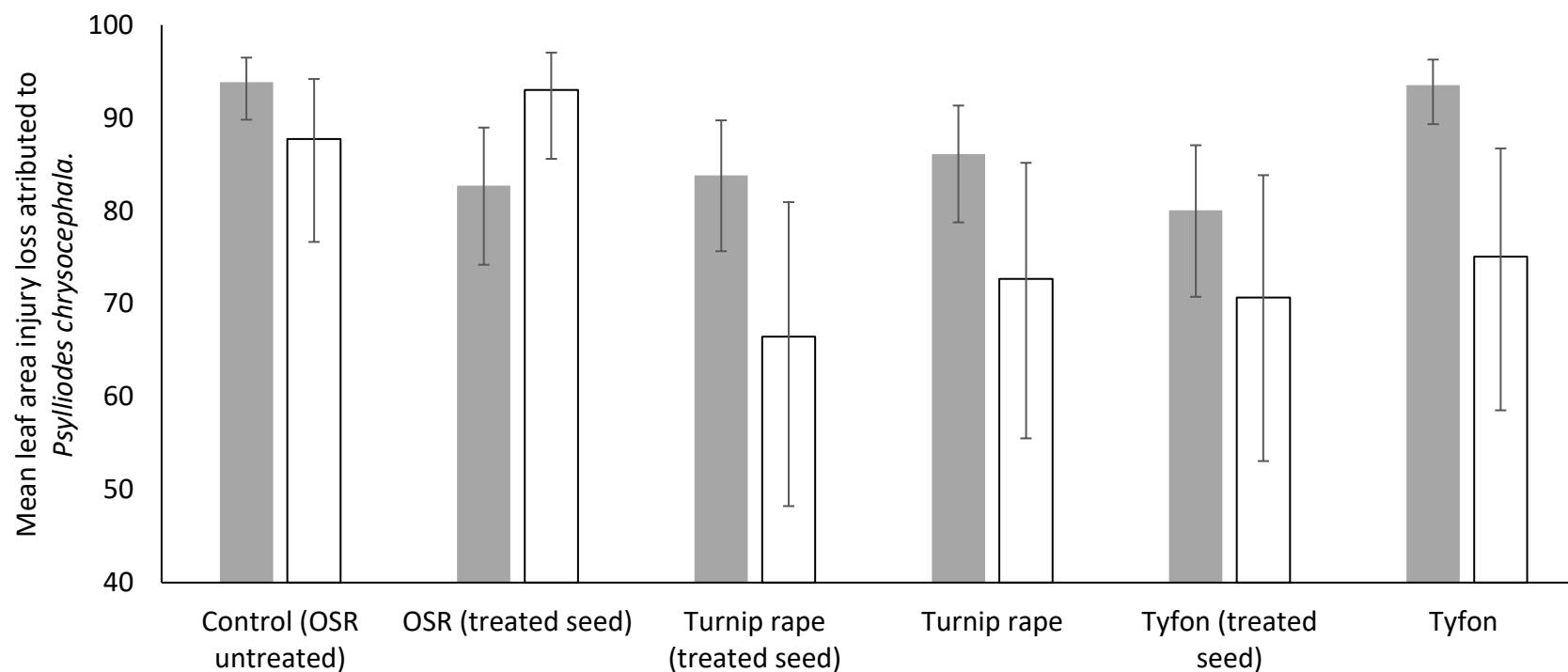
1729 Both turnip rape and Tyfon trap crops were attacked by *P. chrysocephala* (Figure9), with no
1730 difference in the injury levels observed between the trap crop types ($F_{0.37,9.8}=0.37$, $P=0.557$)
1731 or neonicotinoid seed treatment ($F_{2.70,9.8}=2.70$ $P=0.132$).

1732 Due to such high levels of injury, it was not clear if only *P. chrysocephala* damage had
1733 occurred or whether there were other sources of defoliation such as slug and pigeon
1734 damage.



1735

1736 Figure 8. Damage assessment photographs, **A**=untreated oilseed rape (OSR) plant showing
 1737 evidence of shot holing damage indicative of *Psylliodes chrysocephala* feeding activity (plant
 1738 from plot 8). **B**= Neonicotinoid treated OSR plant exhibiting large amounts of feeding
 1739 damage (plant from plot 7) **C**= Untreated turnip rape (*Brassica rapa*) plant exhibiting high
 1740 levels of characteristic shot hole feeding damage (plant from trap crop border of plot 1). **D**=
 1741 Untreated Tyfon plant exhibiting high levels of shot hole damage (plant from trap crop
 1742 border of plot 6). Seed treatment Cruiser, at 15ml/kg. Pictures for both trap crop plants are
 1743 from un-treated seed as there was no significant effect on adult feeding from the seed
 1744 treatment so only one set is represented here. Photographs taken on 24/11/2015, 56 days
 1745 after drilling.



1746

1747 Figure 9. Backtransformed mean percentage leaf area damage to oilseed rape plants (*OSR*, *Brassica napus*) by *Psylliodes chrysocephala*. Grey
 1748 bars = October assessment; Clear bars = November assessment. Treatment codes: A = Untreated OSR; B = Untreated OSR with a turnip rape
 1749 trap crop border; C = OSR with a turnip rape trap crop border sown using neonicotinoid-treated seed; D = Untreated OSR with a Tyfon trap
 1750 crop border; E = OSR with a Tyfon border sown using neonicotinoid treated seed; F = OSR sown using neonicotinoid treated seed. Seed
 1751 treatment Cruiser, at 15ml/kg. Error bars are 95% confidence intervals.

1752 **3.4.4 *Psylliodes chrysocephala* larval infestation:**

1753 Not all treatments could be sampled for larvae due to poor plant survival. Only where a trap
1754 crop boarder was present did any OSR survive to allow destructive sampling for *P.*

1755 *chrysocephala* larvae (Table 2). Collected plants exhibited high levels of injury to both the
1756 OSR and trap crops (Figure9). The number of larvae per plant in the OSR plot centres was

1757 lower in plots with Tyfon trap crop border than Turnip rape; the difference bordered on
1758 significance ($F_{1,2,2}=15.61$, $P=0.052$). Neonicotinoid seed treatment did reduce the numbers

1759 of larvae, but not significantly ($F_{1,2,4} = 2.4$, $P=0.242$, Figure 10).

1760 Trap crop plants were present and available in sufficient numbers for destructive sampling

1761 in all plots (starting seed rate was higher than OSR). Larval infestation of the trap crop plants

1762 was very high; in two plants infestation exceeded 200 larvae in a single plant (one turnip

1763 rape and one Tyfon). Larvae were extracted from all but 8 of the plants dissected ($n=167$)

1764 clearly showing that the larvae will select and use both turnip rape and Tyfon as host plants.

1765 Although more larvae were found per plant in turnip rape than in the Tyfon plants (Figure),

1766 the difference was not significant ($F_{1,9,8}=0.4$, $P=0.543$) and there was no significant effect of

1767 the neonicotinoid treatment on larval numbers ($F_{1,9,8}=0.93$, $P=0.357$, Figure 9).

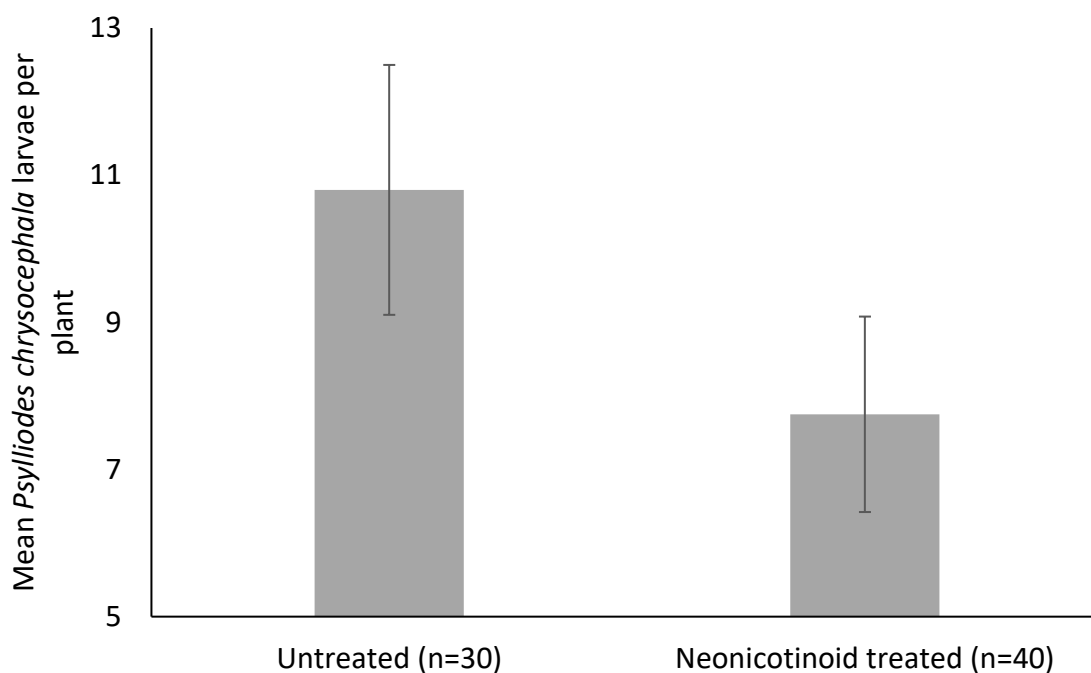
1768

1769

1770 Table 2. The number of oilseed rape (OSR) plants available for destructive sampling (aim to
 1771 achieve 25 per treatment) to determine the effect of treatments on *Psylliodes*
 1772 *chrysocephala* larval infestation. (A) OSR untreated; (B) OSR with turnip rape trap crop
 1773 border; (C) OSR with neonicotinoid seed-treated turnip rape trap crop border; (D) OSR with
 1774 Tyfon trap crop border; (E) OSR with neonicotinoid-treated Tyfon trap crop border; and (F)
 1775 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

1776



1777

1778 Figure 10. Mean \pm SE number of *Psylliodes chrysocephala* larvae per plant in oilseed rape
 1779 (*Brassica napus*) with seed either untreated or treated with neonicotinoid seed dressing

1780

Seed treatment Cruiser, at 15ml/kg.



1781

1782 Figure 11. Oilseed rape (OSR, *Brassica napus*) plants collected for sampling of *Psylliodes chrysocephala* larvae. Plants shown here were taken
1783 from the central crop area of OSR sown grown in association with a Turnip rape trap crop border sown using neonicotinoid-treated seed
1784 (Treatment C). Seed treatment Cruiser, at 15ml/kg. Plants collected 10/2/2016, 146 days after drilling.



1785

1786 Figure 12. Brassica trap crop plants collected for destructive sampling of *Psylliodes*
1787 *chrysocephala* larvae. (A) Turnip rape (*Brassica rapa*, from treatment D), (B), Tyfon, from
1788 treatment B); both treatments sown with untreated seeds. Plants collected 10/2/2016, 146
1789 days after drilling.

1790

1791

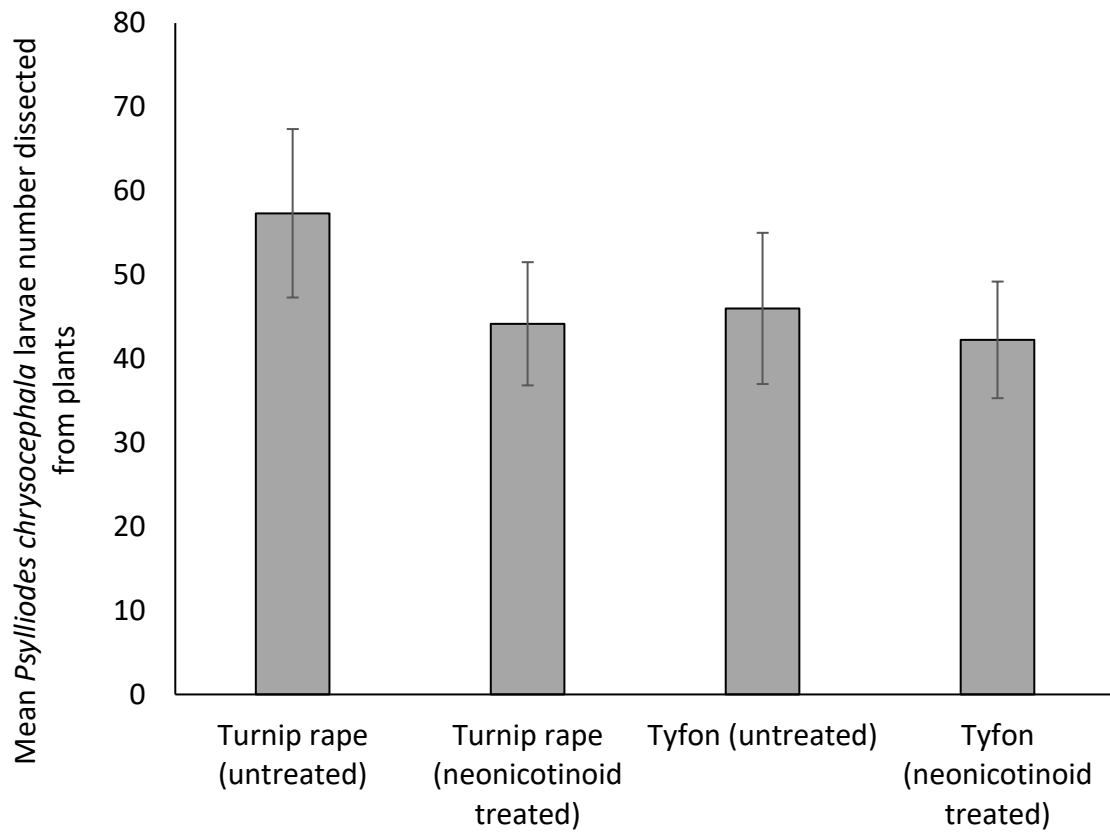
1792



1793

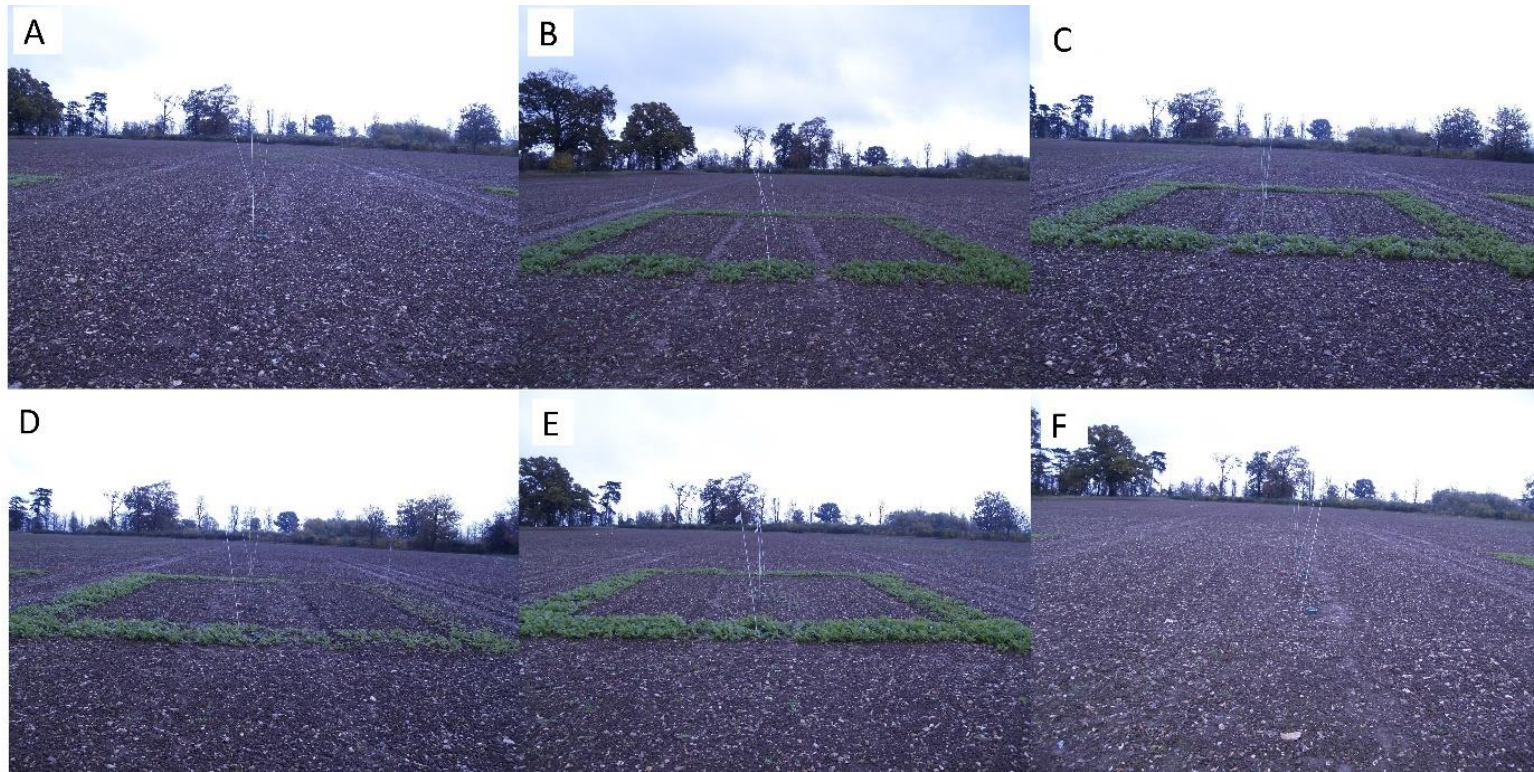
1794 Figure 13. High numbers of *Psylliodes chrysocephala* larvae and larval damage from test
1795 section of a turnip rape plant (untreated seed). Photograph taken 10/02/2016, 146 days
1796 after drilling.

1797



1798

1799 Figure 14. Mean \pm SE number of *Psylliodes chrysocephala* larvae dissected from Brassica trap
 1800 crop plants (Turnip rape and Tyfon) sown using untreated seed or seed treated with Cruiser,
 1801 at 15ml/kg or untreated.



1802

1803 Figure 15. Representative photographs of each treatment tested to protect oilseed rape (OSR) from *Psylliodes chrysocephala* damage. (A) OSR;
1804 (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
1805 border; (E) OSR with neonicotinoid seed-treated Tyfon trap crop border; and (F) OSR with neonicotinoid seed treatment. Seed treatment
1806 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
1807 green leaf material visible is the trap crop borders and the rest of the field is barren.

1808 **3.5 Discussion:**

1809

1810 All methods of crop protection tested, including neonicotinoid seed treatment, were
1811 insufficient to protect the crop beyond winter from high levels of adult feeding and larval
1812 infestation of *Psylliodes chrysocephala* which led to the trial being abandoned. This also
1813 occurred at NIAB Duxford site in the same season (Chapter 2 this thesis) and highlights the
1814 severe damage potential from *P. chrysocephala* when at high abundance, such as observed
1815 in 2015/2016.

1816 Both species of trap crop, turnip rape and Tyfon, did survive the feeding damage by *P.*
1817 *chrysocephala* and although they had very high larval infestation (Figure 10). Both turnip
1818 rape and Tyfon therefore have potential as trap crops for *P. chrysocephala* control
1819 confirming the findings of Barari et al (2005). There is evidence that *P. chrysocephala* exhibit
1820 preference to different Brassica (White *et al.*, 2020). Turnip rape has been suggested as a
1821 preferred host to OSR (Sivčev *et al.*, 2016) with higher adult emergence from turnip rape
1822 than the OSR crop in Serbia. In the present study the OSR was eaten more readily (Figure)
1823 than either the trap crops and could not tolerate the levels of damage. Both the trap crops
1824 show higher tolerance to *P. chrysocephala* feeding than the OSR. This is borne out by the
1825 survival of the trap crops alone while the crop was lost.

1826 Crop establishment (plant density) and lower levels of feeding injury was recorded on OSR
1827 when grown in association with a trap crop; the only OSR plants to survive beyond October
1828 were in the trap crop protected treatments. Further work is required to understand the
1829 underlying mechanism behind these observations. One potential explanation is the trap
1830 crops, which are taller than the OSR crops, are acting as a physical barrier to the beetle's

1831 movement into the central area (Tillman *et al.*, 2015). Another is the higher seed rate in the
1832 trap crop borders and subsequent higher plant density are diluting the level of feeding
1833 away from the OSR in the central area. In the experiment here the area of OSR inside the
1834 trap crop boarder was 9X9m with a 1m border and may disrupt the beetle's movement at
1835 this scale. However, a trap crop planted as a border around the edge of a field may not work
1836 when scaled-up. Unlike pollen beetles, which enters the crop from the field edges (Frearson
1837 *et al.*, 2005; Mauchline *et al.*, 2017). *P. chrysocephala* is known to migrate into the central
1838 area of a field and distribute in patches across the crop (Ferguson *et al.*, 2006; Thioulouse,
1839 Debouzie and Ballanger, 1984). Understanding on the migration pattern of *P. chrysocephala*
1840 would allow better understanding of how and where to locate trap crops. Another option to
1841 be explored would be directional trap crop areas located on in one larger area at the edge of
1842 the crop which see highest migration abundance. This directional migration of *P.*
1843 *chrysocephala* into OSR crop is measured using yellow water traps later in thesis (Chapter 4
1844 and 7).

1845 The number of larvae in both turnip rape and Tyfon were significantly higher than current
1846 thresholds for OSR (Figure10 and Figure 14) i.e., 5/plant; (AHDB, 2019). The extremely high
1847 levels of larvae recorded in the trap crop plants may be an artefact of the lack of alternative
1848 OSR host plants in the field, resulting in larval distributions being concentrated in the small
1849 areas of available plants in the trap crop (Figure 14). The survival of both trap crop types
1850 when infested with high larval numbers suggests they have a high tolerance for larval
1851 infestation. The presence of feeding injury with larval infestation coupled with tolerance to
1852 high abundance of adults and larvae supports the hypothesis that both turnip rape and
1853 Tyfon show traits for a dead-end trap crop, if they were subsequently destroyed
1854 mechanically of through sheep grazing (Shelton and Nault, 2004).

1855 In this study neonicotinoid treatments did not reduce levels of adult *P. chrysocephala*
1856 feeding or significantly alter crop establishment over untreated OSR and they did not save
1857 the crop from total loss. The number of *P. chrysocephala* larvae in the OSR were lower in
1858 treated plots compared to plots sown with untreated seed, but not significantly, nor did
1859 seed treatment significantly reduce the levels of larval infestation in either trap crop species
1860 (Figure). The data presented here suggests that neonicotinoid seed treatments are not
1861 effective in ensuring crop establishment or reductions in *P. chrysocephala* larvae in years of
1862 high pest abundance.

1863 The abandonment of this trial highlights the importance of multi-year studies as the
1864 treatments tested here may be effective in years of lower *P. chrysocephala* numbers. Data
1865 from Sweden (Nilsson, 2002) and Germany (Nilsson, 2002) on the cycles of *P. chrysocephala*
1866 activity suggests they exhibit a cycle of high abundances every seven years. This has not
1867 been examined in the UK and should be considered of high importance when estimating the
1868 effects of *P. chrysocephala* on an experimental trial conducted in only one year. The
1869 methods tested in this trail may be more effective in years of lower pest pressure.

1870

1871 **3.6 Acknowledgements:**

1872

1873 I would like to thank Martin Torrance, Trish Wells, Nigel Watts and Sam Cook for help
1874 collecting field data. I would also like to thank Suzanne Clark for help with statistical
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1876

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1947

1948

1949 **4.0 Comparison of crop protection strategies for *Psylliodes***
1950 ***chrysocephala* in winter oilseed rape (*Brassica rape*): past, present,**
1951 **and future:**

1952

1953 **4.1 Abstract:**

1954

1955 Oilseed rape (OSR, *Brassica napus* L.) is one of the most widely grown crops in UK agriculture. The
1956 use of neonicotinoid seed treatments, used to protect early crop growth and promote
1957 establishment, has been banned in winter OSR since 2013. This restriction has led to increased
1958 pressure from autumn pests such as the cabbage stem flea beetle (*Psylliodes chrysocephala*). Using
1959 two plot-based field experiments the relative effectiveness of past control options i.e. neonicotinoid
1960 seed treatment (Active Ingredient, AI: thiamethoxam) is tested against current control options:
1961 topical sprayed applications of pyrethroid insecticides (AI: lambda cyhalothrin) and increased seed
1962 rates, and potential future options including cultural controls: nurse cropping and trap cropping, and
1963 a future chemical options: an alternative seed treatment, Lumiposa™ (AI: cyantraniliprole), and
1964 neonicotinoid applied as a spray (AI: thiacloprid). Data presented here suggests that in years of
1965 moderate *P. chrysocephala* pressure seed treatments do not significantly reduce pest pressure or
1966 benefit the end yield and that alternatives not based on synthetic insecticides are equally efficient.
1967 The implications for Integrated Pest Management are discussed.

1968

1969

1970 **4.2 Introduction:**

1971

1972 A blanket ban on the use of neonicotinoid insecticide seed treatments (EU, 2018) and the
1973 development of resistance to pyrethroid insecticides in pests species (Brandes and
1974 Heimbach, 2018) has driven an increase in pressure on crop production of oilseed rape
1975 (OSR, *Brassica napus* L., Zhang *et al.*, (2017)). This has led to reductions of the cropping area
1976 in the UK since the restriction came into effect in 2014 (Garthwaite *et al.*, 2019). With pest
1977 pressure particularly from slugs and the cabbage stem flea beetle (*Psylliodes chrysocephala*)
1978 being commonly referenced as the cause of such reductions amid farmer concerns (Collins,
1979 2017; CEH, 2017; Gillbard and Allison, 2019). When giving reasoning for insecticide
1980 application 60% of UK farmers defined pressure from *P. chrysocephala* as a main factor in
1981 applications of insecticides in 2018 (Garthwaite *et al.*, 2019).

1982 With the area of OSR now at its lowest level in over a decade, and the need for imports of
1983 rapeseed, often from countries still allowing the use of neonicotinoid seed treatments
1984 (Harris, 2019) it is important to quantify the contributions that the lost crop protection
1985 methods made to crop protection and yield and to test the relative efficacy of alternative
1986 pest protection measures available to growers now and those that might be available in the
1987 future; research has shown several promising alternative pest protection methods available
1988 to farmers in the post-neonicotinoid era. Here I compare the efficacy of past, present, and
1989 future methods of crop protection against *Psylliodes chrysocephala* in the UK.

1990

1991 **4.2.1 Past control:**

1992 Between 2000 and 2014 the primary method of protecting OSR from *P. chrysocephala* in the
1993 UK was use of neonicotinoid seed treatments to protect the emerging crop from adult
1994 feeding followed by pyrethroid spray applications to control larvae of *P. chrysocephala*
1995 (Redman, 2019; Willis *et al.*, 2020). The neonicotinoid seed treatment was thought to be
1996 effective for the first 6-8 weeks after drilling and provide significant reductions in shot hole
1997 damage (Dewar *et al.*, 2016). To properly inform any amendment to the ban on
1998 neonicotinoid use requires empirical data on the current level of effect as a pest protection
1999 practice.

2000 At the time of this trial (year) the foliar spray Biscaya® (active ingredient: thiacloprid) is
2001 registered for use as an aphicide and for use against pollen beetle in spring so was used as a
2002 model new foliar treatment in the autumn. Since the experiment, the use in spring will also
2003 be banned from 2021 (S. Cook, Personal comment).

2004

2005 **4.2.2 Current:**

2006 Since the ban of neonicotinoid seed dressing in the UK, insecticide applications to OSR have
2007 been predominated by the pyrethroid group, with four of the top five insecticides used
2008 being a pyrethroid (lambda-cyhalothrin, tau-fluvalinate, zeta-cypermethrin, cypermethrin);
2009 the 5th being thiacloprid a neonicotinoid; applied as a foliar spray (Dewar *et al.*, 2016). With
2010 only pyrethroids registered for use in the autumn. This has led to an over reliance in their
2011 use to control *P. chrysocephala* with farmers increasing the frequency of applications
2012 (Hughes, Reay and Watson, 2014). However, growing evidence of widespread (and on some

2013 sites total) resistance of populations of *P. chrysocephala* to pyrethroids, has come to light
2014 (Willis *et al.*, 2020). With target site resistance reported from Denmark (Castberg and
2015 Kristensen, 2018; Højland and Kristensen, 2018), France (Bothorel *et al.*, 2018), Germany
2016 (Brandes and Heimbach, 2018; Heimbach and Brandes, 2016; Zimmer *et al.*, 2014) and the
2017 UK (Foster and Williamson, 2015) and a form of metabolic resistance also reported in
2018 Denmark (Højland and Kristensen, 2018; Castberg and Kristensen, 2018). The evidence for
2019 the UK shows there are areas with 100% resistance but that there are still areas where 0%
2020 of the population exhibits resistance (Syngenta, 2019; Willis *et al.*, 2020). These resistance
2021 levels are probably due to increased pressure from insecticides with one mode of action,
2022 producing ideal conditions for selection for resistance (Mallet, 1989). The level of efficiency
2023 from any insecticide is not 100% a proportion of the population are capable of tolerating the
2024 effect without dying (French-Constant and Bass, 2017). With the use of only a single mode
2025 of action the proportion of the population expressing methods of tolerating the insecticide
2026 effect will increase until there is a higher proportion of resistant individuals than susceptible
2027 ones (Bass *et al.*, 2014). With the ban on use of neonicotinoid seed treatments, pest
2028 resistance to permitted foliar treatments (pyrethroids) and resulting difficulty in preventing
2029 *P. chrysocephala* damage and potential yield loss, the development of alternative pest
2030 protection methods is paramount. The current practice of UK farmers to apply pyrethroids
2031 more frequently to control *P. chrysocephala* has not been clarified to show any appreciable
2032 effect and will be part of this study to determine if this practice is achieving any benefit to
2033 the farmer.

2034 Under current advice farmers are informed to increase seed rate as a measure to reduce
2035 pest pressure from *P. chrysocephala* (AHDB, 2019). In Chapter 2 of this thesis the impact of
2036 OSR seed rate was tested alongside the use of nurse crop mixes. This showed that seed rate

2037 did influence the level of leaf area damage, with lower leaf area loss at higher seed rate
2038 application observed in October (but no effect was seen by November). This suggests that
2039 early *P. chrysocephala* feeding can be diluted amongst plants when at a greater density. In
2040 the present study the influence of seed application rate on levels of *P. chrysocephala*
2041 feeding and larval infestation will be tested. It is not clear whether the dilution of injury will
2042 outweigh the interspecific competition between plants at higher densities (Berry and Spink,
2043 2006).

2044

2045 **4.2.3 Future - cultural:**

2046 Trap cropping is a system whereby the crop species at a more attractive growth stage or a
2047 plant species which is more attractive to the pest than is the crop, is grown alongside the
2048 crop in order to act as a trap for pests, reducing infestation in the main crop (Cook *et al.*,
2049 2007; Hokkanen, 1991; Shelton and Badenes-Pérez, 2006; White, 2016; White, Ellis and
2050 Kendall, 2018). Using trap crops can decrease the need for synthetic chemical application by
2051 reducing the harmful impact of the pest to the desired crop. For example, Bohinc and Trdan
2052 (2013) suggest that trap cropping could be used to protect cabbage from the *Phyllotreta* flea
2053 beetles and therefore reduce the need for insecticide application. Turnip rape (*Brassica*
2054 *rapa*) strips grown at the edge of OSR plots has been shown to reduce numbers of *P.*
2055 *chrysocephala* larvae in the surrounded OSR (Barari *et al.*, 2005). Turnip rape, along with
2056 Tyfon (a stubble rape and Chinese cabbage hybrid) borders have also been shown to lower
2057 levels of adult *P. chrysocephala* feeding on OSR in small plot-based field trials (This Thesis
2058 Chapter 3).

2059 Nurse crops are a class of companion plants, grown among the crop which, unlike intercrops
2060 are not harvested; being either naturally destroyed by frost or by herbicides prior to stem
2061 elongation of the OSR crop. This limited growth time of the companion crop reduces any
2062 competition from the nurse species during the bud-formation and flowering periods of the
2063 crop while being present during the vulnerable early growth stages. Altieri and Gliessman
2064 (1983) showed lower numbers of *Phyllotreta* flea beetles in Californian collard crops with
2065 retained weeds than in weed-free monocultures. Kareiva (1985) demonstrated that
2066 *Phyllotreta* beetles move further when they encounter non-host flora, suggesting greater
2067 movement of the beetle in more diverse cropping systems. Ruck et al (2018) suggests that
2068 using volunteer OSR as an adapted trap crop can reduce *P. chrysocephala* damage in OSR.
2069 This has been shown to have some success in reducing pest injury levels (White *et al.*,
2070 2020). Reductions in *P. chrysocephala* feeding has been observed when OSR is grown with a
2071 nurse crop mix comprising Brassicas (Pak Choi (*Brassica rapa* var. *chinensis*), Chinese
2072 cabbage (*Brassica rapa* subsp. *Pekinensis* (Lour.) Rupr), Salad rocket (*Eruca sativa*) and
2073 Linseed (*Linum usitatissimum*)s see Chapter 2 this thesis.

2074 The defoliation of OSR by mowing or sheep grazing shows potential as a crop husbandry
2075 technique to reduce numbers of *P. chrysocephala* larvae infesting crop plants (Ruck, Cadoux
2076 and Robert, 2018). The later the mowing the greater the reduction in larval numbers (White,
2077 Ellis and Kendall, 2018). Spink (1992) cut OSR with a reciprocating blade mower at 3-6cms
2078 above the ground in January; this did not significantly affect the yield (oil content, seed
2079 weight, pods/branch, thousand grain weight) but defoliation had a definite growth
2080 regulatory effect. Studies in spring OSR show the later timing of defoliation and particularly
2081 after stem elongation the greater the yield deficit (Kirkegaard *et al.*, 2008). Flail cutting is
2082 currently being investigated for *P. chrysocephala* control along with using sheep to graze

2083 the crop as defoliation methods to reduce *P. chrysocephala* larval infestation within plants
2084 (Syngenta, 2019). Here the use of mowing the OSR in March is tested.

2085

2086 **4.2.4 Future - synthetic chemistry:**

2087 At the time of the experiment, the seed treatment Lumiposa™ (active ingredient:
2088 cyantraniliprole) was registered for use in Poland, Hungary and Romania to protect OSR
2089 from *P. chrysocephala* attack, but not in the UK (NFU, 2018). Lumiposa™ is recommended to
2090 protect early growth OSR until BBCH 13-14 from *P. chrysocephala* and is marketed to
2091 “protect seedlings producing uniform and healthy stands” (DuPont, 2017). However, little
2092 data is available on the effectiveness of Lumiposa™ as a protective measure in OSR from *P.*
2093 *chrysocephala* feeding or larval infestation in the UK. Before any registration in the UK, it is
2094 crucial to test how effective a product is in un-biased field trials.

2095

2096 **4.2.5 Aims of this study:**

2097 The relative effects of multiple pest protection options (past, present, and future) available
2098 to farmers for control of *P. chrysocephala* are tested in parallel to help quantify the effects
2099 of the neonicotinoid ban on crop protection and yield in OSR, and the effects on natural
2100 enemies and biodiversity.

2101 It is hypothesised that neonicotinoid seed treatment will give the best control of *P.*
2102 *chrysocephala* and the untreated monoculture OSR will be the most severely effected by
2103 pest pressure. The ranking of other treatments in terms of how they compare in terms of
2104 pest control and in crop yield are the major interest.

2105 **4.3 Methods:**

2106

2107 **4.3.1 Experimental Design & Treatment establishment:**

2108 The experiment tested a variety of control options for *Psylliodes chrysocephala* that were
2109 available in the past (i.e. neonicotinoid seed treatment and pyrethroid sprays), that are
2110 available at the present time (pyrethroid sprays and altering the seed rate) and those that
2111 might be available in the future (new synthetic seed treatments and foliar sprays, and
2112 cultural methods such as trap cropping and nurse cropping, Table 1 and table 2).

2113 The experiment was performed as a replicated plot trial on two fields (Great Field and West
2114 Barnfield) on Rothamsted farm, Harpenden, Herts., UK (Figure 1). Plots were 9m x 9m with
2115 a 2m tram line on all plot edges and between treatment areas and the surrounding crop (DK
2116 Imperial CL).

2117

2118 **4.3.2 West Barnfield:**

2119 In West Barnfield 13 different treatments were tested grouped into past, present and future
2120 control groups as follows - **Past:** neonicotinoids seed dressing, foliar spray, **Current:**
2121 pyrethroid sprayed once or three times, **future cultural:** trap cropping and nurse cropping,
2122 and **future synthetic chemical:** Lumiposa seed treatment (Table 2). The number of
2123 pyrethroid applications was varied to simulate the decline in effectiveness over time (1
2124 application to simulate a single effective treatment of the past and 3 application to
2125 represent the current efficacy levels). A control of untreated OSR (**current**) was replicated
2126 three times to produce three randomized blocks (Figure 1).

2127 **4.3.3 Great Field:**

2128 In Great Field the **Past:** neonicotinoid seed dressing, **current:** increased seed application
2129 rate, and **new cultural** trap cropping and crop defoliation methods were tested; the
2130 experiment comprised 10 treatments in three randomised blocks (Table 3 and Figure 3).

2131

2132 **4.3.4 Link between fields:**

2133 The two experiments were linked by three common treatments (Table 1 and Table 3): **Past:**
2134 OSR with neonicotinoid seed dressing (Treatment E in West Barnfield and treatment J in
2135 Great Field). **Current:** OSR untreated (treatment A both fields), and **new cultural:** OSR with a
2136 trap cop border (Treatment D in West Barnfield and Treatment H in Great Field).

2137

2138 **4.3.5 Seed bed preparation and plot establishment:**

2139 The same plant cultivars were used on both fields: an OSR Clearfield[®] hybrid cultivar DK
2140 Imperial was selected to allow application of specific Clearfield herbicides to kill-off the
2141 nurse crop plants. Clearfield[®] cultivars of OSR exhibit resistance to specific herbicides and
2142 allow applications of herbicides to control brassica weeds with little impact on the crop
2143 (BASF 2018). All cultivars and species used throughout are readily available from multiple
2144 seed suppliers (Table 22). The OSR seed rate was standardised at 100/m² in West Barnfield
2145 and at varying rates of 60, 100 or 120 seeds/m² in Great Field where the impact of seed rate
2146 was tested (Table 3).

2147 Both fields were power harrowed prior to OSR drilling. Great Field plots were drilled on 27th
2148 August 2016 and West Barnfield plots were drilled on 31st August 2016. Both fields were

2149 irrigated to 10mm on 1st September 2016. Other than insecticide applications, the
2150 experiment was managed by Rothamsted Farm according to standard practice.

2151

2152 **4.3.6 Trap crops:**

2153 Trap crops were drilled in a 1m wide strip as a border to plots of the relevant treatments
2154 (Treatments C and D in West Barnfield: 1, and G, H, and I in Great Field; Table 3). The whole
2155 treatment plot remained at 9x9m with the central OSR crop comprising the central 7x7m.
2156 Turnip rape (*Brassica rapa*) cv Jupiter was selected as this species performed well in
2157 previous experiments (Chapter 2) and has the benefit that it can be cropped and can
2158 contribute to seed yield unlike other potential Brassica trap crops such as Tyfon. Seed rate
2159 of 150 seeds/m² as per Chapter 3. Trap crop borders were removed before flowering to
2160 avoid any seed drop and contamination of the field site.

2161

2162 **4.3.7 Nurse crop:**

2163 In **West Barnfield** OSR sown with a nurse crop mix was tested. The mix comprised Pak Choi
2164 (*Brassica rapa* var. *chinensis*), Chinese cabbage (*Brassica rapa* subsp. *Pekinensis* (Lour.)
2165 *Rupr*), Salad rocket (*Eruca sativa*) and Linseed (*Linum usitatissimum*, Table 22). This mix was
2166 used in this study as it showed the most promise as a nurse mix following a previous field
2167 experiment (Chapter 2 this thesis). Seed rate was maintained as in the previous experiment
2168 (Table 22) and was broadcast sown (by myself).

2169

2170 **4.3.8 Defoliation:**

2171 Defoliation of OSR plants to reduce larval infestation of the stems via removal of the leaves
2172 was carried out by mechanical cutting. Cutting was achieved using a manual mower (5 cm
2173 above ground). All cut material was left on the plots, to reduce labour time. Cutting was
2174 performed on 14th March 2017.

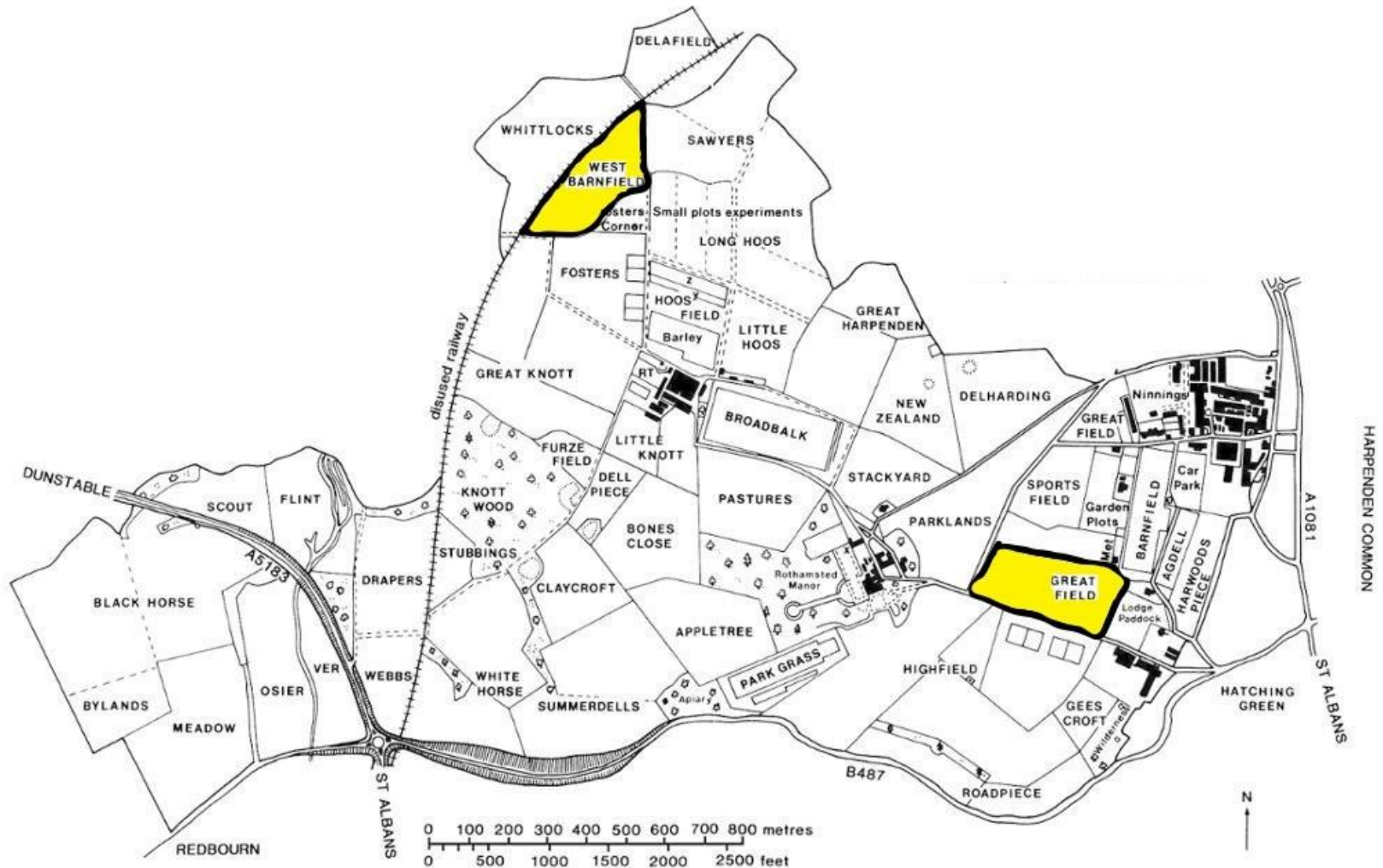
2175

2176 **4.3.9 Synthetic chemical treatments:**

2177 In **West Barnfield** Treatments F and H received one pyrethroid spray and Treatments G and I
2178 received three applications: Table 1). Applications occurred on 30th September 2016, 8th
2179 October 2016, and 17th October 2016; Hallmark Zeon, (75 ml/ha ai lambda cyhalothrin). The
2180 efficacy of applying a neonicotinoid spray, Biscaya[®] (Active Ingredient: thiacloprid 240g/l
2181 (Bayer 2019)), as a potential pest protection measure for autumn *P. chrysocephala* was
2182 tested here on treatments K, L and M (Table); Biscaya[®] was applied on 30th September
2183 2016. Four treatments (E, F, G and L) were sown using seed with neonicotinoid seed
2184 dressing (Cruiser[®] - active Ingredient: thiamethoxam, Syngenta, Table). Also, in West
2185 Barnfield, two treatments were sown using seed treated with Lumiposa[®] seed dressing
2186 (Active Ingredient: cyantraniliprole, DuPont). The Clearfield herbicide Cleravo[®] (AI:
2187 imazamox) was applied to both fields (1Ltr/ Ha) on 8th November 2016 to control weeds and
2188 specifically, the nurse crop.

2189 In **Great Field** application of a single pyrethroid insecticide spray (Hallmark[®] Active
2190 Ingredient: lambda cyhalothrin 100g/l, Syngenta) was made to all plots as the experiment.
2191 This was not part of the treatment design but was needed as the experiment was in danger

2192 of being wiped out by *P. chrysocephala*. A neonicotinoid seed dressing Cruiser® was applied
2193 to treatment J (AI: thiamethoxam, Syngenta, 600g/L at 100ml per 100,000 seeds). No other
2194 insecticide applications were used on treatment plots in Great field.



2195

2196 Figure 1. Field location on Rothamsted farm site at Harpenden UK. Location of experimental fields highlighted in yellow.

2197 Table 1 West Barnfield treatment details testing control options for *Psylliodes chrysocephala*
 2198 in oilseed rape (OSR, *Brassica napus*) on Rothamsted Farm (UK). OSR seed rate was always
 2199 100 seeds/m², trap crop border was Turnip rape (*Brassica rapa* cv. Jupiter) at 150 seeds/m².
 2200 The neonicotinoid seed treatment was Cruiser[®] active ingredient: thiamethoxam; the
 2201 neonicotinoid spray was Biscaya[®] active ingredient: thiacloprid. The pyrethroid spray used
 2202 was Hallmark Zeon[®] active ingredient: lambda cyhalothrin at 75ml/ha; The seed treatment
 2203 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

2204

2205 Table 2 Nurse crop species composition and associated seed rate (number of seeds sown
2206 per m²). This was used in Treatments B (nurse crop) and C (trap crop) in the West Barnfield
2207 field experiment (see Table 1).

Species	Seed rate (m²)
Pak Choi (<i>Brassica rapa</i> var. <i>chinensis</i> cv. Joi choi)	75
Chinese cabbage (<i>Brassica rapa</i> subsp. <i>Pekinensis</i> (Lour.)	75
Salad rocket (<i>Eruca sativa</i>)	150
Linseed (<i>Linum usitatissimum</i>) cv. Abacus	150

2208

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

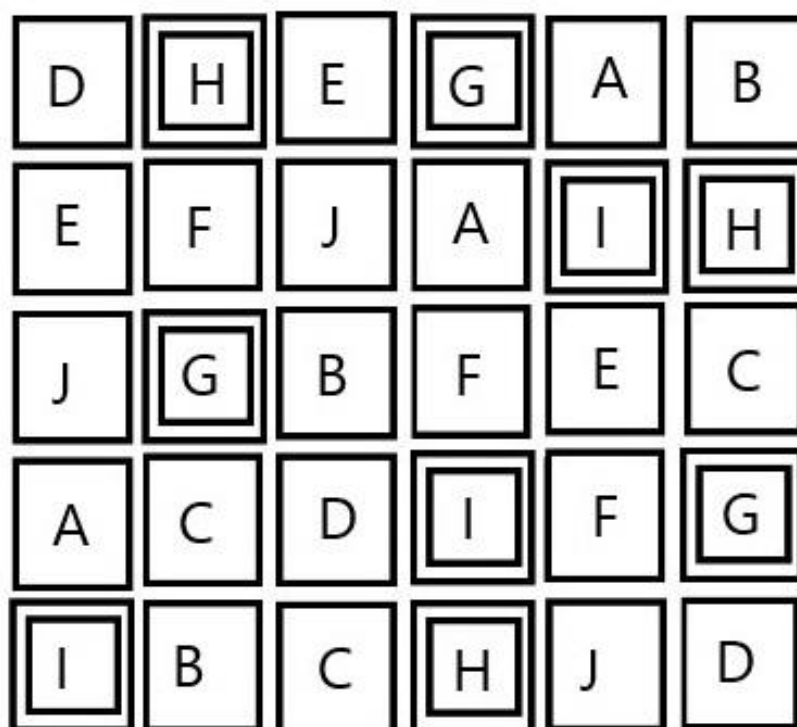
2209

2210 Figure 2. West Barnfield treatment layout as established in the field.

2211 Table 3 Great field treatment details testing control options for *Psylliodes chrysocephala* in
 2212 oilseed rape (OSR, *Brassica napus*) on Rothamsted Farm (UK). Assessing the effect of low
 2213 medium and high OSR seed rate (60, 100 and 120seeds/m²) in combination with defoliation
 2214 (cut) to 5mm plant height. The effect of a 1m trap crop border (Turnip rape *Brassica rapa* at
 2215 150 seeds/m²) 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

2216



2217

2218 Figure 3. Great Field treatment layout.

2219

2220 **4.3.10 *Psylliodes chrysocephala* migration:**

2221 *Psylliodes chrysocephala* migration into the field was recorded using yellow water traps
 2222 (Ringot flora; Nickerson Brothers Ltd, Market Rasen, Lincolnshire, UK) positioned on each
 2223 edge of both fields. Four traps were positioned in a square with two traps 3-meters from the
 2224 crop edge in the headland and two traps 20-meters into the crop. All traps were positioned
 2225 at ground level and 2/3 filled with a water with a drop of detergent (Teepol). Trapping was
 2226 carried out between 26th August until 31st October 2016. Traps were emptied twice/week
 2227 (every Monday and Thursday); *P. chrysocephala* numbers were recorded, and the traps
 2228 were re-set with fresh water and detergent.

2229

2230 **4.3.11 *Psylliodes chrysocephala* resistance to pyrethroids:**

2231 A measure of the percentage of *P. chrysocephala* resistance to the active ingredient of the
2232 pyrethroid Hallmark, lambda-cyhalothrin, was made for populations of beetles collected
2233 from both fields. Adult beetles (c. 50 per field) were collected using an electric aspirator
2234 from 2 meters surrounding experimental plots in each field. Collected beetles were assessed
2235 for resistance using the coated vial bioassay (IRAC 2014). Beetles were randomly assigned to
2236 treatment or control groups and introduced in groups of c.10 to glass vials coated with
2237 either a lambda cyhalothrin (full field rate 7.5g ai/ha) or acetone. Beetles were observed for
2238 movement after 24 and 48 hours and scored as unaffected, affected, or dead. The
2239 percentage population exhibiting resistance (unaffected) was calculated by the survivors in
2240 the treatment vial. This test was performed by S. Foster (Rothamsted Research) on 30th
2241 October 2016.

2242

2243 **4.3.12 Leaf area injury:**

2244 *Psylliodes chrysocephala* feeding damage was assessed on plants positioned both in the
2245 border (1m from the plot edge, on each of the four sides, i.e., North, East, South, and West)
2246 and in the central 7m² area of the plot. Assessments were performed twice in all plots on
2247 22nd September 2016 and 20th October 2016 in West Barnfield and 19th September 2016 and
2248 21st October 2016 in Great Field). Five OSR plants were assessed along each border of each
2249 plot and 20 were assessed in a W-shaped sampling pattern from the crop centre, with 5
2250 plants sampled per leg. Damage was determined by eye, estimated to the nearest 5% leaf
2251 area lost (also see chapters 2 and 3 this thesis). The growth stage of each OSR plant assessed
2252 for damage was also recorded (Lancashire et al. 1991). The leaf area loss of nurse crop

2253 plants (n=10 plants per species) and trap crop plants (five plants per plot edge, total n=20
2254 plants per plot) were also assessed in the relevant treatments.

2255

2256 **4.3.13 *Psylliodes chrysocephala* larval infestation:**

2257 Plants were destructively sampled to assess larval infestation. A sample of five plants for
2258 each species represented within each plot were collected, placed individually into labelled
2259 plastic bags, and transferred to the laboratory. Samples were stored at 4°C and processed as
2260 soon as possible. The stems and petioles of the plants were dissected under a binocular
2261 microscope, using a scalpel and tweezers to cut apart the plant in thin sections (Figure4).
2262 Any larvae found were identified and larval instars determined using the key in Ebbe-Hyman
2263 (1952). Plant samples were taken twice for each plot in both West Barnfield and Great Field
2264 on 3rd November 2016 and 24th February 2017; a third sample was collected on 27th March
2265 in Great Field after cutting the defoliation treatments (D-F).



2266

2267 Figure 4. Photograph of *Psylliodes chrysocephala* 3rd instar larvae found in the stem of an
2268 oilseed rape plant during plant dissections.

2269 **4.3.14 Pitfall trapping for ground-active arthropod diversity:**

2270 To assess the impact of the treatments on the diversity and abundance of ground-active
2271 arthropods, three pitfall traps were set in the centre of each plot approximately 1m apart in
2272 a triangular orientation. Traps catches were combined post-collection; individual trap
2273 catches within plots were not considered to be independent and were used to maximise the
2274 probability of catching representative samples of the active population. Traps in different
2275 plots where at least 10 meters apart are were considered independent. Each trap consisted
2276 of a plastic pot (7cm aperture) buried in the ground, so the pot rim was flush with ground
2277 level, with an inverted plant pot saucer supported with metal wire to restrict excess rain
2278 filling the trap pot and rendering it ineffective. Traps were 1/3 filled with a mixture of anti-
2279 freeze (ethylene glycol) and water to act as a euthaniser and to preserve catches. Traps
2280 were changed weekly from the 1st September 2016 to 3rd November 2016, then fortnightly
2281 until 1st December 2016 and subsequently monthly until May 2017. Samples from each plot
2282 were examined under a light microscope and invertebrates counted and identified to family
2283 (Chinery, 1993; Kirk, 1992), Thrips and Collembola were not counted. All samples were
2284 stored in ethanol post identification.

2285



2286

2287 Figure 5. Photograph of pitfall trap sample processing and identification: arthropods are
2288 sorted into groups and counted.

2289 **4.3.15 Crop density:**

2290 A measure of crop density was carried out shortly before harvest (West Barnfield 16th June
2291 2017, Great Field 15th June 2017). Within each plot, three 0.25m² quadrats were randomly
2292 placed in the central area of each plot and the number of OSR plants present was counted.
2293 From each quadrat, one whole plant was collected. Each plant was cut at ground level and
2294 individually bagged in large paper bags. Samples were returned to the laboratory and kept
2295 at 5°C, until they were processed. Plants were collected when seed pods were still green
2296 (BBCH GS- 79; nearly all pods reached final size (Lancashire et al. 1991)) to avoid pod

2297 shattering. Each plant was measured for height, number of branches, set pods and blind
2298 stalks. Further measures on seed quality were not done as the seeds were not fully ripe.
2299 Collections were carried out on 15th June 2017 and 16th June 2017 from Great Field and
2300 West Barnfield, respectively.

2301

2302 **4.3.16 Crop Harvest and yield measurements:**

2303 All plots were harvested by Rothamsted Research Farm using a plot harvester (Great Field
2304 on 18th June 2017 and West Barnfield on 19th June 2017).

2305 From each plot a subsample of harvested grain was measured for thousand grain weight
2306 (TGW), oil and percentage moisture. This was done using standard procedures at
2307 Rothamsted Research Farm by taking fresh weight then drying the sample to remove
2308 moisture before re-weighing the sample. Seeds were counted for assessment of TGW using
2309 an Elmer C1 grain counter and weighed to calculate the weight of 1000 grains. Oil was
2310 measured using a Bruker, NMR calibrated for Rothamsted Oil and Moisture.

2311

2312 **4.3.17 Statistical analysis:**

2313 In **West Barnfield** data were analysed according to the randomized block design. The
2314 experiment in **Great Field** was analysed using a factorial plus control (neonicotinoid seed
2315 treatment) treatment structure in randomized blocks where the factorial consisted of seed
2316 rate and control management type (trap crop/no trap crop, defoliation cut/ no cut).

2317 **Immigration** Analysis of variance (ANOVA) was used to analyse differences in the numbers
2318 of *P. chrysocephala* caught in yellow water traps on each individual sample date where trap

2319 catches exceeded 10 individuals. Total numbers of beetles were also analysed. For both,
2320 each field edge was defined as a separate block and the two fields were analysed
2321 separately.

2322 **Adult feeding damage** Due to incidences of injury levels scores of 0 and 100%, the data on
2323 percentage leaf area removed by feeding injury were Logit transformed and adjusted, and
2324 analysis of variance (ANOVA) with randomised blocks and nested treatment structure used
2325 to analyse differences between treatments for each sample date (September and October)
2326 separately on each field site.

2327 **Larval infestation:** West Barnfield: - Total numbers of larvae per plant in the November
2328 sample were transformed ($\log_{10}(\text{total larvae} + 1)$) to account for zero counts and analysed by
2329 ANOVA. The February analysis was performed using un-transformed data due to absence of
2330 zero counts.

2331 Great Field: - The total numbers of larvae (all three instars) per plant were analysed using
2332 ANOVA.

2333 **Pitfalls:** Analysis of pitfall trap catches was restricted to Carabidae, Staphylinidae,
2334 Linyphiidae and *P. chrysocephala*. Each group was analysed separately by analysis of
2335 variance (ANOVA) on the grand total of catches over all sampling dates.

2336 **Crop Density:** Plant density differences between treatments was analysed using an analysis
2337 of variance (ANOVA) on the number of plants per quadrat.

2338 **Plant productivity measures:** Plant height, the number of branches, number of pods on the
2339 primary raceme, number of pods on the secondary raceme and total number of pods per
2340 plant was compared using analysis of variance (ANOVA).

2341 **Yield** Thousand Grain Weight (TGW) was compared using analysis of variance (ANOVA). Oil
2342 as percentage yield was analysed using REML.

2343 All statistical analyses were performed using GenStat V18, for windows (VSN International,
2344 Hemel Hempstead).

2345

2346 **4.4 Results:**

2347

2348 **4.4.1 Plot establishment:**

2349 All treatments were successfully established with aerial photographs of each experiment
2350 shown in Figure 6 and Figure 7. The only issue in establishment was the salad rocket in the
2351 nurse mix in West Barnfield which did not successfully germinate. Ultimately, the nurse crop
2352 treatment (and the OSR crop plants among it) had to be manually removed, as the nurse
2353 crop plants were allowed to grow beyond the susceptible growth stage when the Clearfield
2354 herbicide was applied and was only partially effective. Due to fears from Rothamsted farm
2355 staff that the nurse and trap crop plants would become a problem in future years should
2356 they be allowed to flower and set seed; all nurse crop treatments were destroyed on 4th
2357 May 2017. The trap crop treatments had the border removed on the same day before seed
2358 set but crop plants within this treatment were not affected by this operation.

2359

2360



2361

2362 Figure 6. West Barnfield aerial photograph. Image from Google Earth (image date:

2363 25/03/2017).

2364



2365

2366 Figure 7 Great field aerial photograph. Image from Google Earth (image date: 25/03/2017).

2367

2368 **4.4.2 *Psylliodes chrysocephala* migration:**

2369 Catches in yellow water traps showed that *P. chrysocephala* migrated into Great Field
2370 before West Barnfield with catches on the initial day of sampling (Figure 8 and Figure 9).
2371 Beetles were shown to have entered both fields and numbers were increasing at the time of
2372 the first assessment of adult feeding damage (19th September 2016). There was a directional
2373 bias in both fields with a majority of *Psylliodes chrysocephala* being caught on one field
2374 edge. Both fields had high populations to the South side of the crop and West Barnfield was
2375 also high on the East (Figure 8 and Figure 9).

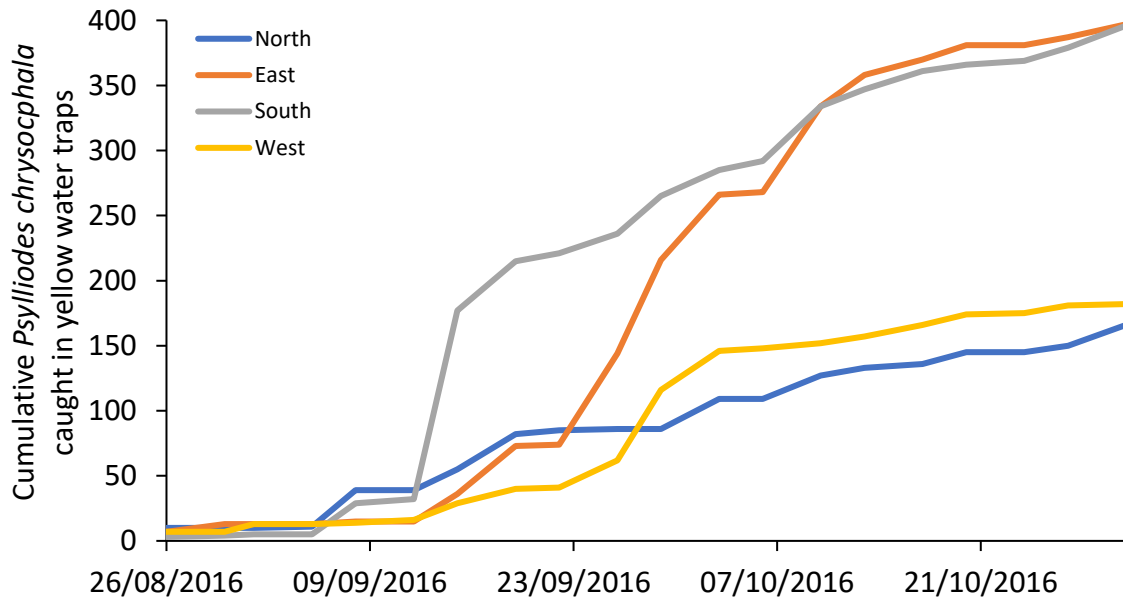
2376

2377 In **West Barnfield**, more beetles were caught in traps placed 20m into the crop compared
2378 with traps placed 3m from the crop edge. The difference was statistically significant on 26th
2379 August ($F_{1,9}=6.76$, $P=0.029$) and 20th October 2016 ($F_{1,9}=11.50$, $P=0.008$). Again, there was
2380 some evidence of an effect on the grand total catches ($F_{1,9}=4.62$, $P=0.060$, Table 4).

2381

2382 In **Great Field** on two dates (10th and 13th October 2016) an effect of trap distance into the
2383 crop was observed with greater beetle numbers caught in traps placed 20m into the field
2384 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).
2385 There was some evidence of the total numbers of beetles caught in traps (accumulated
2386 value of all catches) being affected by distance into field, with those placed 20m onto the
2387 field catching more beetles than those place 3m from the crop edge ($F_{1,9}=4.65$, $P=0.059$,
2388 Table 4).

2389

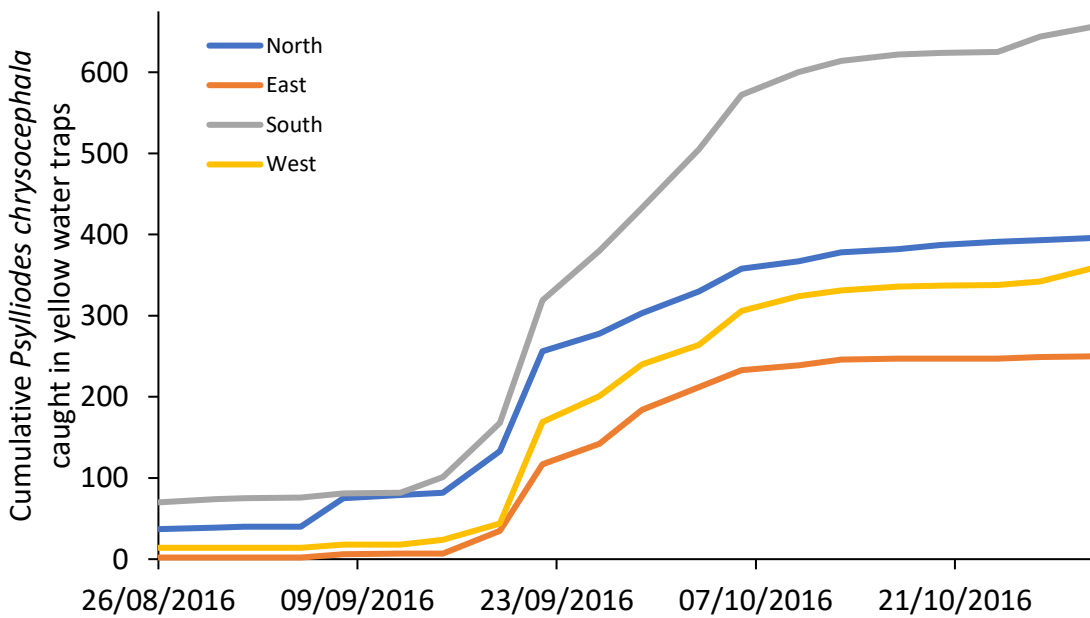


2390

2391 Figure 8. West Barnfield: cumulative yellow water trap counts of *Psylliodes chrysocephala*

2392 on each field edge.

2393



2394

2395 Figure 9. Great field: cumulative yellow water trap counts of *Psylliodes chrysocephala* set at

2396 each field edge.

2397 Table 4 Grand mean and standard error of all *Psylliodes chrysocephala* adults caught in
 2398 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 (\pm 17.62)	90.2 (\pm 17.62)
Great Field	84.9 (\pm 17.68)	123.0 (\pm 17.68)

2399

2400 **4.4.7 *Psylliodes chrysocephala* resistance to pyrethroids:**

2401 There was no control mortality in either sample (Table 5). There was a considerable
 2402 difference in the levels of resistance between the two fields with 48.8% beetles in Great
 2403 Field mobile after 48h exposure to the full field rate (7.5 g ai/ha) and 84.9% resistant in
 2404 West Barnfield.

2405

2406 Table 5 Resistance testing of adult *Psylliodes chrysocephala* to the pyrethroid lambda-
 2407 cyhalothrin at the full field rate (7.5 g ai/ha). Number of beetles active (unaffected) after 48
 2408 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%

2409

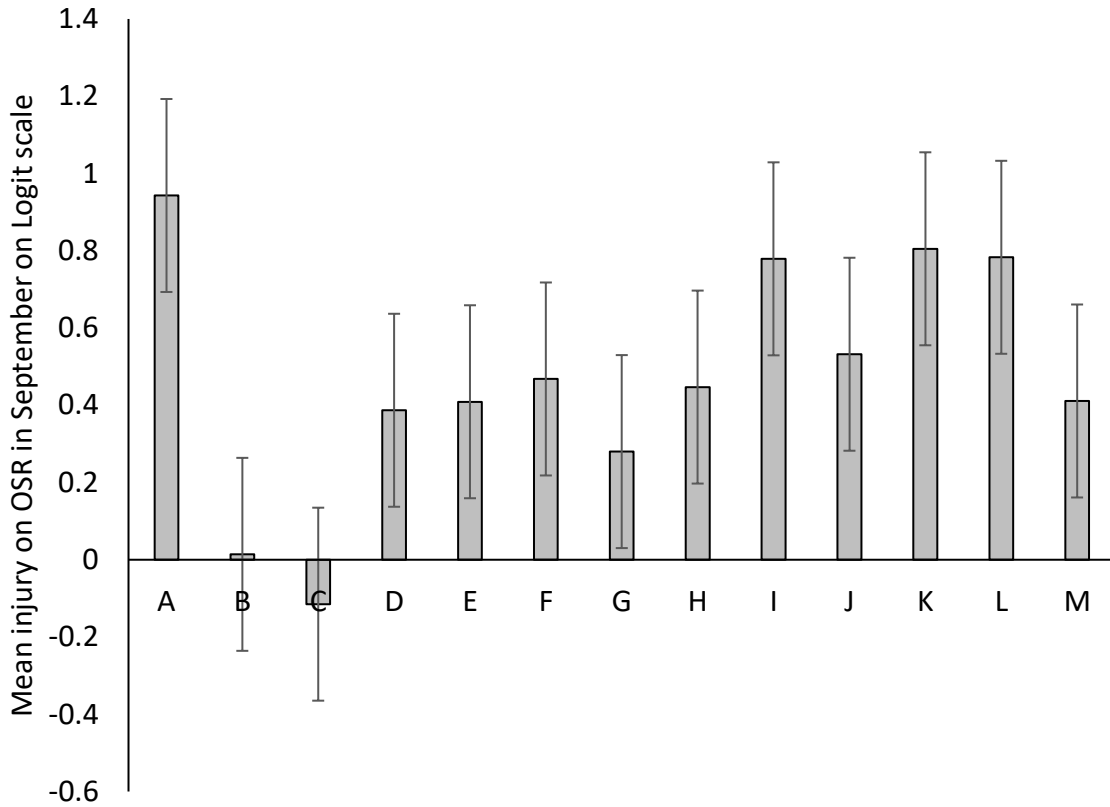
2410 **4.4.3 *Psylliodes chrysocephala* adult feeding:**

2411 In **West Barnfield**, within the nurse crop treatments, no injury was observed on Linseed at
 2412 any point during assessments. Salad rocket seed did not germinate and was never recorded
 2413 in the treatments. Pak Choi and Chinese Cabbage were injured to lower levels than the OSR
 2414 with a maximum percentage damage of 60% for Pak Choi and 50% for Chinese Cabbage

2415 (Table 6). Trap crop borders were heavily damaged by adult beetles, with feeding injury
2416 observed on all assessed plants, with a maximum leaf area loss recorded of 65%. Injury
2417 levels on OSR exceeded 60% in all treatments. One incidence of 100% leaf area loss was
2418 observed in Treatment J (Lumiposa™ treated OSR).

2419 Figure 10 and Figure 11 show the leaf area injury levels of the different treatments in
2420 September and October, respectively. Nurse crops reduced injury on the OSR (September:
2421 $F_{1,8}=26.44$, $P < 0.001$, October: $F_{1,8}=11.11$, $P = 0.002$) as did trap crop borders in September
2422 (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
2423 was reduced by neonicotinoid seed treatment in September ($F_{1,6}=9.52$, $P = 0.005$) but no
2424 difference between controls by the October assessment ($F_{1,6}=26.9$, $P = 0.948$). The Lumiposa
2425 treatment also reduced leaf injury in September ($F_{2,6}=6.27$, $P = 0.006$) but not October
2426 ($F_{2,6}=26.6$, $P = 0.606$). The spray applications did not reduce injury in September, although it
2427 was close to significant ($F_{2,6}=2.97$, $P = 0.069$), and there was a reduction in injury in the
2428 October assessment ($F_{2,6}=12.59$, $P < 0.001$). There was no effect of treatment on the growth
2429 stage of OSR (September: $F_{12,29}=1$, $P = 0.473$; October: $F_{12,30}=0.50$, $P = 0.895$, Table 7).

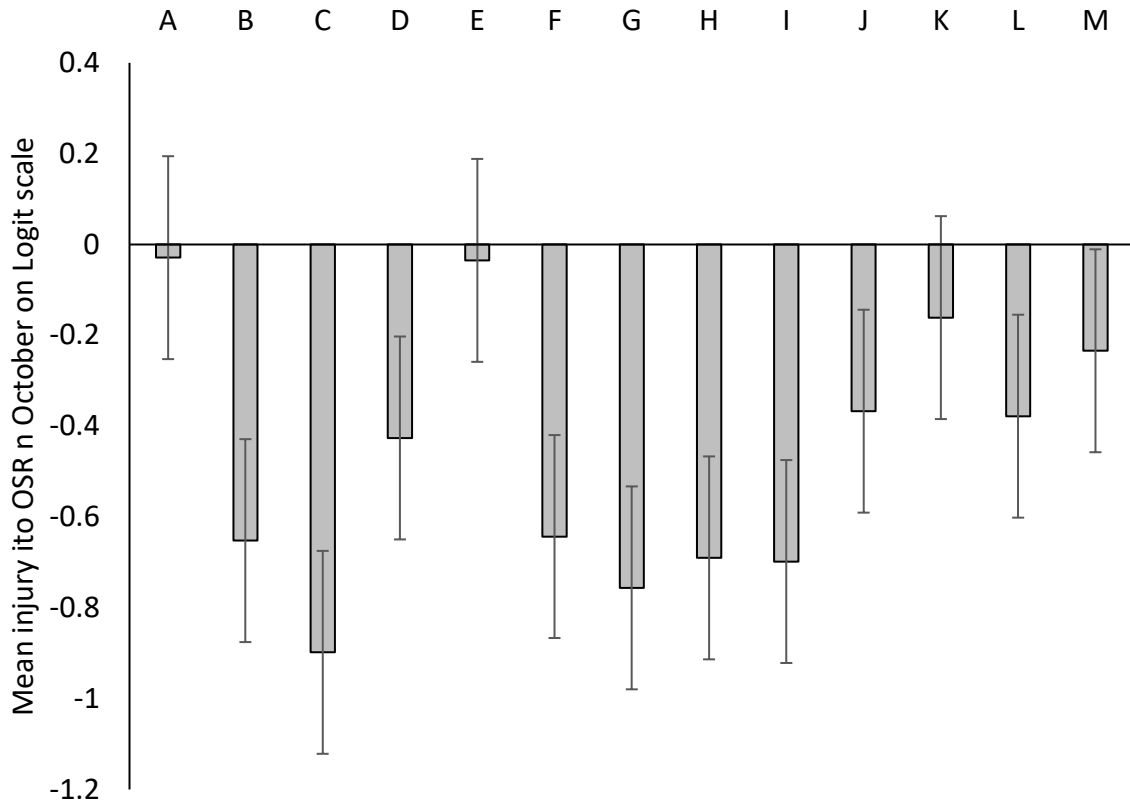
2430



2431

2432 Figure 10. West Barnfield: Mean injury in September to oilseed rape (*Brassica napus*) from
 2433 *Psylliodes chrysocephala* under multiple pest protection methods Treatment code: (A) OSR –
 2434 untreated control, (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap
 2435 crop border (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment–
 2436 Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid spray
 2437 (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high
 2438 pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low
 2439 pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high
 2440 pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing,
 2441 (K) OSR treated with Lumiposa™ seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR
 2442 no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid
 2443 seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE
 2444 shown.

2445



2446

2447 Figure 11. West Barnfield: Mean injury in October to oilseed rape (*Brassica napus*, OSR)
 2448 from *Psylliodes chrysocephala* under multiple pest protection methods Treatment code: (A)
 2449 OSR – untreated control, (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip
 2450 rape trap crop border (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed
 2451 treatment– Cruiser, (F) OSR with neonicotinoid seed treatment - Cruiser + low pyrethroid
 2452 spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing - Cruiser + high
 2453 pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low
 2454 pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high
 2455 pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing,
 2456 (K) OSR treated with Lumiposa™ seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR
 2457 no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid
 2458 seed dressing - Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE
 2459 shown.

2460

2461

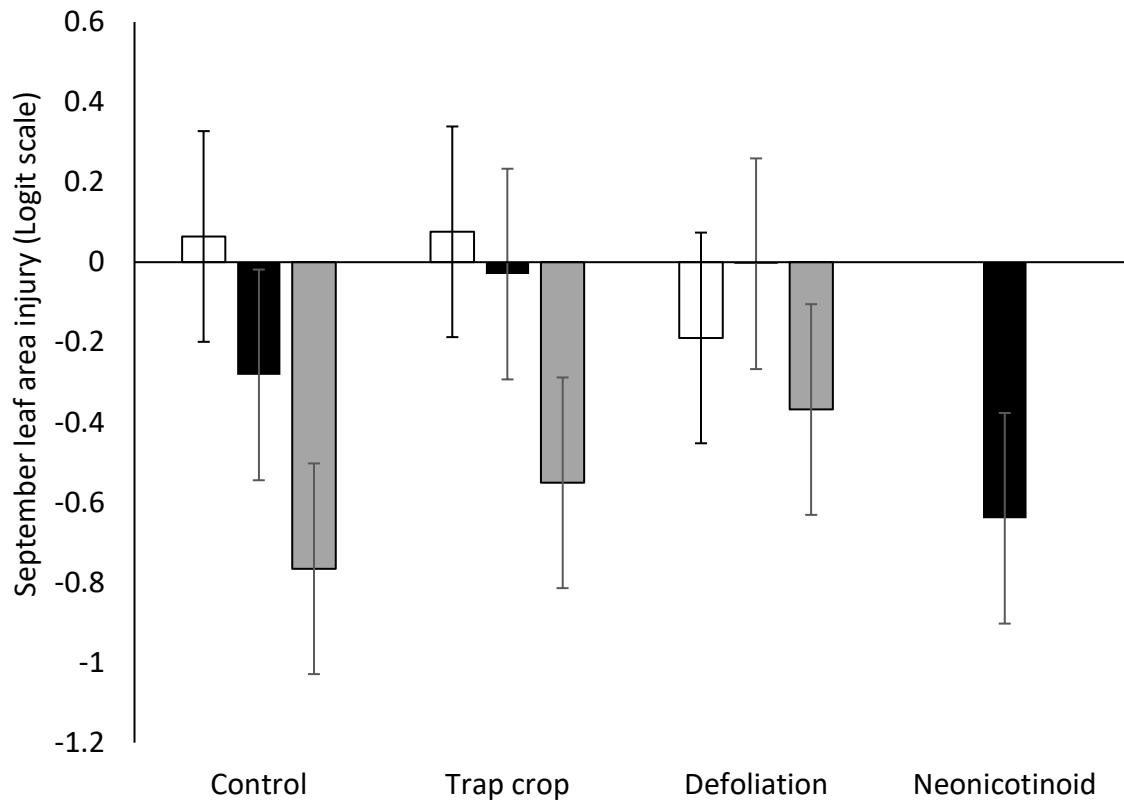
2462 Table 6. West Barnfield: percentage of leaf area lost with standard error on nurse crop
 2463 species because of adult *Psylliodes chrysocephala* injury in September (20/09/2016) and
 2464 October (20/10/2016) assessments. No Linseed shown as no leaf area loss was observed
 2465 and no salad rocket 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)

2466

2467 In **Great Field** no effect of management type (control, defoliation, or trap crop) was evident
 2468 for feeding injury on the OSR plants in September ($F_{2,4}=0.44$, $P=0.651$, Figure 12) or October
 2469 samples ($F_{2,4}=0.72$, $P=0.502$, Figure 13). A significant effect of seed rate was apparent in
 2470 September ($F_{2,4}=5.04$, $P=0.021$) with decreasing injury levels as seed rate increased. This was
 2471 not significant in the October assessment ($F_{2,4}=0.72$, $P=0.072$). Reductions in the amount of
 2472 injury on OSR within the 1m trap crop border was observed (September: $F_{2,4}=30.76$, $P<0.001$
 2473 and October: $F_{2,4}=7.43$, $P=0.006$).

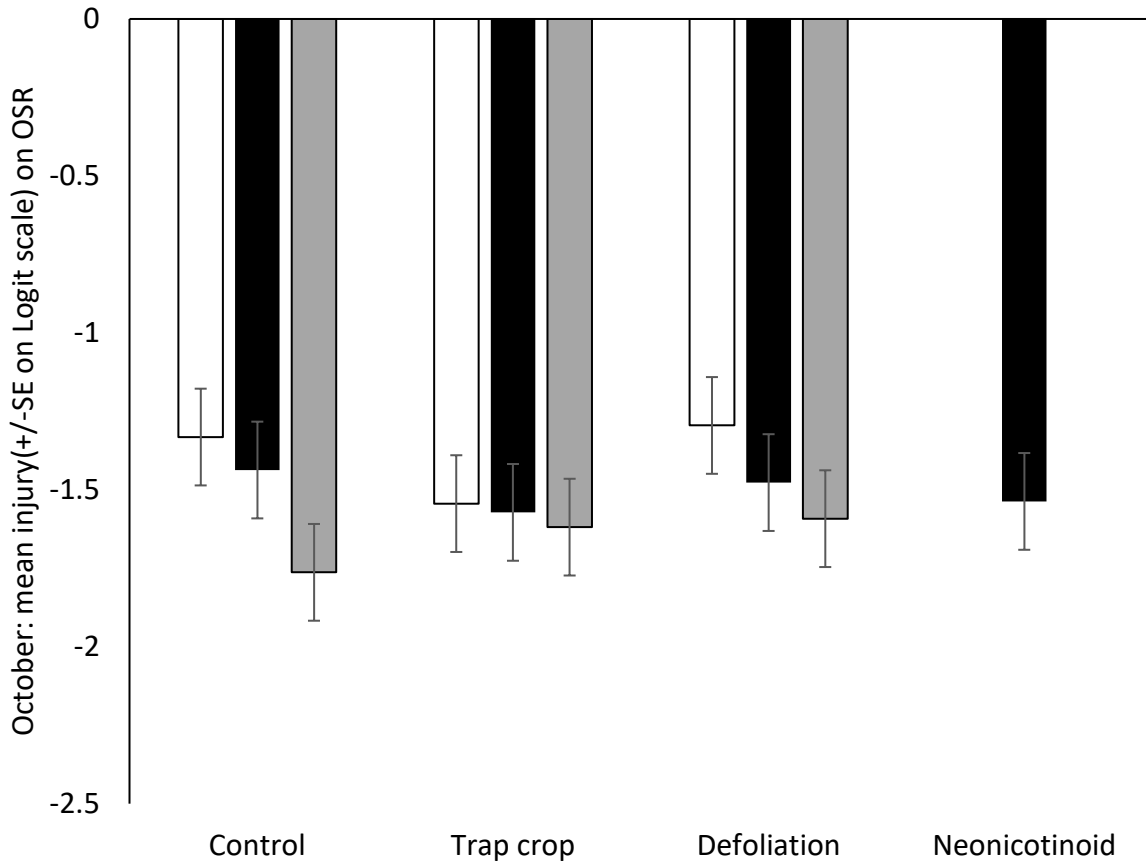
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2475

2476 Figure 12. Great Field: Mean (+/-SE, on Logit scale) leaf area injury (attributed to *P.*
 2477 *chrysocephala*) in September to central oilseed rape (OSR, *Brassica napus*) sown at 3 seed
 2478 rates and under different pest protection methods. Control – OSR monoculture; Trap crop –
 2479 OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before
 2480 stem elongation (14/03/2017); Neonicotinoid = OSR seed treated with neonicotinoid
 2481 (Cruiser). OSR seed rates: clear – 60 seeds/m²; grey – 100 seeds/m² and black – 120
 2482 seeds/m².

2483



2484

2485

Figure 13. Great Field: Mean (+/-SE, on Logit scale) leaf area injury (attributed to *P.*

2486

chrysocephala) in September to central oilseed rape (OSR, *Brassica napus*) sown at 3 seed

2487

rates and under different pest protection methods. Control – OSR monoculture; Trap crop –

2488

OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before

2489

stem elongation (14/03/2017); Neonicotinoid = OSR seed treated with neonicotinoid

2490

(Cruiser). OSR seed rates: clear – 60 seeds/m²; grey – 100 seeds/m² and black – 120

2491

seeds/m².

2492

2493

Growth stage (GS) of the OSR plants in the plot centres in Great Field was not affected by

2494

treatment or seed rate in either sample (treatment September $F_{2,18}=1.19$, $P=0.326$ and

2495

October $F_{2,18}=0.26$, $P=0.776$ and seed rate September $F_{2,18}=2.12$, $P=0.149$; October

2496 $F_{2,18}=0.08$, $P=0.920$, Table 7). No effect on the GS of OSR within the border metre of plots
 2497 was observed in September ($F_{2,4}=0.57$, $P=0.387$). However, in the October assessment OSR
 2498 plants within the trap crop border were at an earlier GS than other treatments ($F_{2,4}=47.10$,
 2499 $P<0.001$).

2500

2501 Table 7 Growth stage of oilseed rape (OSR, *Brassica napus*) on the BBCH scale (Lancashire *et*
 2502 *al.*, 1991) in the central area of experimental plots (see Table 1). Mode values have been
 2503 rounded to the nearest whole value.

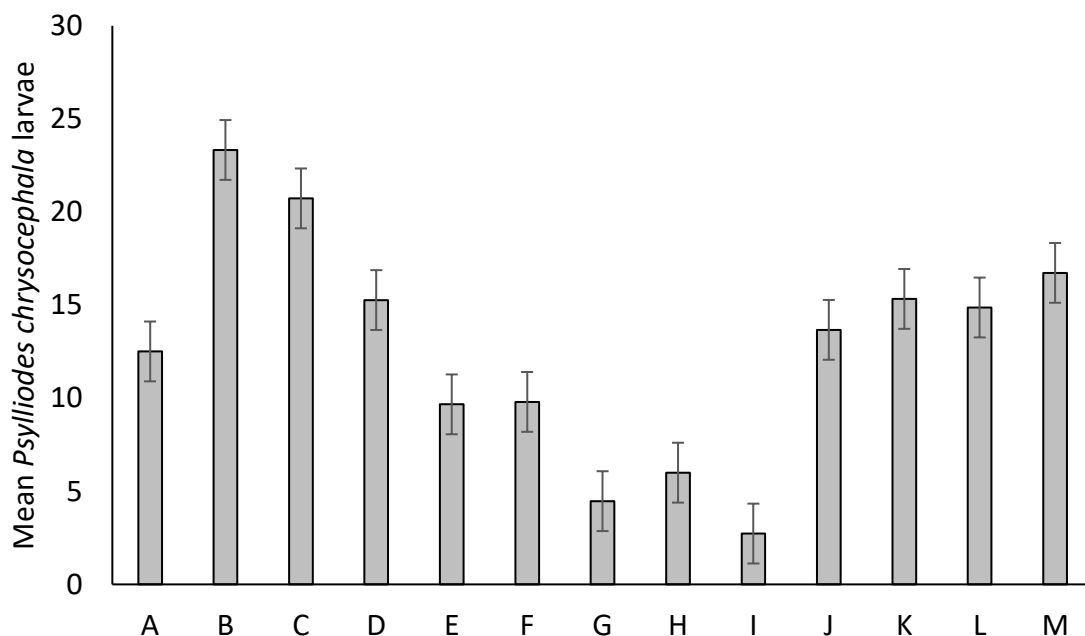
	September	October
West Barnfield	12	15
Great field	12	14-15

2504

2505 **4.4.4 *Psylliodes chrysocephala* larval infestation:**

2506 **West Barnfield:** A total of 20 OSR plants were not infested with *P. chrysocephala* larvae,
 2507 with a maximum of 54 in a single plant. A total of 258 plants exceeded the 5 larvae/plant
 2508 threshold level for spray application (total n=470). In the November assessment turnip rape
 2509 trap crop borders did not affect larvae loading in the OSR ($F_{1,8}=1.79$, $P=0.191$) neither did
 2510 the nurse crop ($F_{1,8}=0.34$, $P=0.566$). Pyrethroid sprays applications did not reduce larvae
 2511 loading ($F_{2,6}=2.40$, $P=0.109$) neither did neonicotinoid spray application ($F_{1,6}=1.36$, P
 2512 $=0.252$). Seed treatment with Lumiposa™ did not affect larvae numbers ($F_{2,6}=0.69$, P -
 2513 $=0.509$), whereas neonicotinoid seed treatment did reduce the number of larvae ($F_{1,6}=4.70$,
 2514 $P=0.038$). In the February assessment the number of larvae per plant was significantly

2515 different between treatments ($F_{12,180}=19.06$, $P<0.001$) with higher numbers of larvae in both
 2516 nurse crop and trap crop treatments and reduced in neonicotinoid seed treatments (figure
 2517 14).



2518
 2519 Figure 14. West Barnfield: Grand means (+/-SE) of *Psylliodes chrysocephala* larvae extracted
 2520 from oilseed rape (OSR, *Brassica napus*) plants from the central area of plots with different
 2521 control measures against P/ chrysocephala: Treatment code: (A) OSR – untreated control,
 2522 (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D)
 2523 OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with
 2524 neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G)
 2525 OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3
 2526 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1
 2527 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3
 2528 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™
 2529 seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing +
 2530 neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing -
 2531 Cruiser + neonicotinoid spray (Biscaya).

2532

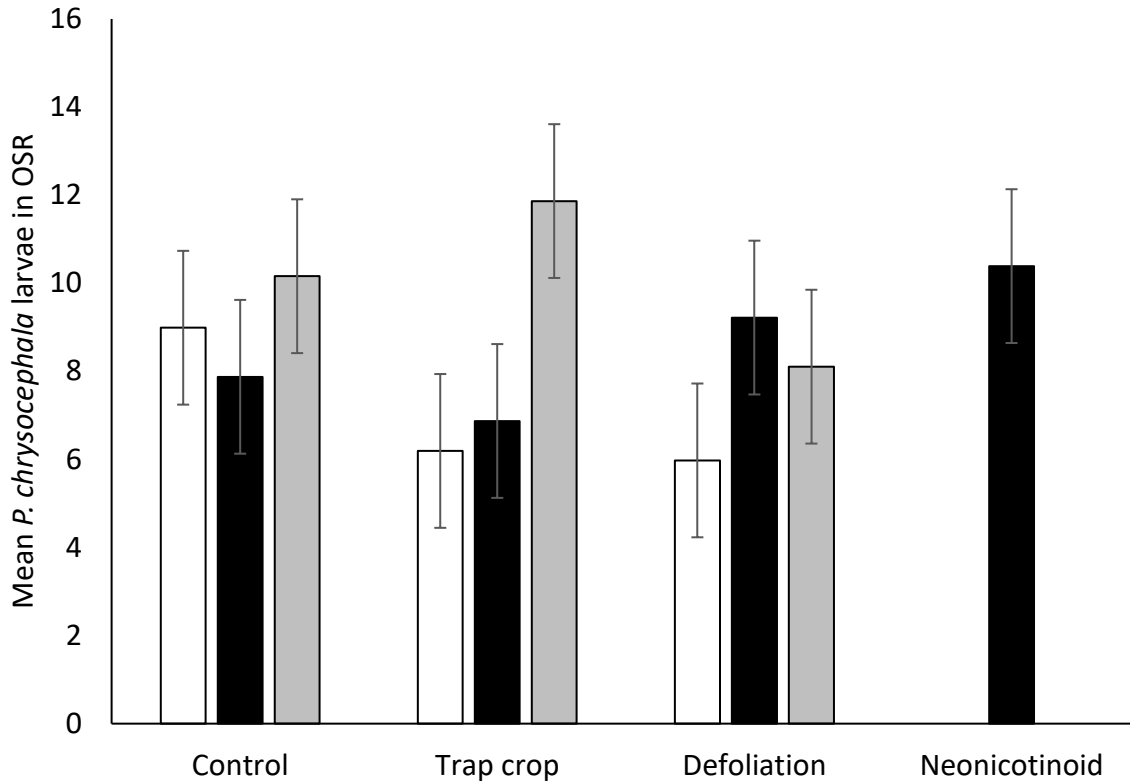
2533 **Great Field:** In the November assessment, the larval numbers were reduced with increasing
2534 OSR seed rate but were not significantly different ($F_{2,4}=2.62$, $P=0.103$) and the presence of a
2535 trap crop also had no effect on larval numbers ($F_{2,4}=0.46$, $P=0.640$, see Figure 14).

2536 At the February assessment only six individual OSR plants avoided infestation with *P.*
2537 *chrysocephala* larvae (total dissected N= 481). Reductions in larval numbers were seen as
2538 seed rate increased ($F_{2,4}=14.18$, $P < 0.001$, Figure 15) there was no effect from the different
2539 control measures ($F_{2,4}=0.81$, $P=0.445$, Figure 15).

2540 At the final assessment in March seed rate once again showed significant effects on total
2541 larval numbers per plant with numbers reducing as seed rate increased (Table 8, $F_{2,4}=9.74$,
2542 $P=0.002$). The defoliation treatment showed a significant reduction in larvae infection
2543 numbers in plots ($F_{2,4}=90.37$, $P < 0.001$). With defoliation reducing the per plant larvae to
2544 below the action threshold whereas all other treatments were much higher (Figure 16).

2545 The number of *P. chrysocephala* larvae extracted from OSR plants increased between each
2546 assessment with highest numbers in the March assessment (Figure 17). Defoliation had
2547 clear effects, reducing the numbers within the defoliated plants on the March assessment
2548 (Figure 17). When looking at the numbers of larvae in relation to the OSR seed rate, no
2549 consistent pattern was observed with larval number decreasing as seed rate increased in
2550 November which was reversed in February and not continuous in March but showing lower
2551 numbers of larvae at higher seed application rate (Table 9).

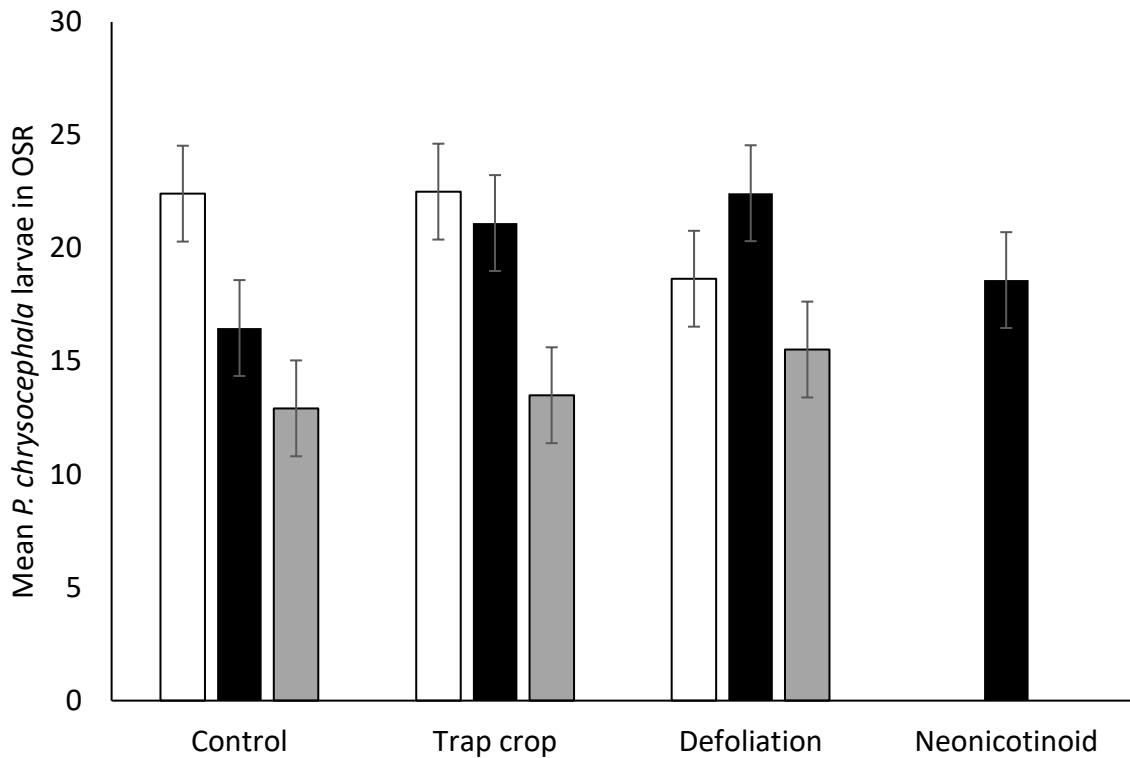
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2553

2554 Figure 15. Great Field: Mean number of *Psylliodes chrysocephala* larvae dissected in
 2555 November from oilseed rape (OSR, *Brassica napus*) sown at 3 seed rates and under different
 2556 pest protection methods. Control – OSR monoculture; Trap crop – OSR with turnip rape
 2557 (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation
 2558 (14/03/2017); Neonicotinoid = OSR seed treated with neonicotinoid (Cruiser). OSR seed
 2559 rates: clear – 60 seeds/m²; grey – 100 seeds/m² and black – 120 seeds/m².SE shown.

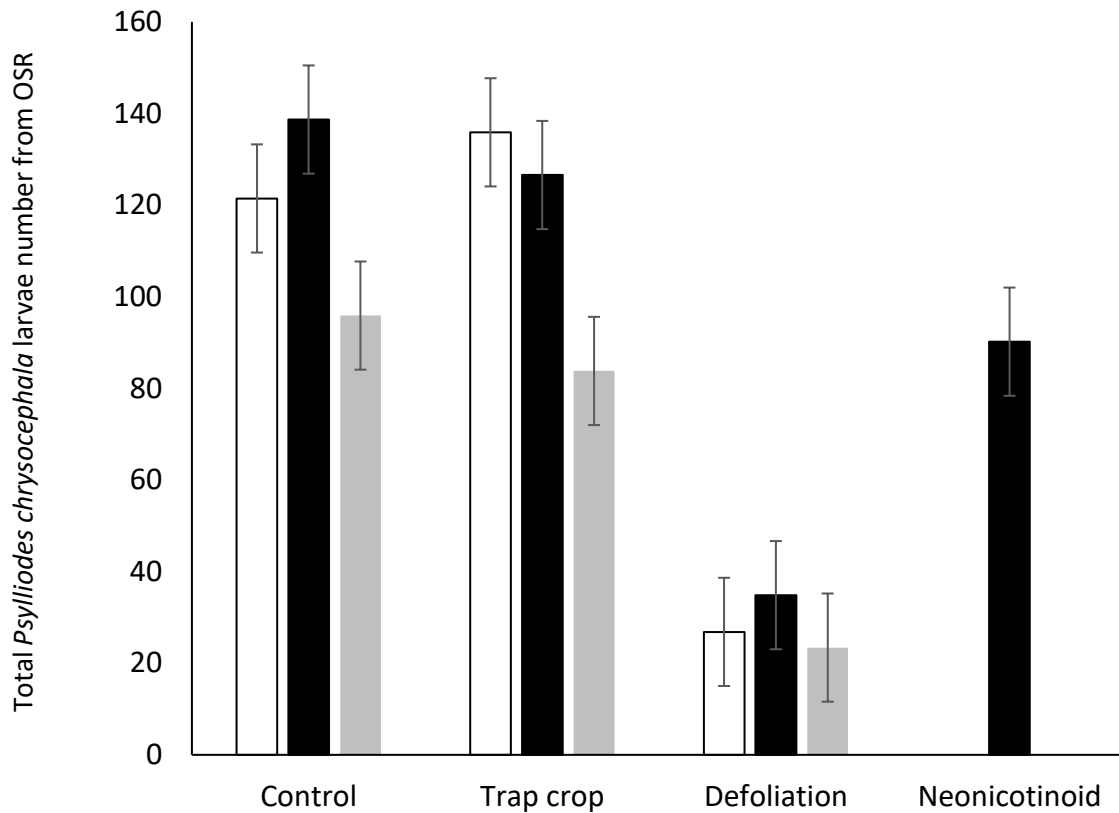
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2561

2562 Figure 16 Great Field: Mean *Psylliodes chrysocephala* larvae dissected in February from
 2563 oilseed rape (OSR, *Brassica napus*) sown at 3 different seed rates and under different pest
 2564 protection methods. Control – OSR monoculture; Trap crop – OSR with turnip rape (*Brassica*
 2565 *rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation (14/03/2017);
 2566 Neonicotinoid = OSR seed treated with neonicotinoid (Cruiser). Showing for OSR seed rates:
 2567 clear – 60 seeds/m²; grey – 100 seeds/m² and black – 120 seeds/m². SE shown.

2568



2569

2570 Figure 17. Great field: Mean *Psylliodes chrysocephala* larvae removed from 10 dissected
 2571 oilseed rape (OSR, *Brassica napus*) plants in March 2016. Control – OSR monoculture; Trap
 2572 crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm
 2573 before stem elongation (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid
 2574 (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m²; grey – 100 seeds/m² and black –
 2575 120 seeds/m². Data shown is for plot totals larvae counts from 10 dissected plants with SE
 2576 shown.

2577

2578

2579 Table 8 Great field: mean (+/- SE) *Psylliodes chrysocephala* larvae dissected from 10 oilseed
 2580 rape (OSR, *Brassica napus*) plants. Values are means of all replicates and all seed rates of
 2581 each treatment, n=9 for control, defoliation cut and turnip rape trap crop border and n=3
 2582 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)

2583

2584 Table 9 Great field: mean numbers of *Psylliodes chrysocephala* larvae dissected from oilseed
 2585 rape (OSR, *Brassica napus*) plants at three seed rate applications: low 60s/m², medium
 2586 100s/m² and high 120s/m². Values are means of all replicates, control, defoliation cut and
 2587 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)

2588

2589

2590 **4.4.5 Larval loading in nurse and trap crop species:**

2591 Due to concerns from Rothamsted farm all nurse and trap crop plants were removed in May
2592 (04/05/2017) before they could flower. Therefore, the only measurement of *Psylliodes*
2593 *chrysocephala* in the companion plants was taken in November.

2594

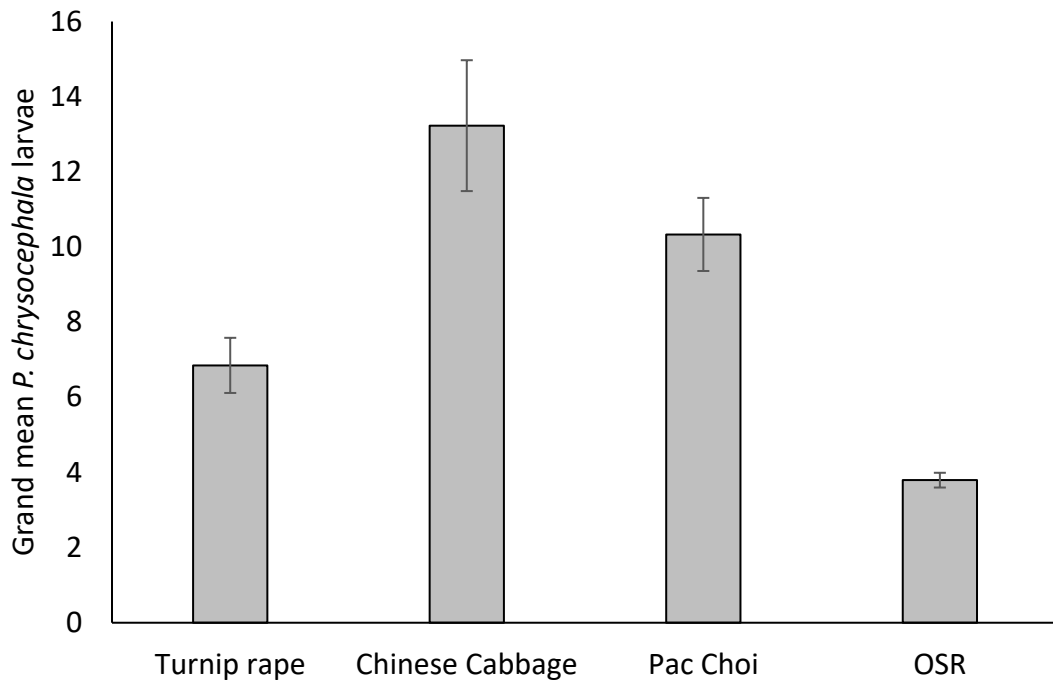
2595 **West Barnfield:** Larvae were retrieved from all Chinese Cabbage (n= 35) and Pac Choi (n=
2596 30) nurse crops dissected and in all but 2 of the turnip rape trap crop plants (n= 34), there
2597 was no evidence of any infestation in Linseed (Figure 18).

2598

2599 **Great Field:** Comparable numbers of larvae were extracted from both the OSR crop and the
2600 Turnip rape trap crop plants (Figure 19).

2601

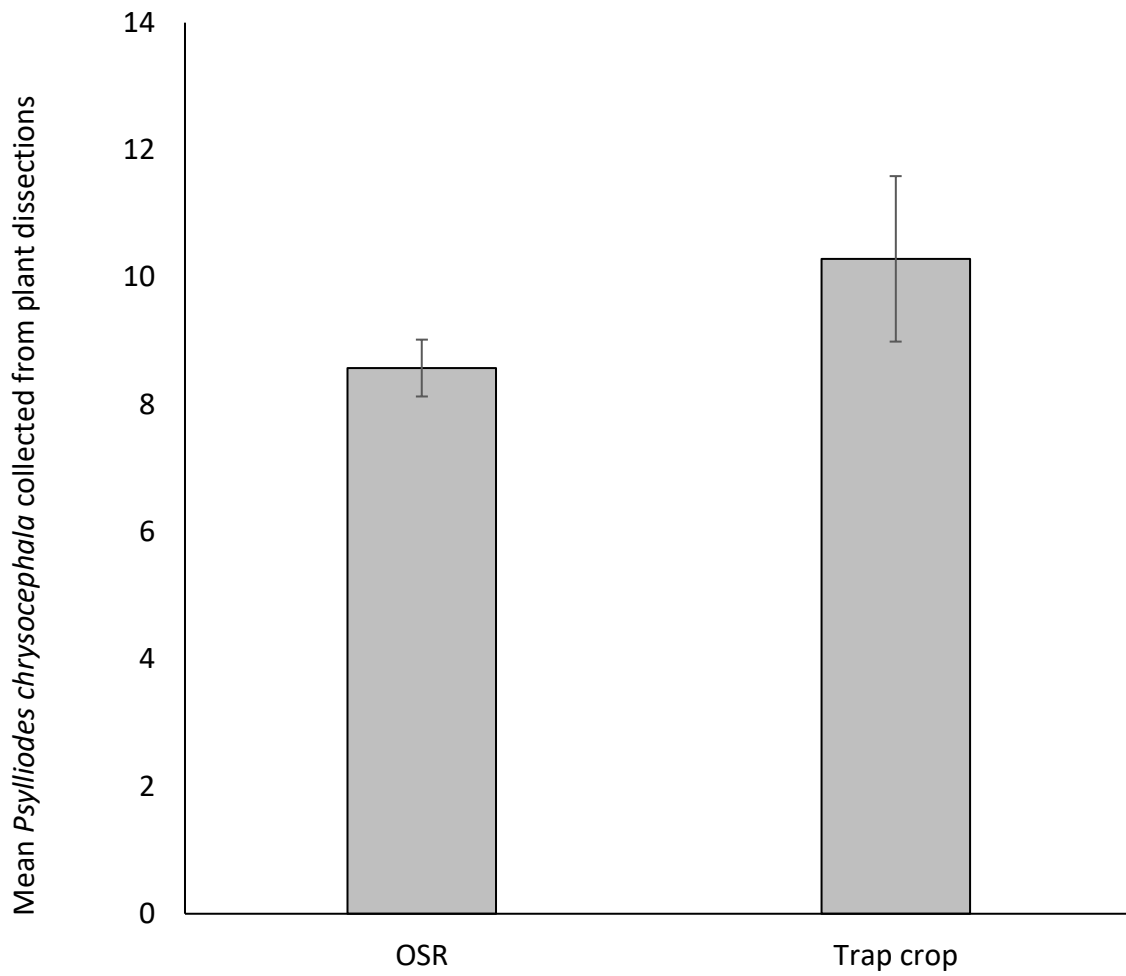
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2603

2604 Figure 18. West Barnfield. Plant dissections for *Psylliodes chrysocephala* larvae. Showing
 2605 grand mean (+/SE) for the trap crop (Turnip rape, *Brassica rapa*), nurse crops Chinese
 2606 Cabbage (*Brassica rapa* var. *chinensis* cv. *Joi choi*) and Pac Choi (*Brassica rapa* subsp.
 2607 *pekinensis* Lour.) alongside OSR (Oilseed rape, *Brassica napus*).

2608



2609

2610 Figure 19. Great field. *Psylliodes chrysocephala* larvae dissected from Oilseed rape (OSR,
2611 *Brassica napus*) and trap crop (Turnip rape, *Brassica rapa*) plants. SE shown.

2612

2613

2614 **4.4.6 Pitfall trapping for ground-active arthropod diversity.**

2615 **West Barnfield:** No difference in the total numbers of *P. chrysocephala* was observed
2616 ($F_{12,30}=1.18$, $P=0.339$). Carabidae numbers were not influenced by trap crop borders
2617 ($F_{1,8}=0.45$, $P=0.506$) or nurse cropping ($F_{1,8}=2.41$, $P=0.132$) but greater numbers were
2618 caught in plots which received pyrethroid applications ($F_{2,6}=5.77$, $P=0.008$) but did not differ
2619 between any other chemical treatment ($F_{2,6}=1.31$, $P=0.287$). Staphylinidae numbers did not
2620 vary between treatments ($F_{12,30}=0.80$, $P=0.649$) neither did numbers of Linyphiidae
2621 ($F_{12,44}=0.83$, $P=0.621$, see Table 10).

2622

2623 Table 10. West Barnfield: grand means of pitfall traps within oilseed rape (*Brassica napus*)
 2624 under various autumn pest protection practices. Treatments: (A) OSR – untreated control,
 2625 (B) OSR - with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D)
 2626 OSR – trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with
 2627 neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G)
 2628 OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3
 2629 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1
 2630 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3
 2631 application), (J) OSR treated with Lumiposa[®] seed dressing, (K) OSR treated with Lumiposa[®]
 2632 seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing +
 2633 neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing -
 2634 Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE shown.

Treatment	<i>P. chrysocephala</i> (+/-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

2635

2636

2637 **Great field:** When comparing the grand total counts of *P. chrysocephala* caught in pitfall
 2638 traps there were no effects of treatment from seed rate ($F_{2,4}=0.25$, $P=0.783$) or
 2639 management type ($F_{2,4}=1.06$, $P =0.370$). Carabidae numbers were greater in defoliation plots
 2640 than other treatments ($F_{2,4}=4.40$, $P =0.033$). No effect of seed rate was apparent ($F_{2,4}=0.16$,
 2641 $P =0.855$). Staphylinidae numbers were also greater in defoliation plots but not significantly
 2642 different from the control ($F_{2,4}=3.27$, $P =0.063$) with no other treatments showing variations
 2643 ($F_{2,4}=2.44$, $P =0.137$). No effect of seed rate ($F_{2,4}=0.94$, $P=0.413$) or management ($F_{2,4}=2.19$, P
 2644 $=0.149$) was seen on Linyphiid although numbers were lower in neonicotinoid treated plots
 2645 and higher in defoliation treatments than in OSR control (Table 11).

2646

2647 Table 11 Great field: grand means (+/- SE) of pitfall catches according to treatment in
 2648 oilseed rape (*Brassica napus*). * denote significant differences.

Table of means	<i>P. chrysocephala</i> (+/-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

2649

2650

2651 **4.4.8 Crop plant density at harvest:**

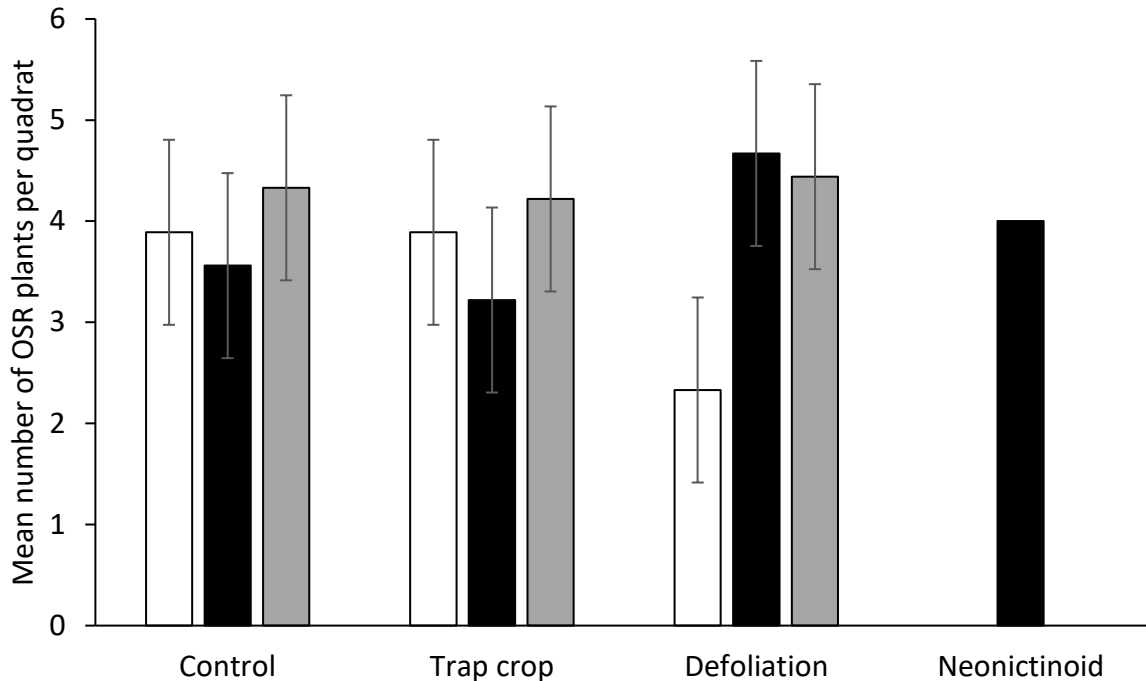
2652 **West Barnfield:** There was no effect of treatment on crop plant density shortly prior to
2653 harvest ($F_{10,26}=0.41$, $P=0.932$, grand mean: 6.22 range: 5.11-7.00 plants per quadrat).

2654

2655 **Great Field.** There was an increase in plant density in plots sown with increased seed rate,
2656 as would be expected, but perhaps surprisingly, the difference was not significant ($F_{2,4}=1.56$,
2657 $P=0.238$, Figure 20). There was no significant effect of the neonicotinoid seed treatment on
2658 crop plant density compared with the density in the agronomic treatments ($F_{1,4}=0.06$,
2659 $P=0.945$). No effect of defoliation was observed on the number plants surviving to harvest
2660 ($F_{1,4}=0.03$, $P=0.876$).

2661

2662



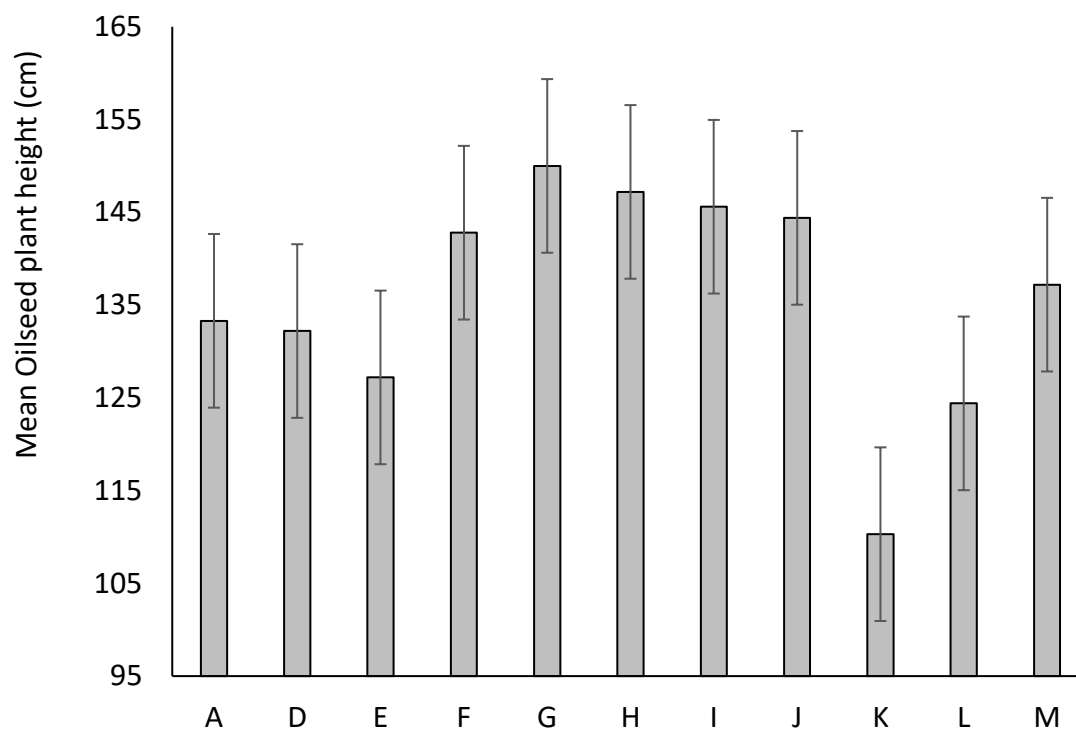
2663

2664 Figure 20. Great field mean number of oilseed rape (OSR, *Brassica napus*) sown at three
 2665 different seed rates and with different control measures for *Psylliodes chrysocephala*:
 2666 Control – OSR monoculture; trap crop – OSR with turnip rape trap crop border; Defoliation –
 2667 OSR cut to 5cm before stem elongation; neonicotinoid – OSR treated with neonicotinoid
 2668 (Cruiser). Oilseed rape seed rate: clear – 60 seeds/m²; grey – 100 seeds/m² and black – 120
 2669 seeds/m². SE shown.

2670

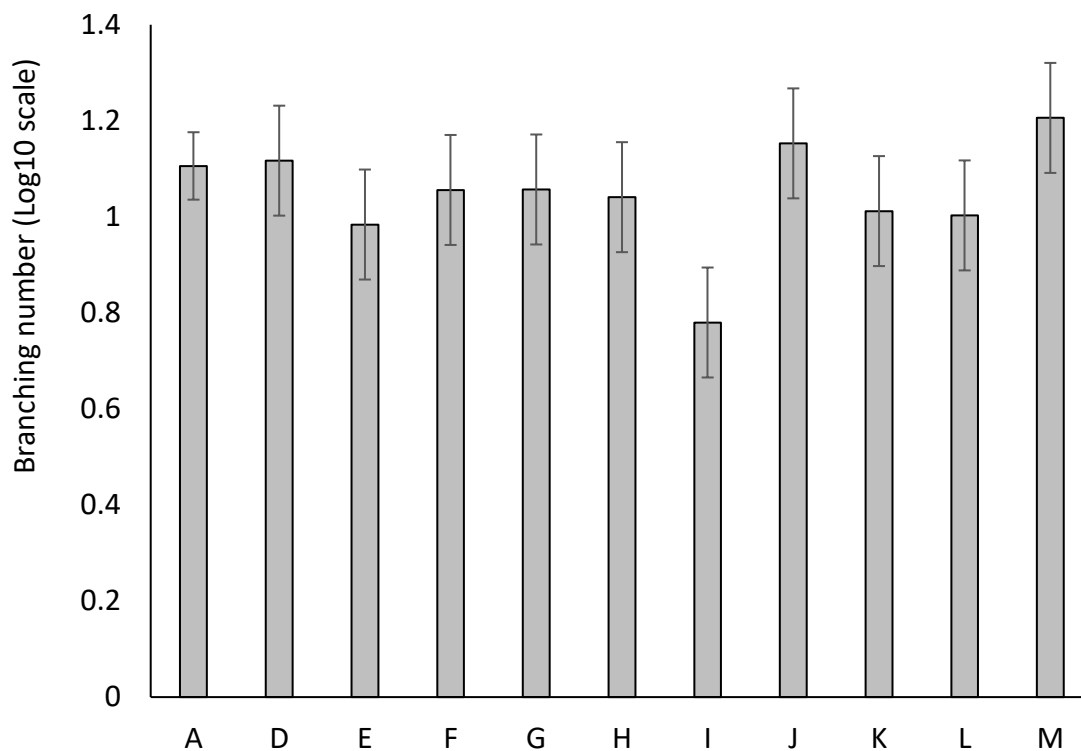
2671 4.4.8. Plant growth (height, branching and pod production).

2672 **West Barnfield:** The only treatment to show a difference from the control treatments was
 2673 the application of Lumiposa combined with a neonicotinoid spray (treatment K); these
 2674 plants were shorter than those in all other treatments ($F_{2,4}=3.76$, $P=0.037$, Figure 21).
 2675 Branching was not affected by treatment ($F_{10,26}=1.06$, $P=0.429$, Figure 21 and Figure 22)
 2676 neither was the total number of pods produced per plant ($F_{10,78}=1.14$, $P=0.370$, Figure 22).



2678

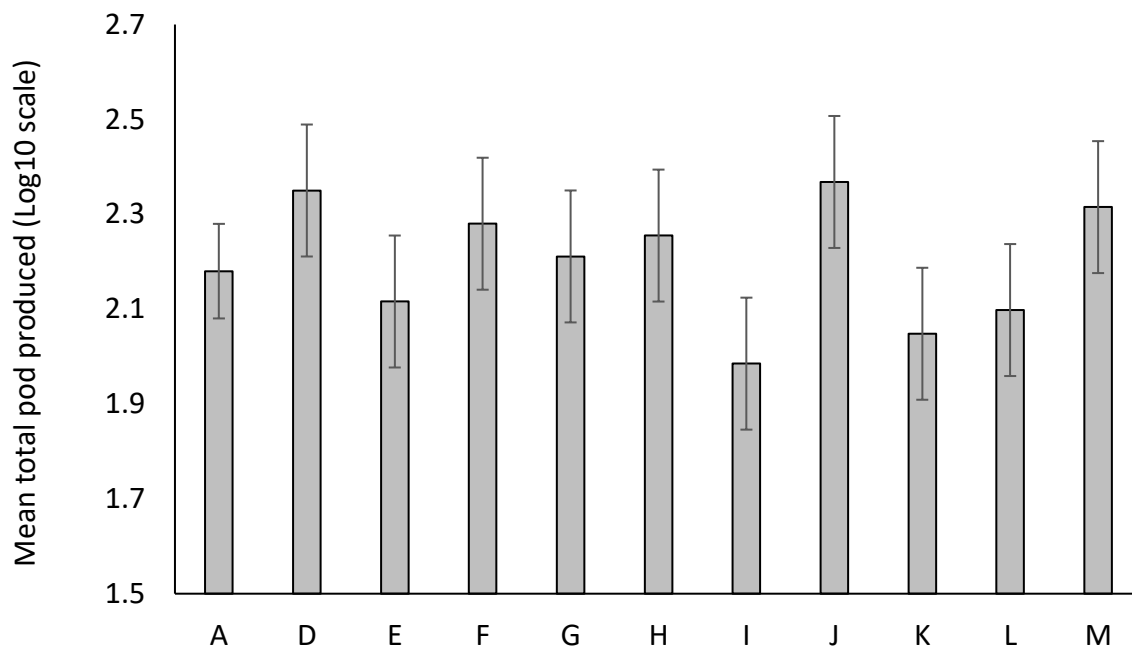
2679 Figure 21. West Barnfield: Mean oilseed rape (*Brassica napus*) plant height (cm) under
 2680 different pest protection methods Treatment code: (A) OSR – untreated control, (D) OSR –
 2681 trap crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with
 2682 neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G)
 2683 OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3
 2684 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1
 2685 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3
 2686 application), (J) OSR treated with Lumiposa[®] seed dressing, (K) OSR treated with Lumiposa[®]
 2687 seed dressing + Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing +
 2688 neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing -
 2689 Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with standard error.



2691

2692 Figure 22. West Barnfield: Branching of oilseed rape (*Brassica napus*) under different pest
 2693 protection methods. Treatment code: (A) OSR – untreated control, (D) OSR – trap crop
 2694 border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with neonicotinoid
 2695 seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR with
 2696 neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H)
 2697 OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR
 2698 untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR
 2699 treated with Lumiposa[®] seed dressing, (K) OSR treated with Lumiposa[®] seed dressing +
 2700 Neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray
 2701 (Biscaya), and (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid
 2702 spray (Biscaya). Data presented on Log10 scale to account for plants which did not produce
 2703 side branches with standard error.

2704



2705

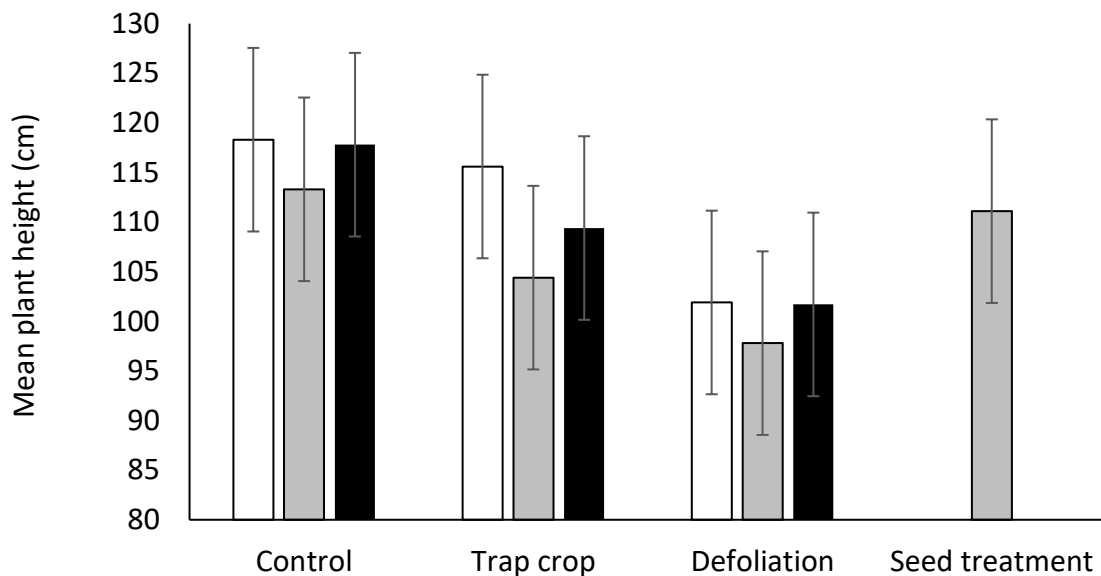
2706 Figure 23. West Barnfield: Mean total pod production of oilseed rape (*Brassica napus*) under
2707 different pest protection methods Treatment code: (A) OSR – untreated control, (B) OSR -
2708 with nurse crop, (C) OSR – with nurse crop and turnip rape trap crop border (D) OSR – trap
2709 crop border, (E) OSR – with neonicotinoid seed treatment– Cruiser, (F) OSR with
2710 neonicotinoid seed treatment - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G)
2711 OSR with neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3
2712 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1
2713 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3
2714 application), (J) OSR treated with Lumiposa[®] seed dressing, (K) OSR treated with Lumiposa[®]
2715 seed dressing + neonicotinoid foliar spray (Biscaya), (L) OSR no seed dressing +
2716 neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing -
2717 Cruiser + neonicotinoid spray (Biscaya). Data present on Logit scale with SE shown.

2718

2719 **Great field:** Plant height at maturity was not affected by seed rate ($F_{2,4}=0.86$, $P=0.422$) but
 2720 was reduced by defoliation ($F_{2,4}=4.43$, $P=0.012$, Figure 24). Neonicotinoid seed treatment
 2721 did not differ from control plants ($F_{1,18}=0.1$, $P=0.754$, Figure 24). There was no effect of
 2722 management on the amount of branching ($F_{2,18}=0.99$, $P=0.394$, Figure 25) nor was there any
 2723 effect of seed rate ($F_{2,18}=0.23$, $P=0.794$, Figure 25).

2724 The total number of pods set were reduced by defoliation ($F_{2,18}=4.68$, $P=0.023$, Figure 26)
 2725 but was unaffected by seed rate ($F_{2,18}=0.16$, $P=0.856$, Figure 26).

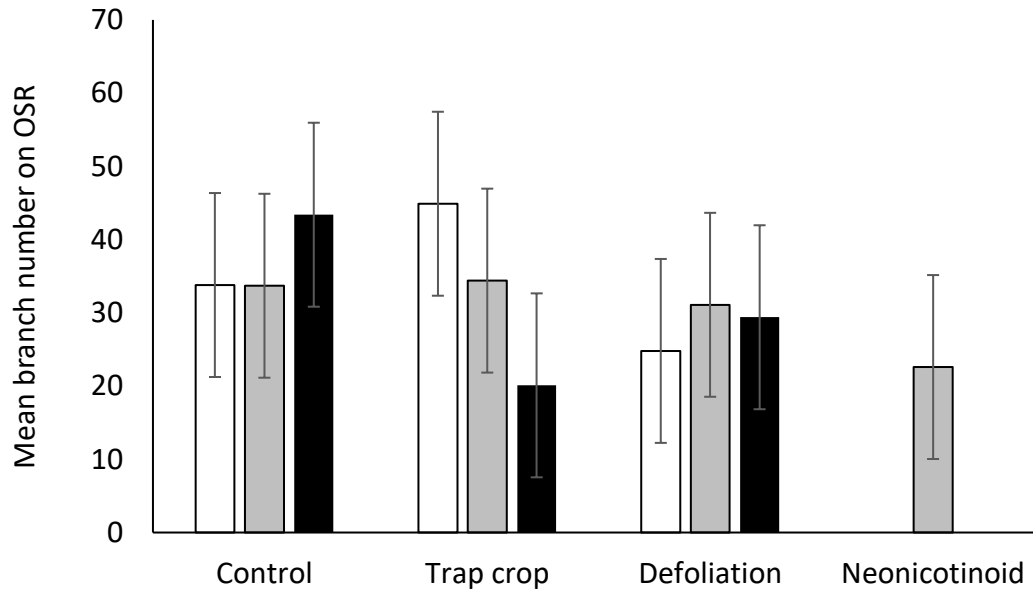
2726



2727

2728 Figure 24. Great field: Mean height of oilseed rape (OSR, *Brassica napus*). Control – OSR
 2729 monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation
 2730 – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with
 2731 neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m²; grey – 100
 2732 seeds/m² and black – 120 seeds/m². SE shown.

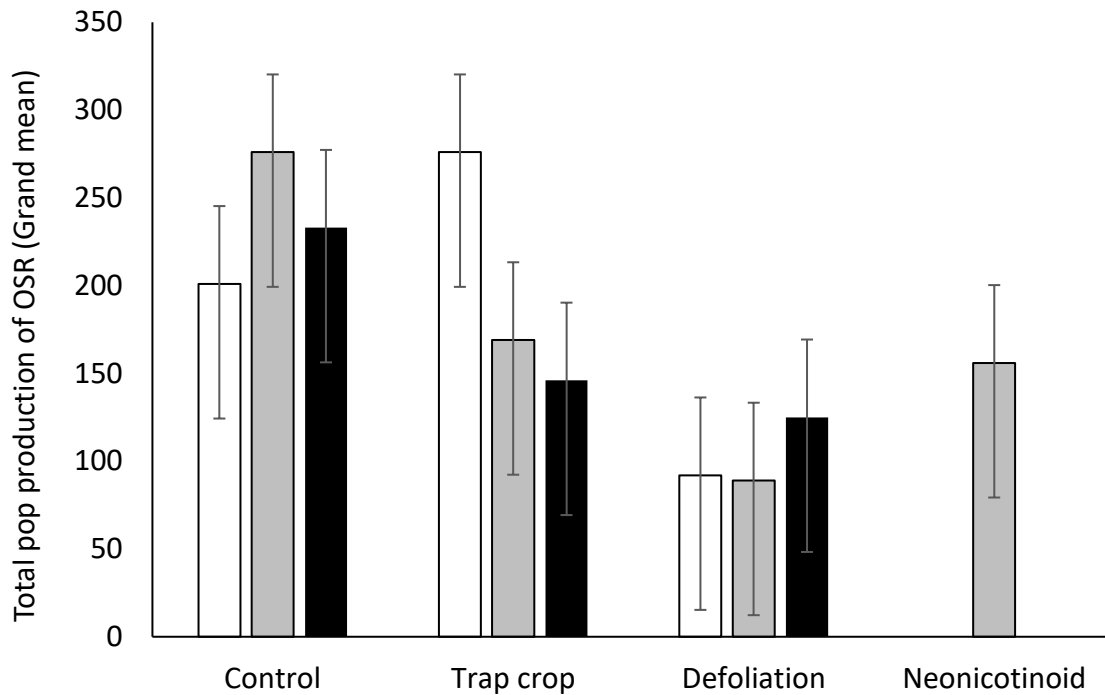
2733



2734

2735 Figure 25. Great Field: Mean branching number of oilseed rape (OSR, *Brassica napus*) under
 2736 differing pest protection methods. Control – OSR monoculture; Trap crop – OSR with turnip
 2737 rape (*Brassica rapa*) trap crop border; Defoliation – OSR cut to 5cm before stem elongation
 2738 (14/03/2017); Neonicotinoid = OSR treated with neonicotinoid (Cruiser). Showing for OSR
 2739 seed rates: clear – 60 seeds/m²; grey – 100 seeds/m² and black – 120 seeds/m². SE shown.

2740



2741

2742 Figure 26. Great field average total pod set on oilseed rape (OSR, *Brassica napus*). Control –

2743 OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border;

2744 Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR

2745 treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m²; grey

2746 – 100 seeds/m² and black – 120 seeds/m². SE shown.

2747

2748 4.4.9. Crop Harvest and yield measurements:

2749

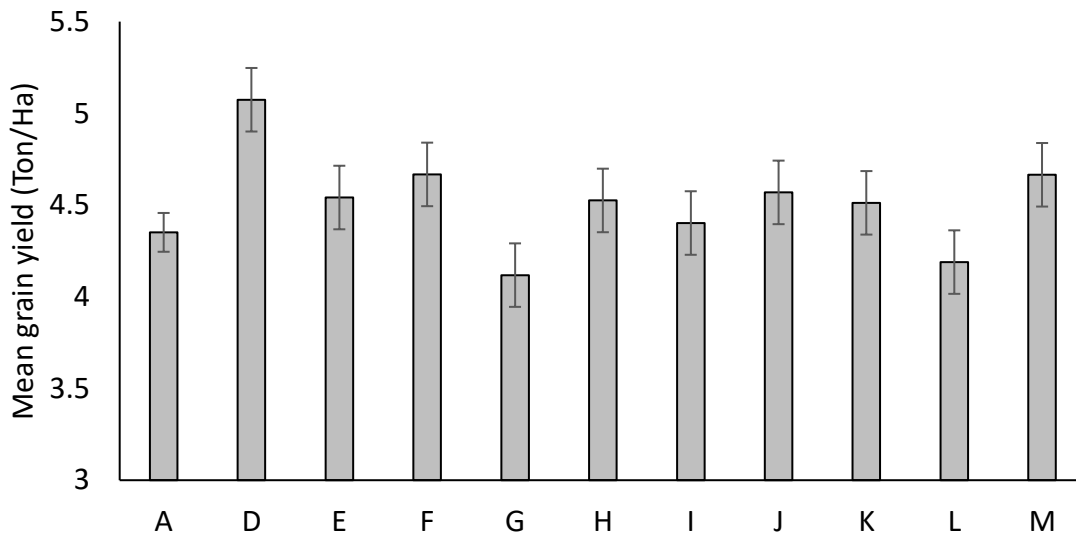
2750 4.4.9.1 Grain yield:

2751 In **West Barnfield** the trap crop treatment showed increased grain yield compared to the

2752 control (tons/ha, $F_{1,8}=14.09$, $P < 0.001$). No effect was apparent from any insecticide

2753 applications (pyrethroid sprays: $F_{2,4}=2.05$, $P = 0.151$, neonicotinoid seed dressing: $F_{1,4}=0.13$, P

2754 =0.723, neonicotinoid spray: $F_{1,4}=0.01$, $P=0.904$, Lumiposa™ seed treatment: $F_{2,4}=2.51$, P
 2755 =0.103, Lumiposa™ and neonicotinoid spray $F_{2,4}=0.37$, $P=0.694$). See Figure 27.

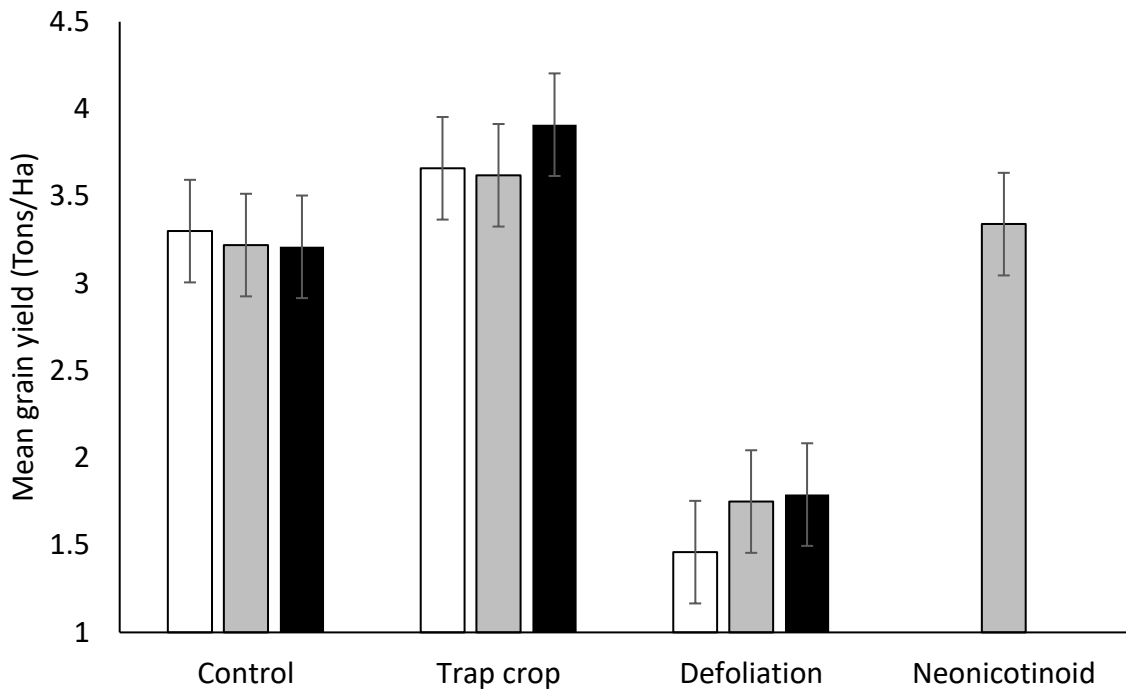


2756

2757 Figure 27. West Barnfield: Mean grain yield of oilseed rape under different treatments for
 2758 control of *Psylliodes chrysocephala*. Treatment code: (A) OSR – with nurse crop mix, (D) OSR
 2759 – trap crop border, (E) OSR – with neonicotinoid seed treatment – Cruiser, (F) OSR treated
 2760 with neonicotinoid seed dressing - Cruiser + low pyrethroid spray (Hallmark, x1 application),
 2761 (G) OSR treated with neonicotinoid seed dressing - Cruiser + high pyrethroid spray
 2762 (Hallmark, x3 application), (H) OSR untreated (no seed dressing) + low pyrethroid spray
 2763 (Hallmark, x1 application), (I) OSR untreated (no seed dressing) + high pyrethroid spray
 2764 (Hallmark, x3 application), (J) OSR treated with Lumiposa™ seed dressing, (K) OSR treated
 2765 with Lumiposa™ seed dressing + neonicotinoid spray (Biscaya), (L) OSR no seed dressing +
 2766 Neonicotinoid spray (Biscaya), and (M) OSR treated with neonicotinoid seed dressing -
 2767 Cruiser + neonicotinoid spray (Biscaya). Treatment (B) OSR - with nurse crop and (C) OSR –
 2768 with nurse crop and trap crop border are not represented due to abandonment of
 2769 treatments before harvest. SE shown.

2770 **Great Field:** Reductions in grain yield were observed for the defoliation treatments (Grain
 2771 tons/Ha: $F_{2,18}=80.95$, $P < 0.001$ see Figure 28). Seed rate did not affect the amount of grain
 2772 produced ($F_{2,18}=0.49$, $P = 0.622$).

2773



2774

2775 Figure 28. GreatField: Mean grain yield expressed as tonnes per hectare. Control – OSR
 2776 monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation
 2777 – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with
 2778 neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m²; grey – 100
 2779 seeds/m² and black – 120 seeds/m². SE shown.

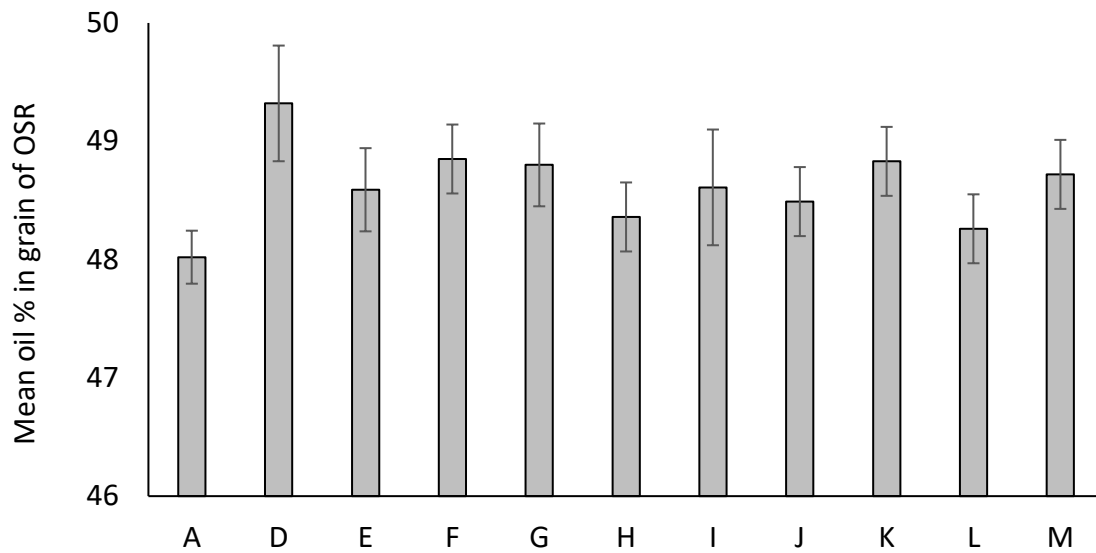
2780

2781 4.4.9.2 Oil percentage in grain:

2782 **West Barnfield:** The oil percentage in grain was higher in the trap crop treatment ($F_{1,8}=6.77$;
 2783 $P=0.019$) and tended towards higher in the neonicotinoid seed dressing ($F_{1,4}=4.14$, P
 2784 $=0.061$). There was no difference between control and pyrethroid spray ($F_{2,4}=2.59$, P

2785 =0.109), neonicotinoid spray ($F_{1,4}=2.80$, $P=0.114$) or the use of Lumiposa™ seed treatment
2786 ($F_{2,4}=3.02$, $P=0.080$) was observed (Figure 29).

2787



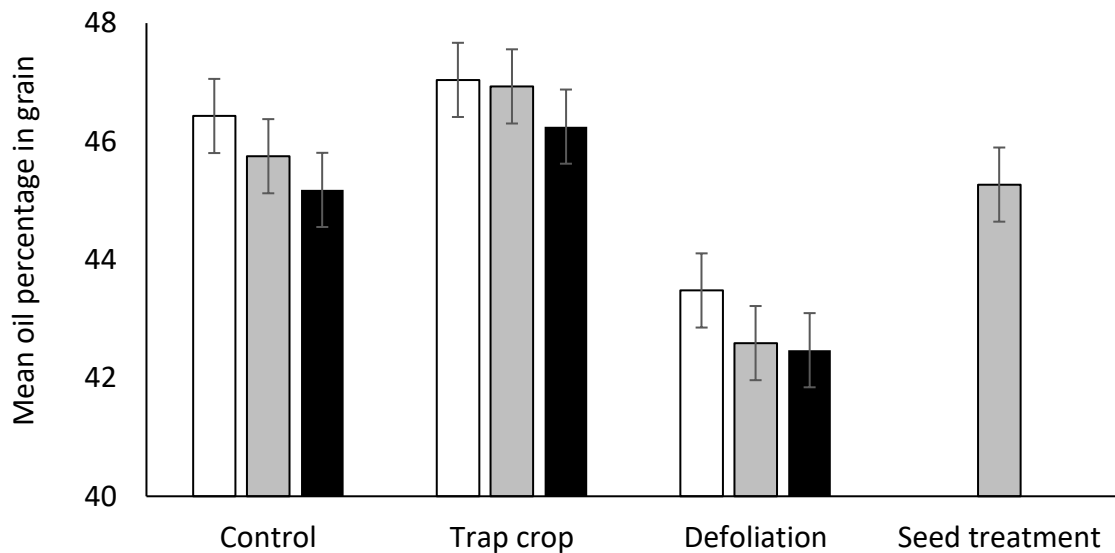
2788

2789 Figure 29. West Barnfield, mean (\pm SE) oil percentage of oilseed rape seeds for each
2790 treatment. Treatment code: (A) OSR – with nurse crop mix, (D) OSR – trap crop border, (E)
2791 OSR – with neonicotinoid seed treatment – Cruiser, (F) OSR treated with neonicotinoid seed
2792 dressing - Cruiser + low pyrethroid spray (Hallmark, x1 application), (G) OSR treated with
2793 neonicotinoid seed dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H)
2794 OSR untreated (no seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR
2795 untreated (no seed dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR
2796 treated with Lumiposa™ seed dressing, (K) OSR treated with Lumiposa™ seed dressing +
2797 neonicotinoid spray (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and
2798 (M) OSR treated with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya).
2799 Treatment (B) OSR - with nurse crop and (C) OSR – with nurse crop and trap crop border are
2800 not represented due to abandonment of treatments before harvest. SE shown.

2801

2802 **Great Field:** Defoliation showed a reduction in the oil percentage in grain ($F_{2,18}=4.01$, P
 2803 $=0.036$). There was evidence of inverse relationship between seed rate and oil % in grain;
 2804 with the lower seed rate showing significantly greater yield ($F_{2,18}=4.01$, $P=0.036$, Figure 30).

2805



2806

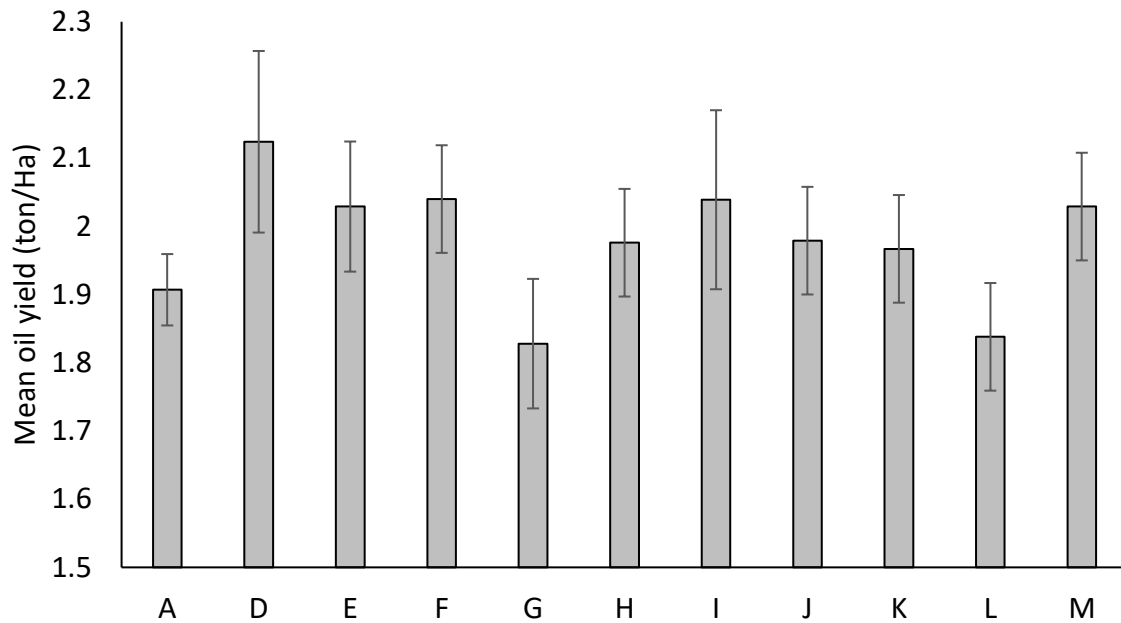
2807 Figure 30. Great field mean oil % in grain of oilseed rape (OSR, *Brassica napus*). Control –
 2808 OSR monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border;
 2809 Defoliation – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR
 2810 treated with neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m²; grey
 2811 – 100 seeds/m² and black – 120 seeds/m². SE shown.

2812

2813 4.4.9.3 Oil yield tonnes per hectare:

2814 **West Barnfield:** The total oil yield when expressed as tonnes per hectare was unaffected by
 2815 any treatment (Trap crop: $F_{1,8}=1.90$, $P=0.186$, pyrethroid spray: $F_{2,4}=0.76$, $P=0.484$,
 2816 neonicotinoid seed dressing: $F_{2,4}=0.30$, $P=0.592$, neonicotinoid spray: $F_{1,4}=0.01$, $P=0.943$,

2817 Lumiposa™: $F_{2,4}=1.93$, $P=0.175$, or Lumiposa and neonicotinoid spray: $F_{2,4}=0.12$, $P=0.884$,
 2818 Figure 31).



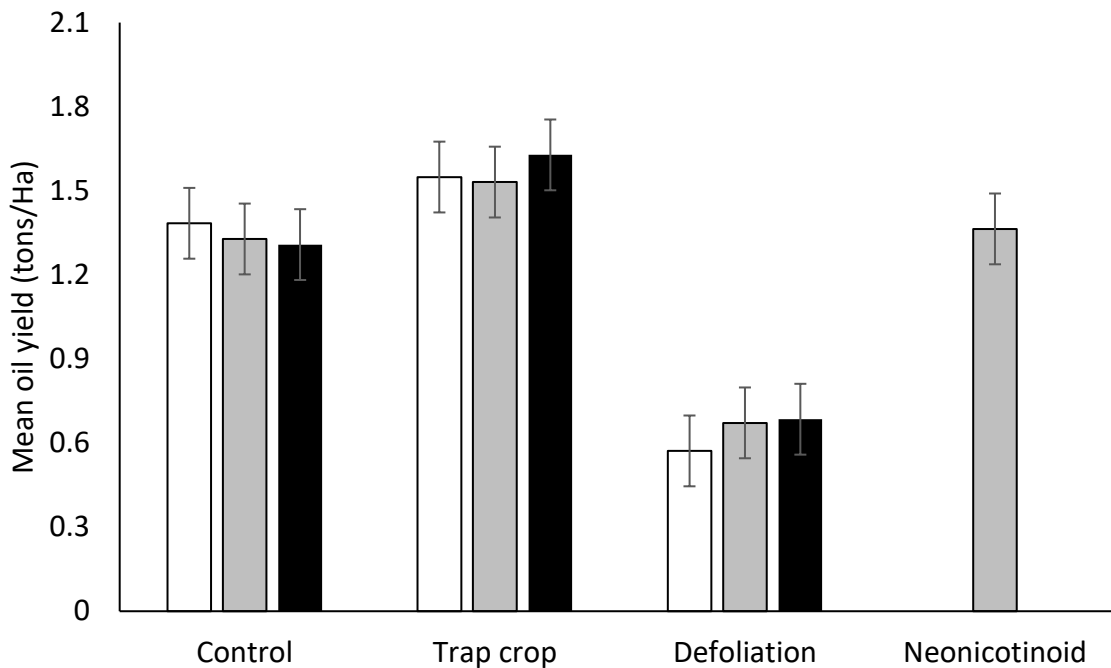
2819

2820 Figure 31. West Barnfield: Mean oil yield in terms of tons per hectare. Treatment code: (A)
 2821 OSR – with nurse crop mix, (D) OSR – trap crop border, (E) OSR – with neonicotinoid seed
 2822 treatment – Cruiser, (F) OSR treated with neonicotinoid seed dressing - Cruiser + low
 2823 pyrethroid spray (Hallmark, x1 application), (G) OSR treated with neonicotinoid seed
 2824 dressing - Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no
 2825 seed dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed
 2826 dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR treated with Lumiposa™
 2827 seed dressing, (K) OSR treated with Lumiposa™ seed dressing + neonicotinoid spray
 2828 (Biscaya), (L) OSR no seed dressing + neonicotinoid spray (Biscaya), and (M) OSR treated
 2829 with neonicotinoid seed dressing - Cruiser + neonicotinoid spray (Biscaya). Treatment (B)
 2830 OSR - with nurse crop and (C) OSR – with nurse crop and trap crop border are not
 2831 represented due to abandonment of treatments before harvest. SE shown.

2832

2833 **Great Field:** The oil yield expressed as tonnes per hectare was not affected by seed rate
 2834 ($F_{2,4}=0.56$, $P=0.588$) but was reduced by defoliation ($F_{2,4}=183.57$, $P<0.001$), see Figure 32.

2835



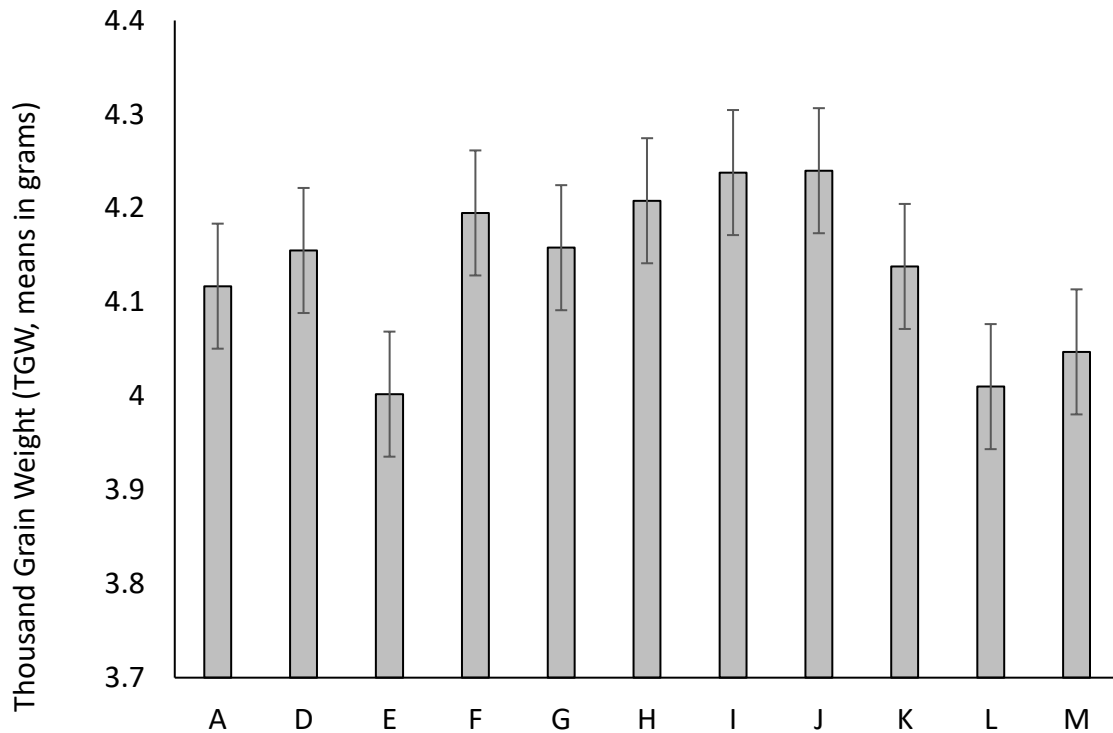
2836

2837 Figure 32. GreatField: Mean oil yield expressed as tonnes per hectare. Control – OSR
 2838 monoculture; Trap crop – OSR with turnip rape (*Brassica rapa*) trap crop border; Defoliation
 2839 – OSR cut to 5cm before stem elongation (14/03/2017); Neonicotinoid = OSR treated with
 2840 neonicotinoid (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m²; grey – 100
 2841 seeds/m² and black – 120 seeds/m². SE shown.

2842

2843 4.4.9. 4. Thousand Grain Weight:

2844 **West Barnfield:** There was a significant effect of treatment on thousand grain weight
 2845 ($F_{10,26}=2.22$, $P=0.05$) with E, L and M (OSR – neonicotinoid seed treated, OSR neonicotinoid
 2846 spray and OSR – neonicotinoid seed treated and neonicotinoid spray) showing lower
 2847 weights than the other treatments (Figure 33).



2848

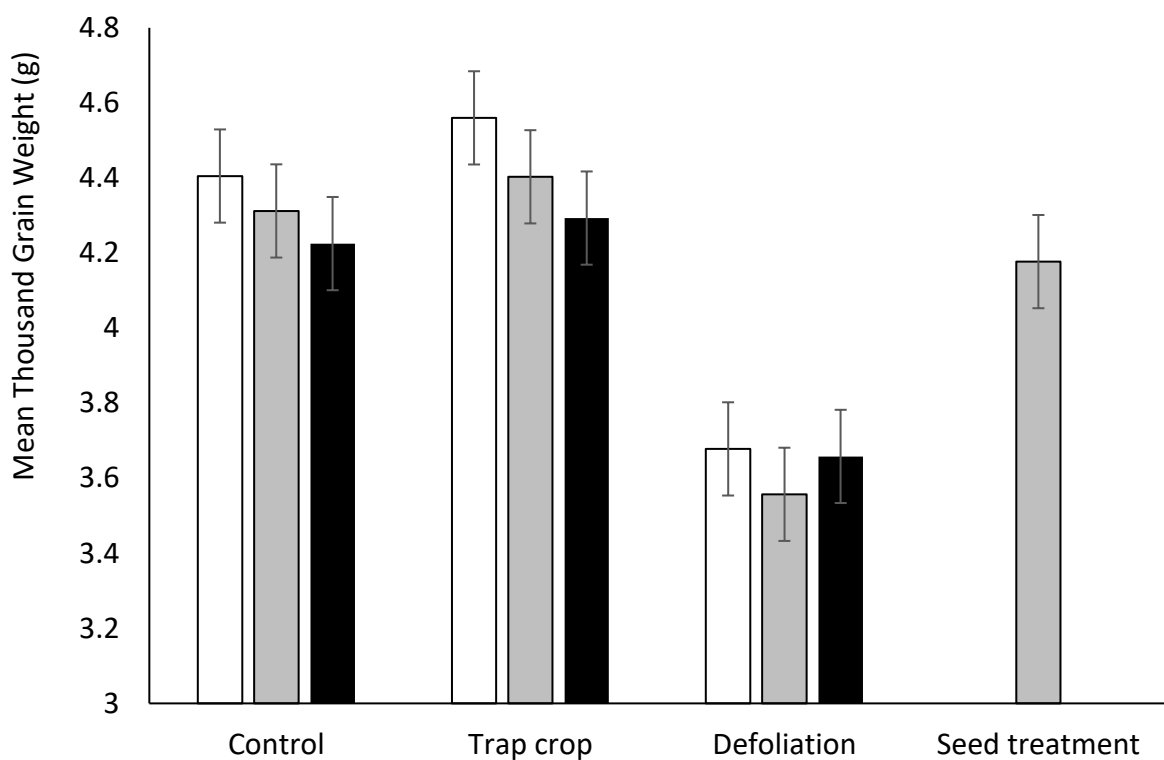
2849 Figure 33. West Barnfield: Mean Thousand Grain Weight (TGW) for oilseed rape (OSR).
 2850 Treatment code: (A) OSR – with nurse crop mix, (D) OSR – trap crop border, (E) OSR – with
 2851 neonicotinoid seed treatment – Cruiser, (F) OSR with neonicotinoid seed dressing - Cruiser +
 2852 low pyrethroid spray (Hallmark, x1 application), (G) OSR with neonicotinoid seed dressing -
 2853 Cruiser + high pyrethroid spray (Hallmark, x3 application), (H) OSR untreated (no seed
 2854 dressing) + low pyrethroid spray (Hallmark, x1 application), (I) OSR untreated (no seed
 2855 dressing) + high pyrethroid spray (Hallmark, x3 application), (J) OSR with Lumiposa™ seed
 2856 dressing, (K) OSR with Lumiposa™ seed dressing + neonicotinoid spray (Biscaya), (L) OSR no
 2857 seed dressing + neonicotinoid spray (Biscaya), and (M) OSR with neonicotinoid seed dressing
 2858 - Cruiser + neonicotinoid spray (Biscaya). Note: Treatment (B) OSR - with nurse crop and (C)
 2859 OSR – with nurse crop and trap crop border are not presented due to abandonment of
 2860 nurse crop treatments before harvest.

2861

2862

2863 **Great Field:** There was a significant effect of management on TGW with reduction in
 2864 defoliation treatments ($F_{2,4}=95.22, P<0.001$). There was no significant effect from seed rate
 2865 ($F_{2,4}=2.60, P=0.134$) although a trend of reducing TGW with increasing seed rate was
 2866 observed (Figure Figure 334). There was no effect of neonicotinoid seed treatment
 2867 ($F_{1,4}=0.00, P=0.989$).

2868



2869

2870 Figure 34. Great field Thousand Grain Weight (TGW) of oilseed rape (OSR). Control – OSR
 2871 monoculture; Trap crop – OSR with turnip rape trap crop border; Defoliation – OSR cut to
 2872 5cm before stem elongation (14/3/2017); Neonicotinoid – OSR treated with neonicotinoid
 2873 (Cruiser). Showing for OSR seed rates: clear – 60 seeds/m²; grey – 100 seeds/m² and black –
 2874 120 seeds/m². SE shown.

2875

2876 **4.4.10. Ranking treatment:**

2877 **West Barnfield:** To better interpret the findings of the West Barnfield experiment, each
2878 measurement (leaf injury, larval loading, and yield measurements) was given a relative score
2879 for each treatment. Using the treatment means derived from the statistical analyses, the
2880 relative rank for each treatment was calculated. The level of pest control was ranked lowest
2881 to highest leaf area injury and number of larvae per plant. All harvest measurements were
2882 ranked from highest to lowest. This produced a relative rank for each treatment whereby
2883 the lower the score the lower the pest pressure and the higher the yield (Table 12).

2884 Table 12. West Barnfield. Relative ranking of treatment effects on pest control and end oilseed rape (OSR, *Brassica napus*) when under
 2885 multiple autumn pest protection practices. Values taken from data and set-in numerical ranking from best performance (lowest pest injury and
 2886 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
 2887 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	

2888

2889 **4.5 Discussion:**

2890

2891 **4.5.1 Overview:**

2892 No method of pest protection used in this study was significantly different in the end yield
2893 to the untreated control monoculture. This included the use of neonicotinoid seed dressing
2894 and would suggest that the ban on its use in the EU is justified as it provided little actual
2895 crop protection. The use of pyrethroid sprays did reduce the larval infestation levels in
2896 February, suggesting there is still some level of larval control if not control of the adults of *P.*
2897 *chrysocephala*. The defoliation of OSR did show reductions in larval numbers and did not
2898 reduce the number of harvestable plants, however timing issues lead to secondary pest
2899 problems in this study, but there is evidence that this method can reduce larvae numbers
2900 without increasing plant mortality and further work is needed to avoid secondary pest
2901 issues.

2902

2903 **4.5.2 *Psylliodes chrysocephala* migration:**

2904 When measuring migration of *Psylliodes chrysocephala* into the field a bias towards greater
2905 catches of beetles further into the field was observed (Figure 8 and Figure 9). The within
2906 field distribution of *P. chrysocephala* has been shown to be patchy within the central area of
2907 the crop (Thioulouse, Debouzie and Ballanger, 1984). Suggesting that *P. chrysocephala*
2908 migrated into the central area of the crop before distributing within the crop (Ferguson *et*
2909 *al.*, 2006; Warner *et al.*, 2003).

2910 It was also apparent in both fields that the beetles do not enter the crop from all sides
2911 equally (Figure 8 and Figure 9). A clear bias in terms of number of beetles captured along
2912 the southern edge in GreatField and the southern and eastern edges in West Barnfield.
2913 From the data collected during this experiment it is not possible to determine if this
2914 directional bias to migration is consistent between years. With multiple-year sampling of
2915 OSR fields and directionality of migration it would be possible to determine if in a given field
2916 the beetles enter on a known edge each year. With this information a more informed
2917 decision on the location of a trap crop border could be applied along the edges of main
2918 migration routes. A single trap crop border may be able to be placed in the direct line of
2919 primary migration and at a larger block area and not a strip boarder. Further recordings on
2920 *P. chrysocephala* migration into crops is required to develop appropriate trap cropping
2921 systems. The field migration of pollen beetles (*Brassicogethes aeneus*) has been shown to be
2922 heavily effected by wind direction (Mauchline *et al.*, 2017). This knowledge can allow trap
2923 crop boarders to be located in the best location to disrupt pests entering the crop, as a
2924 boarder functions with *B. aeneus* (Cook *et al.*, 2007).

2925

2926 **4.5.3 Past: neonicotinoid seed treatment and foliar spray:**

2927 In the trial reported here there was no benefit to treating seed either with a neonicotinoid
2928 (AI: thiamethoxam) or with Lumiposa™ (AI: cyantraniliprole) as there was little to difference
2929 when compared to untreated control plants in terms of autumn pest levels and cropping
2930 yield. The level of protection bestowed on OSR by neonicotinoid seed treatment was not
2931 enough to protect OSR in 2015 at Rothamsted (chapter 3 this thesis). The concerns of
2932 farmers that OSR cannot be grown without neonicotinoids (UK, 2020) are unsubstantiated

2933 by this trial. With no yield increase or pest protection increases seen compared to controls
2934 the extra cost of insecticide application would be unnecessary in the 2016-17 season.
2935 Recent reviews on the loss of neonicotinoid from the OSR highlights the concerns of farmers
2936 on the issues regarding implementation and seed bed preparation of OSR being inhibited by
2937 *P. chrysocephala* (Kathage *et al.*, 2018). Here the perceived efficacy of neonicotinoid seed
2938 dressing is brought into doubt and any further examination of its use should be based on
2939 empirical evidence of actual pest control. With the current ban imposed primarily to limit
2940 exposure to pollinating insects (Goulson, 2013) the limited benefit to the crop reported here
2941 requires further investigation.

2942

2943 **4.5.4 Current: Pyrethroid foliar spray insecticides:**

2944 The level of resistance in both experimental fields was shown to be high (49% and 85%).
2945 However, these measures were taken after pyrethroid application in the fields and suggest
2946 that non-resistance beetles were killed during applications. The application of spray
2947 insecticides (pyrethroid) did not significantly affect the level of leaf area injury during the
2948 autumn. However, reductions in *P. chrysocephala* larvae loading were observed for
2949 pyrethroid spray applications in the following year. This reduction in a field which was
2950 shown to have up to 84.9% resistance in adults (Table 54) suggests that pyrethroids are less
2951 affective against adult beetles but do still show some control of larval stages of *P.*
2952 *chrysocephala*.

2953

2954 **4.5.5 Current: Seed application rate:**

2955 A significant effect of seed rate was apparent in September ($F_{2,4}=5.04$, $P=0.021$) with
2956 decreasing injury levels as seed rate increased. This was not significant in the October
2957 assessment ($F_{2,4}=0.72$, $P=0.072$). The effect of seed rate has shown to be highly variable
2958 between space and time (White *et al.*, 2020). Increases in yield were apparent as the seed
2959 rate applied reduced, recording declining yield return with increasing seed rate. The
2960 mechanism behind this observation may be due to competition between conspecific plants
2961 (Berry and Spink, 2006). These data are in line with current recommendations of increasing
2962 seed rate as a pest prevention measure (AHDB, 2019). However, in years of lower *P.*
2963 *chrysocephala* pressure the benefit of increased seed rate may be outweighed by the
2964 increase in plant-plant competition. In years of high *P. chrysocephala* reductions in
2965 individual plant injury can be reduced by increased seed application (chapter 2 this thesis).
2966 In the season of these experiments the *P. chrysocephala* population was not as large or as
2967 damaging as seen in other years (chapter 2 and 3, this thesis). It is suggested here that
2968 improved yields can be achieved at low seed rate and that recommendations of increasing
2969 seed rate as a pest protection measure can reduce levels of per plant injury as reported
2970 here. However, it is not enough in years of high pest abundance as seen in 2015 (chapter 2
2971 this thesis). In years where pressure is not so severe as in that reported here the increase in
2972 seed rate did not show a significant yield penalty. This may be partly down to reductions in
2973 later pest pressure from *B. aeneus* from increased plants/m² (AHDB, 2021).

2974

2975 **4.5.6: Future cultural: Defoliation:**

2976 Reductions in larval loading from defoliation has been reported when the crop is topped
2977 and when grazed by sheep (White *et al.*, 2020). In the trial reported here the defoliation
2978 did result in a significant reduction in larvae number but reduced the end yield. The
2979 defoliation did not affect the final plant density, supporting the hypothesis that OSR can
2980 tolerate high levels of injury. The OSR defoliation treatment plots were mown in March (14th
2981 March 2017) just before stem extension. This was to maximise the number of larvae
2982 removed by the treatment but could have been too late and may have contributed to the
2983 yield reduction in this treatment (Clarke, 1978; Freyman, Charnetski and Crookston, 1973;
2984 Kirkegaard *et al.*, 2008; Seymour *et al.*, 2015). In other studies, defoliation was performed
2985 earlier and did not result in yield loss (Kirkegaard *et al.*, 2012; McCormick, Virgona and
2986 Kirkegaard, 2013; Spink, 1992; Susko and Superfisky, 2009).

2987 The treatments which were defoliated exhibited a delay in the start of flowering compared
2988 to the un-cut treatments and surrounding crop. This delay led to the defoliated plants being
2989 at bud stage when the rest of the crop was in full flower. *Brassicogethes aeneus* are known
2990 to enter flower buds to access pollen and that this can cause bud abortion (Seimandi-Corda,
2991 Jenkins and Cook, 2021). The yield reductions reported here may be due to high levels of
2992 pollen beetle causing greater pod aborting in the defoliated plants. Previous studies on the
2993 impact of defoliation to OSR yield have been carried out on a larger scale (White *et al.*,
2994 2020; Syrový, Shirtliffe and Zarnstorff, 2016; Seymour *et al.*, 2015). The hypothesis of
2995 reducing *P. chrysocephala* larvae through defoliation and the crop surviving the injury has
2996 been shown here and in other studies in the UK (White *et al.*, 2020) and on spring OSR in
2997 Canada and Australia (Kirkegaard *et al.*, 2012). Further examination on the timings on

2998 grazing in spring and winter OSR in Australia demonstrate the importance of livestock
2999 removal before stem elongation (Sprague *et al.*, 2014). The method of defoliation was also
3000 an important consideration for farmers surveyed on their opinions of novel pest protection
3001 methods; these farmers raised concerns about using machinery on the crop in winter/ early
3002 spring on wet soil and damaging the soil surface (chapter 8 this thesis); farmers tending to
3003 prefer the method of sheep grazing to reduce damage to soil structure (chapter 8 this
3004 thesis).

3005

3006 **4.5.7 Future cultural: trap cropping and nurse cropping:**

3007 The nurse crop treatments were removed after winter (May) due to a mild winter and
3008 limited effect of the Clearfield herbicide to remove the nurse species. The farm
3009 management were concerned about nurse species and the turnip rape trap crop seeding
3010 and causing potential problems with weed contamination of the field site in future years.
3011 The nurse crops did provide a level of crop protection at early growth with reductions on
3012 the level of leaf area injury on the OSR crop when in association with a nurse crop. This
3013 provided evidence that nurse cropping can reduce levels of early growth injury to OSR crop
3014 plants as shown in chapter 2 (this thesis). However, the difficulty to successfully control the
3015 nurse crops without removing the OSR crop highlights the complexity of nurse cropping with
3016 Brassicas. Prior to its removal the nurse crop did demonstrate that Pak choi and Chinese
3017 cabbage are suitable host plants for *P. chrysocephala*. However, this did result in higher
3018 numbers of larvae in the nurse crop treatment.

3019 The trap crop borders did reduce the levels of *P. chrysocephala* injury to the OSR crop. They
3020 were shown to contain more larvae than the OSR confirming the findings of Barari *et al.*,

3021 (2005). This supports the hypothesis that turnip rape can be used as a means of
3022 manipulating *P. chrysocephala* feeding and interactions with the OSR crop. However, the
3023 boarders were removed in early spring, due to farm concerns about seed drop and
3024 subsequent volunteers. Therefore, any benefit from the trap was only conferred during the
3025 autumn and winter. This reduced the potential positive effect of trap crops on reducing
3026 infestation in the OSR plots by subsequent pests such as pollen beetle (Cook and Denholm,
3027 2008; Cook *et al.*, 2007; Gotlin Čuljak *et al.*, 2016). This increased the open area (1m strip)
3028 around the central treatment area which were then exposed to higher levels of sunlight. The
3029 increased yield observed in this treatment may be due to increased photosynthetic
3030 potential, a primary factor in yield formation (Diepenbrock, 2000), which cannot be
3031 confirmed or discounted in this study.

3032

3033 **4.5.8. Future synthetic control:**

3034 Lumiposa™ did not significantly reduce leaf area damage or infestation of *P. chrysocephala*
3035 larvae over untreated-seed control. Larval infestation was actually found to be higher in
3036 Lumiposa™ treated seed than in plants sown using untreated seed. No significant
3037 improvement in yield was observed over untreated seed. However, like all treatments in this
3038 study effects may have been masked due to the small plot size used. Larger field trials are
3039 required to understand the levels of protection Lumiposa™ can provide an OSR crop. The use
3040 of a neonicotinoid foliar spray also converted little benefit to the crop in levels of pest control
3041 and in the end crop yield.

3042

3043 **4.5.7 Conclusions:**

3044 Data presented here suggests that alternative non-synthetic chemical-based pest protection
3045 methods are equal to the task of defending OSR from *P. chrysocephala* and show
3046 comparable yield returns to both types of synthetic insecticides used in this experiment.
3047 This suggests that further research into the functionality of trap cropping as an autumn pest
3048 protection method should prove fruitful. Issues with pollen beetle restricted any meaningful
3049 measurements to be taken from the cutting treatments tested here. However, *P.*
3050 *chrysocephala* larval levels supports previous studies showing OSR can recover via
3051 compensatory growth from cutting with reduced internal larvae loading. The data presented
3052 here suggest that growing OSR in the post-neonicotinoid era is possible in the UK.

3053

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3062

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3131 [statistics-reveal-jump-](https://www.fwi.co.uk/arable/harvest/harvest-statistics-reveal-jump-in-cereal-yields-and-osr-woes?name=harvest-statistics-reveal-jump-in-cereal-yields-and-osr-woes&category_name=arable%2Fharvest)
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3228
3229

3230 **5.0 The impact of injury caused by *Psylliodes chrysocephala* L.**
3231 **adults and larvae on flower and yield production in *Brassica rapa* L:**

3232

3233 **5.1 Abstract:**

3234

3235 Protecting crops from pest attack is a major undertaking in modern agriculture. Under
3236 current EU legislation the use of neonicotinoid seed dressing is banned in oilseed rape (OSR,
3237 *Brassica napus* L.), an important oil crop and break crop in the UK. This restriction and the
3238 reduction in efficiency of other synthetic chemical alternatives has exacerbated pest
3239 pressure from the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) a pest which can
3240 threaten establishment in OSR. In two experiments, the ability of OSR to compensate for
3241 injury caused by adult and larval stages of *P. chrysocephala* was assessed. Leaves on
3242 experimental plants were injured by either simulated shot holing or inoculating plants with
3243 larvae at different densities. Both experiments showed that leaf area loss at early growth of
3244 OSR can be compensated for. Whilst there was no effect of inoculating with <5 larvae,
3245 significant impacts were observed when 25 were introduced. The data presented here
3246 suggest that higher numbers of larvae reduce plant productivity in terms of number of
3247 flowers produced and the harvestable yield. The implications for flower visiting insects and
3248 farming productivity are discussed.

3249

3250

3251 **5.2 Introduction:**

3252

3253 Oilseed rape (OSR, *Brassica napus* L.) is the second most produced vegetable oil crop
3254 globally (Shahbandeh, 2020) and the primary oil crop in Europe (Commission, 2020) where it
3255 forms an important component of the agricultural rotation with cereals (Redman, 2019).

3256 *Psylliodes chrysocephala* L. (cabbage stem flea beetle) is one of the most important autumn
3257 pests of winter OSR in the UK and coastal areas of Europe causing direct injury in two
3258 distinct ways: (i) adult beetles feeding on the cotyledons and leaves of plants in the early
3259 stages of crop establishment cause characteristic 'shot-holing' injury which can threaten
3260 crop establishment, and (ii) the stem-boring larvae which feed within the leaf petioles and
3261 stem, weakening the plant and increasing its susceptibility to secondary infections, e.g. stem
3262 canker *Leptosphaeria maculans* Ces & Not (Williams, 2010).

3263 Since the new millennium *P. chrysocephala* has typically been controlled through the use of
3264 neonicotinoid insecticides; these act systemically, entering all plant tissues (Bass and Field,
3265 2018), and can be applied as seed coatings to protect the crop from germination.

3266 Neonicotinoid seed treatments are considered to confer effective pest control during the
3267 early growth stages of the crop (Dewar *et al.*, 2016) and were designed to protect crop
3268 establishment (Bass and Field, 2018). If pest pressure is still evident after the efficacy of
3269 seed treatment has reduced the application of spray insecticide in the pyrethroid group was
3270 recommended for further protection from *P. chrysocephala* pressure. However, due to the
3271 systemic nature of neonicotinoids there has been growing global concern about their impact
3272 on non-target species (Blacquiere *et al.*, 2012; Pereira, Diniz and Takasusuki, 2020). In 2013,
3273 this led the EU to impose restrictions in their use on crops used by bees, including OSR. This

3274 was extended (2018) to a blanket ban on their use outside (EU, 2018) and calls to review of
3275 their use in the USA, Canada, Australia, New Zealand (Government, 2019; Zealand, 2018) and
3276 some parts of Africa (NASAC). In the absence of neonicotinoids, growers are increasingly
3277 relying on the use of the pyrethroid insecticides alone (Nicholls, 2016). This overdependence
3278 of a single insecticide group has in effect stalled any insecticide resistance management
3279 strategy and exacerbated the development and spread of resistance to pyrethroid
3280 insecticides, a phenomenon already being reported in *P. chrysocephala* across the EU
3281 (Bothorel *et al.*, 2018; Brandes and Heimbach, 2018; Foster and Williamson, 2015; Gavloski
3282 *et al.*, 2000; Højland and Kristensen, 2018; Højland *et al.*, 2015; Zimmer *et al.*, 2014; Willis
3283 *et al.*, 2020). This originally raised concerns that OSR cultivation may be threatened by injury
3284 from *P. chrysocephala* in the UK and on the European continent without adequate synthetic
3285 control options (Zhang *et al.*, 2017). This has been realised in the UK with reductions in the
3286 area of winter OSR grown from 621,000 ha in 2014 before the ban (DEFRA, 2014a) to
3287 337,000 ha in 2020 (DEFRA, 2020), with reluctance to grow OSR attributed to *P.*
3288 *chrysocephala* now causing potential loss of OSR from rotations (Dyer, 2019). With no
3289 alternative synthetic control options, it is crucial to better understand what level of injury
3290 the crop can compensate for to ensure the minimum level of application and thus reduce
3291 exposure of the beetle reducing the pressure leading to resistance. The decision for
3292 application is often based on an economic action threshold, i.e. the abundance of a pest
3293 above which yield deficit is greater than the cost of management implementation (Pedigo,
3294 Hutchins and Higley, 1986).

3295 Economic action thresholds for pyrethroid insecticides against *P. chrysocephala* exist in
3296 Europe and vary slightly between countries; of these, the UK has the most conservative
3297 thresholds (14). Growers are advised to apply insecticide: (i) against adult injury at the first

3298 sign of attack if the risk is high during emergence of cotyledons or when >25% of leaf area
3299 has been lost between cotyledons unfolding and the 2 leaf stage or when 50% of the leaf
3300 has been eaten at the 3-4 leaf stage or when the crop is growing more slowly than it is being
3301 eaten (Oakley, 2003; AHDB, 2019) or (ii) against larvae when the mean number exceeds five
3302 larvae per plant; this threshold was recently increased from two larvae per plant and was
3303 initially set due to the low cost of application (Green, 2008) but was increased due to the
3304 high incidence and spread of resistance (AHDB, 2019).

3305 Data on the level of larval infestation of OSR in the UK has shown an increasing trend in
3306 occurrence and abundance of *P. chrysocephala* larvae in the UK following the ban on
3307 neonicotinoid seed treatments in 2013, ranging from 88% of plants with scaring (related to
3308 larval density) in the East of England 59% in the North, although the national average
3309 remains below the five larvae per plant action threshold (FERA, 2020). This trend for
3310 increasing abundance has raised the concern for the pest and requires more research to
3311 understand the carrying capacity of OSR for *P. chrysocephala* larvae without suffering loss in
3312 yield.

3313 Oilseed rape has been shown to have high levels of compensation capacity to leaf area loss
3314 or defoliation, given time (Ellis, 2015a; Nowatzki. T and Weiss. M, 1997). Research on early
3315 growth OSR tolerance to injury has focused on rate of biomass accumulation (Nowatzki. T
3316 and Weiss. M, 1997; Ellis, 2015a; DEFRA, 2014b; White *et al.*, 2020) or production of
3317 glucosinolate as a response to injury (Döring and Ulber, 2020; Koritsas, Lewis and Fenwick,
3318 1991; Koritsas, Lewis and Fenwick, 1989). These studies did not take the OSR to yield and
3319 have not recorded any long-term compensation capacity. It has been shown that OSR can

3320 tolerate high levels of defoliation (from grazing by sheep or mowing) if the injury occurs
3321 prior to stem elongation (Kirkegaard *et al.*, 2008a; Kirkegaard *et al.*, 2012).

3322 The threshold of 5 larvae is derived from Purvis (1986) where the efficacy of
3323 organophosphates to control *P. chrysocephala* was tested, however, as noted by the author,
3324 the yield responses seen at a mean of 5 larvae/plant may have been due or partially due to
3325 the benefits from insecticidal action on reducing virus vectors (aphids). More recent studies
3326 have been done assessing larval development (Döring and Ulber, 2020) and OSR
3327 glucosinolate production (Koritsas, Lewis and Fenwick, 1989; Koritsas, Lewis and Fenwick,
3328 1991), but none have taken the OSR to maturity and direct relationship between injury and
3329 larval infection on yield is still lacking from the literature.

3330 Previous studies on *P. chrysocephala* larval feeding have used complex collection techniques
3331 and specialised equipment to rear the larvae (Döring and Ulber, 2020) or have not provided
3332 detailed information on how the larvae were collected (Koritsas, Lewis and Fenwick, 1991).

3333 This study is the first the authors are aware of that manipulates in a controlled way *P.*
3334 *chrysocephala*- adult-induced leaf injury and infestation using field collected larvae to
3335 quantify their combined effects on flower production, floral rewards, and seed production
3336 in OSR.

3337 **5.2.1 Aims of study:**

3338

3339 This study aimed to quantify the direct impact of *P. chrysocephala* injury on the productivity
3340 of OSR in terms of seed quantity and quality. The effects were assessed on potted plants in
3341 semi-field conditions over two harvest years. In experiment 1 (2017 cropping season), the
3342 leaves of the OSR plants were manually injured to varying degrees using a hole punch to
3343 simulate adult *P. chrysocephala* feeding in a consistent manner. In experiment 2 (2018
3344 cropping season), in addition to controlled leaf area injury, plants were inoculated with
3345 known numbers of *P. chrysocephala* larvae to test the interaction between the level of leaf
3346 area loss and levels of larval infestation. In experiment 2, measurements were made to test
3347 whether injury or larval infestation impacts flowering time, abundance, or floral resource
3348 quality. In both experiments the OSR was pot-grown and kept in pest-free mesh cages
3349 outdoors to maturity in order to assess the impact of injury on final yield.

3350

3351 **5.3 Methods:**

3352

3353 **5.3.1 OSR pot experiment 1 (2017): Leaf injury:**

3354 For experiment 1 (harvest: 2017) Winter OSR (cv. DK Imperial) plants were sown in
3355 staggered batches in individual plant plugs (19mm², 30mm depth) using a standard compost
3356 mix (Petersfield Products, Leicester, UK) and kept in an unlit, unheated glasshouse until
3357 germination had occurred. The plants were then transplanted to 18cm pots (13/02/2017),
3358 ensuring all plants had equal amounts of substrate, then placed in an outdoor net cage (4m
3359 x 4m x 2m, with a mesh gauge of 2mm (Garratt *et al.*, 2018)) to exclude pests and
3360 pollinators.

3361 Four levels of simulated leaf injury were applied (0%, 25%, 50% or 90% leaf area loss) to
3362 plants at two early growth stages (GS) expressed according to the BBCH scale (Lancashire *et*
3363 *al.*, 1991): cotyledons expanded (GS9) and the first true leaf extended (GS10). Simulated
3364 injury was applied 25 days after sowing (18/02/2017). A hole punch (3mm diameter) was
3365 used to remove a controlled amount of leaf area from plants, applying simulated injury as
3366 comparable as possible to actual *P. chrysocephala* feeding i.e. shot hole injury, as opposed
3367 to the cutting action caused by caterpillars; it has been shown in spring OSR that defoliation
3368 by cutting produced less biomass re-growth and less pods than hole-punch defoliated plants
3369 (Susko and Superfisky, 2009). Simulated injury was used as a proxy for adult feeding to allow
3370 careful control of the level of leaf area removal and to ensure standardization between
3371 replicates and treatments.

3372

3373 Following plant injury, two grids of 100 plants were set out; each comprised 10 randomized
 3374 blocks of 10 plants, with one plant of each level of leaf area loss (25%, 50% and 90%) at both
 3375 growth stages (GS9 and GS10) and two un-injured (0% injury) control plants for each GS in
 3376 each block. There were therefore eight treatments overall (Table 1), with each injury level
 3377 replicated 20 times for each growth stage group (GS9 and GS10) and the two control
 3378 treatments (0% injury) each replicated 40 times. The extra control treatments were added
 3379 to aid blocking structure and achieve the desired plant density. Plants were equally
 3380 distributed within each grid over an area of 2m², thus simulating a density of 50 plants/m²,
 3381 common for OSR crops (Roques and Berry, 2016), with a 1m gap between the grids. The
 3382 plants were placed on a metal mesh supported ~10cm from the ground and slug pellets
 3383 were spread on the ground underneath to prevent slug feeding. Plants were hand watered
 3384 until 19th of May 2017, when automatic irrigation was set up. Plants were maintained until
 3385 harvest (see section 2.3).

3386

3387 Table 1 Treatments to test the effect of *Psylliodes chrysocephala* feeding injury on oilseed
 3388 rape (*Brassica napus* OSR) plants in 2017. Feeding injury was simulated using a hole punch
 3389 to remove varying amounts of leaf material (0, 25, 50 and -90%) at two different growth
 3390 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

3391



3392

3393 Figure 1. Experiment in 2017, layout of treatments inside field cage at University of Reading,
3394 nearside 'grid 1' and far side 'grid 2'. All plants were laid out to a plant density of 50
3395 plants/m². Plant pots were supported off the ground on a metal grid to prevent slug injury.

3396 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%
 3397 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
 3398 (columns) with two controls (treatments 2 and 5) for each growth stage tested. Layout produced 50plants/m².

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

3399

3400 **5.3.2 Oilseed rape pot experiment 2 (2018): Leaf injury and larval**

3401 **infestation:**

3402 For experiment 2 (harvest 2018), Winter OSR (cv. DK Imperial) plants were grown in the
3403 same conditions as experiment 1 to reduce variation between years. Plants were sown on
3404 5/10/2017. Leaf area loss treatments were combined with subsequent controlled infection
3405 with *P. chrysocephala* larvae. As experiment 1 showed no effect of growth stage on effects
3406 of leaf area loss (see section 3.11), plants at the 1-2 true leaf stage were used (GS11-12) as
3407 these plants are more likely to infested with larvae than cotyledons (personal observation).

3408 Adult *P. chrysocephala* feeding was simulated using the same method as experiment 1 with
3409 0%, 25% and 90% leaf area removed, 55 days post sowing (29/11/2017). These were each
3410 followed 48 hours later by infection with *P. chrysocephala* larvae at zero, low or medium
3411 (current action threshold) levels of infestation (0, 1 and 5 larvae, respectively). Non-injured
3412 plants were also subjected to a high level of infestation (25 larvae). This sequence of
3413 simulated adult injury followed by larval infestation mimics the usual order of injury as it
3414 occurs in the field. Each of the resulting 10 treatments (Table 3) was replicated 12 times,
3415 with plants arranged in randomized blocks split into neighbouring grids comprising six blocks
3416 of 10 plants. Guard rows of additional OSR plants were grown around the experiments to
3417 allow each of the experimental plants to be within a density of 50 plants per m² and to allow
3418 comparable growth restrictions across all blocks, i.e., no experimental plants were located
3419 on the edges of the grids.

3420

3421 Table 3 Treatments in 2018 to test the impact of varying amounts of adult *Psylliodes*
 3422 *chrysocephala* feeding injury (0, 25, and 90% leaf area injury), varying larval infestation (0, 1,
 3423 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

3424

3425 Table 4 Layout of pots for second experiment (2018). Numbers represent the treatment
 3426 type; (1) 0 larvae/ 0 injury, (2) 1 larvae/ 0 injury, (3) 5 larvae/ 0 injury, (4) 25 larvae/ 0 injury,
 3427 (5) 0 larvae/ 25 injury, (6) 0 larvae/ 90% injury, (7) 1 larvae/ 25% injury, (8) 1 larvae/ 90%
 3428 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

3429



3430

3431 Figure 2. Second experiment (2018) layout of treatments inside field cage at University of
3432 Reading, nearside Grid 1 and far side Grid 2. All plants were laid out to a plant density of 50
3433 plants/m². Plant pots were supported off the ground on a metal grid to prevent slug injury.

3434

3435 **5.3.3 Simulation of shot holing leaf injury (experiments 1 and 2):**

3436 In both experiments 1 and 2 the OSR plants were injured using a leather punch to simulate
3437 the effects of *P. chrysocephala* feeding activity (3mm diameter hole punch, Figure3). The
3438 required level of injury was estimated by eye and all injury was done by the same individual
3439 (myself) to ensure consistency between treatments and comparability with assessment of
3440 adult feeding injury in the field (Chapters 2, 3 and 4, this thesis). It was important to ensure
3441 that the simulated injury was as comparable as possible to real insect feeding as it has been

3442 shown that the method of defoliation can have significant impacts on OSR growth (Susko
3443 and Superfisky, 2009).

3444 In experiment 1 for group 1 (GS: 10), the simulated feeding injury was applied as a total area
3445 of both the cotyledons, whereas for group 2 (GS: 11) the injury was applied to the first true
3446 leaf.

3447 In experiment 2 the simulated feeding injury was applied to the first two true leaves (GS 10-
3448 11) on 29th November 2017, and this was 48 hours prior to larval inoculation. This sequence
3449 of adult feeding injury of leaves followed by larval infestation of the stems mimics the usual
3450 order of injury as it occurs in the field.

3451



3452

3453 Figure 3. Simulated injury to winter oilseed rape (*Brassica napus*) (A) and the leather punch

3454 (B) set to 3mm to mimic *Psylliodes chrysocephala* adult feeding injury.

3455

3456 **5.3.4 Infestation of plants with *Psylliodes chrysocephala* larvae**

3457 **(experiment 2):**

3458 Larvae of *P. chrysocephala* were obtained from an untreated crop of OSR (cv. Campus) on
3459 Rothamsted Farm, Harpenden, UK in December 2017. Live larvae were carefully extracted
3460 from the plants by cutting open the stems and petioles using a scalpel under a light
3461 microscope and removing the larvae with a paint brush. The 2nd instar larvae (determined
3462 using the key by Ebbe-Nyman (1952)) were transferred to Petri dishes lined with damp filter
3463 paper and kept in a dark fridge at 3-4°C prior to plant infection. Second instar larvae were
3464 selected as they were more robust (less prone to desiccation) and had a higher infection
3465 success rate than either 1st or 3rd instars (unpublished preliminary trials); they were also
3466 caught most frequently in pitfall traps set in OSR crops (personal observations), suggesting
3467 that they actively move between plants.

3468 Plants were infested with known numbers of larvae (0, 1, 5 or 25 per plant) by carefully
3469 placing them at the base of the stem (hypocotyl); larvae were left to locate and enter the
3470 plant naturally. To make the rate of infestation comparable between treatments, and more
3471 realistic of field conditions, the larvae were introduced over a nine-day period with 20% of
3472 the total number of larvae added every other day (1-9 December 2017). This allowed time
3473 for the collection of larvae and ensured that larvae were added to experimental plants
3474 within 24 hours of being extracted from field-collected plants. Staggered infestation also
3475 simulates the infestation process in the field, where it occurs over time. Plant fleece was
3476 placed over the plants during the infection period as freezing conditions were forecast. No
3477 larval mortality was observed.

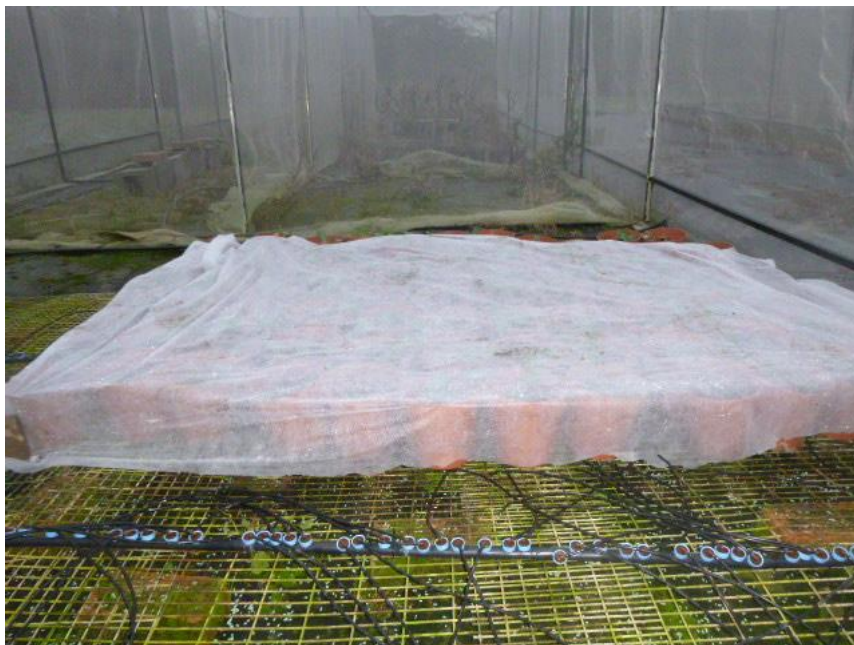
3478



3479

3480 Figure 4. Introduction of *Psylliodes chrysocephala* larvae to the base of oilseed rape,
3481 *Brassica napus*. A total of four larvae can be seen close to the OSR stem.

3482



3483

3484 Figure 5. Experiment 2 (2018) showing plants covered with horticultural fleece to protect
3485 from frost damage and to reduce mortality of *Psylliodes chrysocephala* larvae that were
3486 artificially introduced.

3487 **5.3.5 Confirmation of larval infestation rates in the plants**

3488 **(experiment 2):**

3489 To confirm that larvae had entered the plants and to assess the proportion of larvae which
3490 entered the plants successfully, destructive sampling was performed on two of the blocks in
3491 spring (103 days after introduction, 21-23 March 2018; GS14), and plants were dissected to
3492 locate the larvae. A further four blocks were left to flower and were removed before pod
3493 ripening (GS77-83) to assess larval survival and development to adulthood. The plants were
3494 dissected (154 days after infection, 21-22 May 2018) as described above and scaring and the
3495 number of larvae found in the stems was recorded. The plant pot (with the compost) was
3496 bagged to capture emerging adults after pupation. Bagged plants were observed every 2-3
3497 days for 5 weeks until 27th June; any adult *P. chrysocephala* captured in the bags following
3498 emergence were recorded and removed.

3499



3500

3501 Figure 6. A) Evidence of stem scarring on OSR; this injury is indicative of *Psylliodes*
3502 *chrysocephala* larvae entering the stem to feed. B) *Psylliodes chrysocephala* Larvae
3503 extracted from one oilseed rape plant (treatment 4; 25 larvae introduced) on 21st March
3504 2018 - confirming that larvae did actively enter the plants after inoculation.

3505 **5.3.6 Floral longevity (experiment 2):**

3506 The dates of the start (first flower open) and end (last flower senesced) of flowering were
3507 recorded for whole plants and the duration of flowering (days) was calculated for each plant
3508 in experiment 2. The longevity of flowering is affected by levels of pollinator activity (Bell
3509 and Cresswell, 1998), and, as flower-visiting insects were excluded from the cage, the
3510 flowering period would expected to be longer than it would be in field conditions
3511 (Carruthers *et al.*, 2017). Therefore, assessments were only considered as relative measures
3512 of treatment effects.

3513

3514 **5.3.7 Nectar and Pollen measurements (Experiment 2):**

3515 Six flowers were removed from each plant in experiment 2 for analysis of nectar (3 flowers)
3516 and pollen productivity (3 flowers). All six flowers were collected from the main raceme and
3517 were of approximate age i.e., between flower number 20-35 counting from the first flower
3518 up, to maximise comparability between flowers and plants. Flowers produced early were
3519 chosen for analysis so not to affect the yield; removal of later-produced flowers (but not
3520 early-produced flowers) has been shown to affect yield (Tommev and Evans, 1992). Whole
3521 flowers were cut at the stem (base of pedicle) with sharp scissors to minimise plant injury,
3522 disturbance and leave a clean uniform wound. This was done 24 hours after first opening to
3523 allow nectar secretion to occur and the pollen to dehisce (Nedic *et al.*, 2013), and between
3524 11:00 -13:00 hrs to ensure consistency in daily fluctuation of these resources (Carruthers *et*
3525 *al.*, 2017).

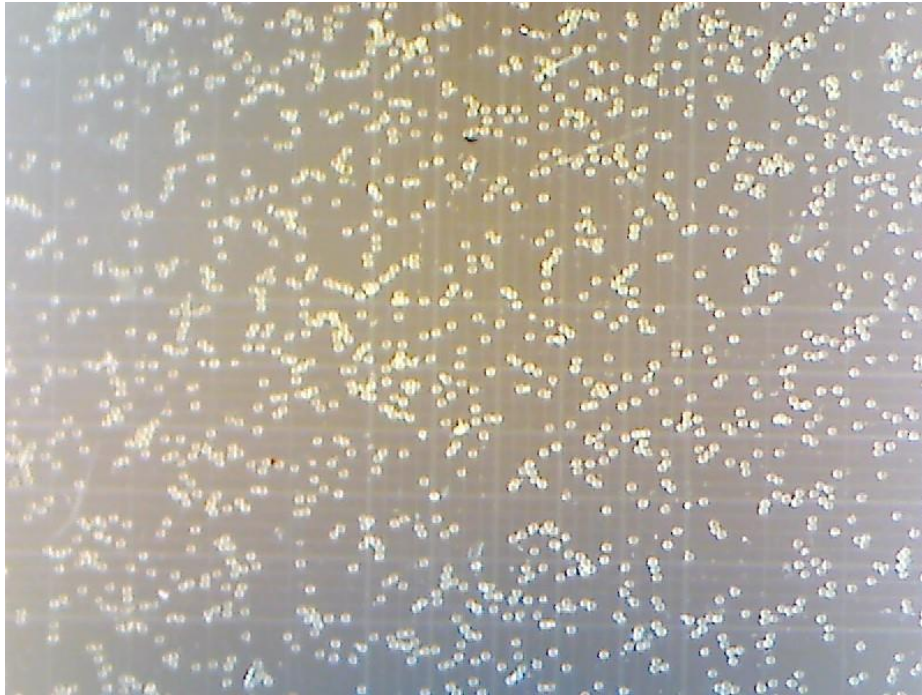
3526 Nectar was extracted by inserting a micropipette (10µL, Drummond, Broomall, PA, USA) into
3527 a single nectary and the percentage of sugar was measured using an eye refractometer
3528 (Bellingham and Stanley Ltd, Tunbridge Wells, UK. 0-50% and 40-85%). Due to time
3529 constraints during flowering, it was not possible to accurately record nectar volume and as
3530 such the values obtained are relative measures of nectar sugar concentration, expressed as
3531 a relative treatment effect.

3532 Flowers collected to measure pollen were stored immediately after collection in 99.9%
3533 ethanol for later analysis. Quantification of the number of pollen grains per ml was
3534 determined based on methods adapted from Hicks *et al* (2016). Anthers were removed from
3535 the flower using fine scissors and placed in an Eppendorf tube. Pollen was extracted by
3536 sonication and vortex spinning samples. Once all the pollen grains were in suspension, the
3537 anthers were removed, and the tubes were spun in a centrifuge to form a pollen pellet. The
3538 samples were dried at 60°C overnight. Each dry pellet was re-suspended in ethanol (60-
3539 120µl), vortex spun and sonicated to evenly distribute the pollen grains. A subsample was
3540 then transferred to a haemocytometer and the grains counted under a light microscope.
3541 The counts were converted to estimated number of pollen grains per ml using the following
3542 equation:

3543

$$3544 \quad \text{pollen grains per ml} = ((\text{pollen count}/\text{cell number}) * \text{cell volume}) * \text{dilution factor}$$

3545



3546

3547 Figure 7. Sample of *Brassica napus* pollen on haemocytometer.

3548

3549 **5.3.8 Harvest and yield measurements (experiments 1 and 2):**

3550 All plants were hand-harvested, when pods were dry to the touch (GS 89), on the 1st of
3551 September 2017 for experiment 1 and on 12th of July 2018 for experiment 2, and all set pods
3552 stored in paper bags, one for the main raceme and one for the side branches. The bags were
3553 stored in dry conditions prior to seed analysis. Plant height (to nearest cm) was recorded
3554 using a tape measure, and the number of side branches, pods set, and number of blind
3555 stalks were counted and recorded. Blind stalks are pod-less stalks with remnants of flowers
3556 that failed to set pods (Williams, Martin and White, 1986; Seimandi-Corda, Jenkins and
3557 Cook, 2021). By adding the number of pods and number of blind stalks, an estimated flower
3558 number per plant was calculated.

3559 From each plant, a set of 10 pods were randomly selected from the main raceme and split
3560 to extract the seeds. The seeds were analysed using a Near InfraRed Analyser (Perten DA
3561 7250 NIR Analyser) to measure the oil percentage. The same set of seeds were then
3562 processed through an electronic seed counter (Elmor applied electronics, C3 Counter) to
3563 determine the number of seeds per sample and the thousand grain weight as measures of
3564 seed yield.

3565

3566 **5.3.9 Statistical Analyses:**

3567 All statistical analyses were performed using Genstat for Windows 18th Edition
3568 (International, 2016).

3569 All responses from Experiment 1 were analysed using multi-stratum ANOVA (with strata
3570 corresponding to whole grids, blocks within grids, and pots within blocks within grids). After
3571 adding an offset of 1 to account for zeros, the number of secondary branches was
3572 transformed to logarithms (base 10), and the number of flowers to square roots, to remove
3573 variance heterogeneity; all other responses were untransformed. Plant height, number of
3574 secondary branches, primary pods, thousand grain weight and percentage oil content were
3575 unrecordable for 8, 9, 16, 16, 12 and 10 plants (i.e. a maximum of only 8% of the total of
3576 200), respectively, and were hence set to missing and estimated using the method of Healy
3577 & Westmacott (Healy and Westmacott, 1956) as implemented in Genstat. Experiment 2 was
3578 analysed using a combination of multi-stratum ANOVA and where responses were zeros
3579 REML was used with transformation on LOG scale where necessary. For nectar a Wald test
3580 was performed due to restrictions in sample numbers. The pollen analysis was done using a
3581 REML to take missing samples into account and retain the blocking structure.

3582 **5.4 Results:**

3583

3584 **5.4.1 Confirmation of larvae introduction:**

3585 Dissections carried out in Experiment 2 showed that larvae successfully entered the
3586 experimental plants. Although the target infestation levels were not achieved, especially for
3587 the 25 larvae/plant treatment, increased introduction rates led to increased infestation,
3588 with significant differences between treatments ($F_{6,8.54}; P=0.027$, Table 5). Therefore, each
3589 treatment returned low (<1), medium (c. 2), and high (>5) larval infestation.

3590

3591 Table 5. Mean numbers \pm SE of *Psylliodes chrysocephala* larvae re-captured 103 days post
3592 introduction to oilseed rape plants. No larvae were found in 0 plants with SE derived from
3593 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

3594

3595 Larval development and survival assessment in summer (GS 77-83, 154 days post
3596 introduction, 21-22nd/05/2018) showed a total of 19 out of 28 plants exhibited evidence of
3597 *P. chrysocephala* feeding activity (external scaring and internal feeding tunnels) with 13
3598 adults collected from eight plants (range 1-3) with the highest capture from treatment with
3599 25 larvae introduced). This provides evidence that *P. chrysocephala* larvae were capable of
3600 surviving the artificial infestation method and completing their development to adults.

3601 **5.4.2 Plant Mortality (experiments 1 & 2):**

3602 A total of eight plants died in experiment 1 (leaf area loss): five controls and three from the
3603 90% leaf area loss treatment. In experiment 2 (leaf area loss x larval infestation) a total of
3604 five plants died from different treatments with no clear trend with treatment (Table 6). All
3605 other plants survived and produced harvestable pods.

3606

3607 Table 6. Plants which did not survive to harvest in experiment 2: experiment to determine
3608 effect of varying leaf area loss (0, 25 or 90%), varying larval infestation (0, 1, 5 or 25 larvae)
3609 and their interaction. Details show treatments of plants that died (n=1 plant each).

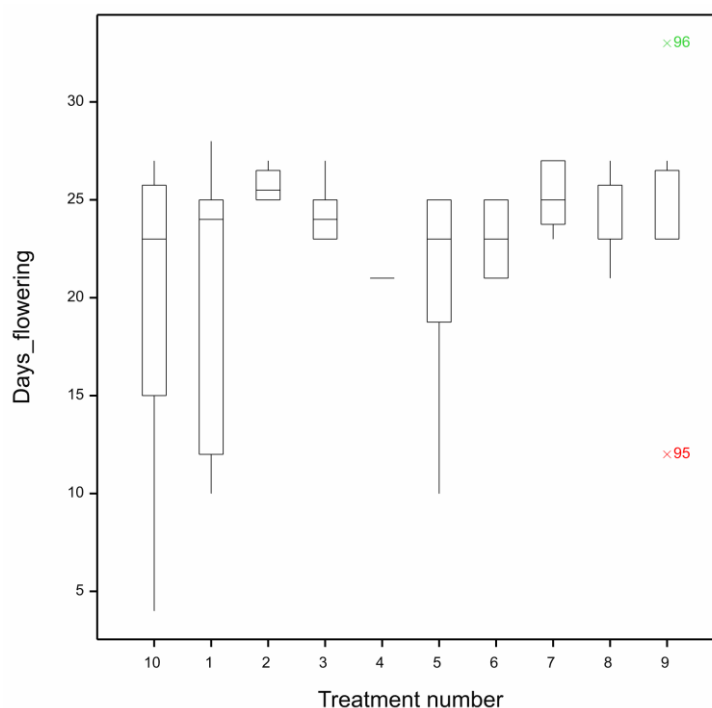
Treatment	Larvae introduced	Level of injury applied
3	5	0
5	0	25
7	1	25
8	1	90
9	5	25

3610

3611 **5.4.3 Flowering period (experiment 2):**

3612 There was no effect of leaf injury level on the period of flowering ($F_{4,55}=1.27$; $P=0.29$, mean:
3613 22 days, range: 4-33 days Figure 8). However, there was a statistically significant reduction
3614 in flowering duration for the 25 larvae treatment (treatment 4, $F_{2,55}=3.81$; $P=0.028$, Figure
3615 8).

3616



3617

3618 Figure 8. Experiment 2 (2018). Number of days the OSR plants were in flower after receiving
 3619 leaf area injury and introduction of *Psylliodes chrysocephala* larvae. Treatment numbers: (1)
 3620 0 larvae/ 0 leaf injury, (2) 1 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0
 3621 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/ 25% leaf
 3622 injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury, (10) 5 larvae/ 90% leaf
 3623 injury.

3624

3625 **5.4.4 Nectar and pollen (experiment 2):**

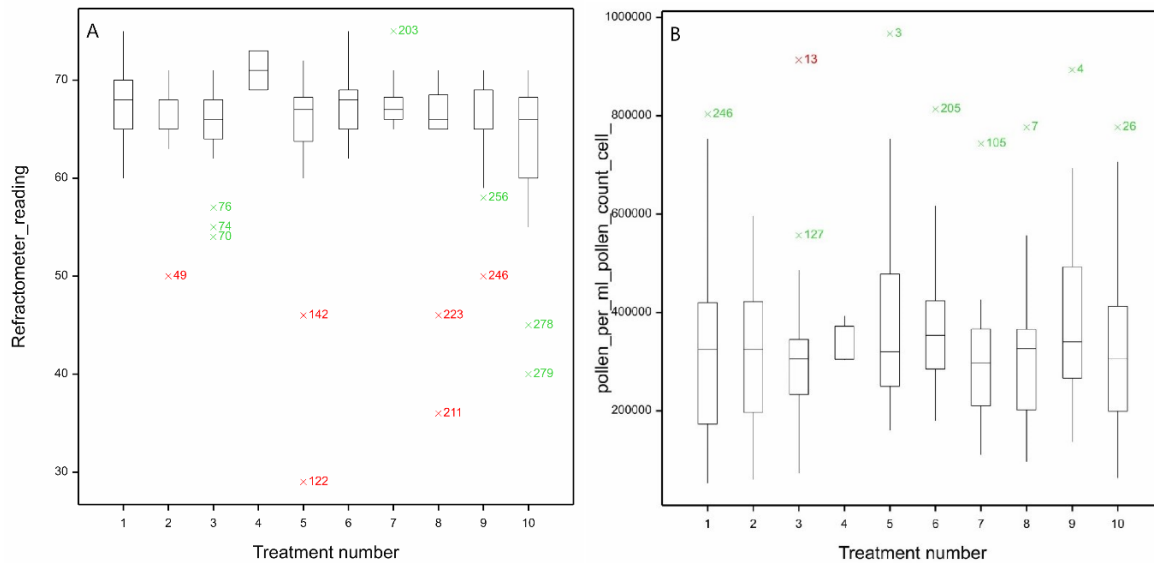
3626 A total of 13 plants did not produce flowers in the desired location for sampling according to
 3627 the protocol (See section 2.3.2) in grid 1 to measure pollen or nectar. Of these, five plants
 3628 had died prior to flowering (Table 6). Of those which were alive but did not produce flowers
 3629 in the right place on the main raceme, five were from treatment 4 (25 larvae introduced)
 3630 and the other three plants were from different treatments (2, 5 and 10). Of these only 2

3631 (treatment 4) produced any pods on the main raceme, while all surviving plants produced
3632 pods on secondary branches. In grid 2, 16 plants could not be measured for pollen and
3633 nectar, four from treatment 4 (25 larvae introduced) three from 10, two from treatments 9,
3634 3 and 6 and one from treatment 2. All plants in grid 2 were destructively sampled for
3635 evidence of *P. chrysocephala* activity and were not measured for yield. Pod set data was
3636 collected and showed pods were produced on all except one plant but outside of the pollen
3637 and nectar sampling window.

3638 In treatment 4 (25 larvae introduced) only two flowers were available at the correct age on
3639 the main raceme for analysis (desired n=24). The same was true for pollen, with only three
3640 samples being achieved, all from the same plant.

3641 The percentage of sugar in the nectar was highly variable (mean: 63.39%, range: 29-75%
3642 sugar) with no effect of leaf area loss ($\chi^2_2 = 0.88$, $P=0.644$) or larval infestation ($\chi^2_2=3.03$,
3643 $P=0.220$) and there was no interaction ($\chi^2_4=7.91$, $P=0.095$, Figure 9). The same was found for
3644 numbers of pollen grains (mean: 30,0081, range: 53,333- 9,666,666 grains/ml), with no
3645 effect of variation in leaf area loss ($F_{2,50.7}=0.71$, $P=0.496$) or larval infestation ($F_{2,52}=0.91$,
3646 $P=0.410$), with no interaction ($F_{4,51}=0.38$, $P=0.821$, Figure 9). It should be noted that no
3647 pollen beetles (*Brassicogethes aeneus* F.) or other insects were observed on collected
3648 flowers, thus confirming the efficacy of the mesh cages in excluding insects.

3649



3650

3651 Figure 9. Experiment 2 (2018). (A) Refractometer readings of percentage sugar content of
 3652 nectar and (B) number of pollen grains per ml. Treatments: (1) 0 larvae/ 0 leaf injury, (2) 1
 3653 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf
 3654 injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury,
 3655 (9) 5 larvae/ 25% leaf injury, (10) 5 larvae/ 90% leaf injury.

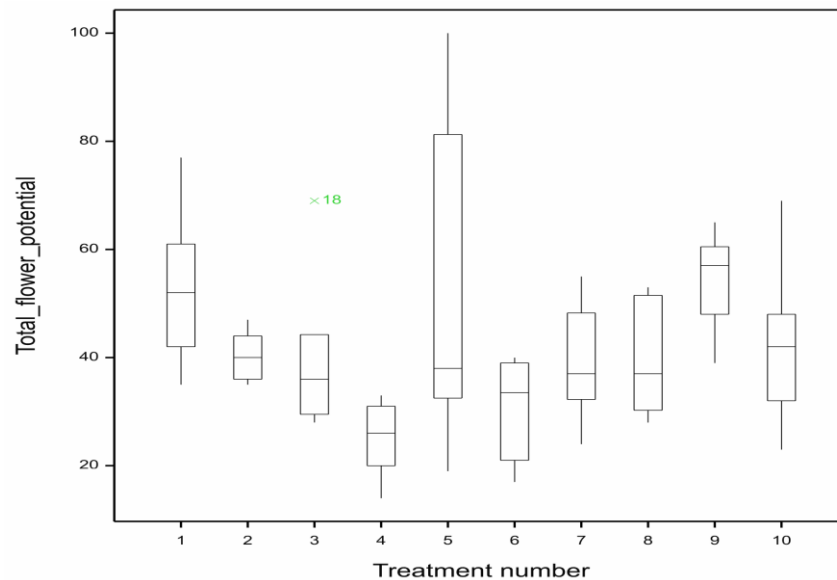
3656

3657 Summing the total number of pods and the number of blind stalks gave the total number of
 3658 flowers produced by each plant. In Experiment 1 (analysis on square root scale), the total
 3659 number of flowers produced (range 0 – 542, overall mean (square root scale): 9.51 (back-
 3660 transformed mean: 90.44)) was not affected by injury level ($F_{3,172}=1.26$, $P=0.291$) or growth
 3661 stage ($F_{1,172}=1.51$, $P=0.221$), and there was no interaction between these factors ($F_{3,172}=2.24$,
 3662 $P=0.085$). In Experiment 2 the only treatment to differ from controls was the 25 larvae
 3663 introduction showing significant reductions in flower potential ($F_{1,42}=8.15$, $P=0.007$, Table 7
 3664 and Figure 10). All other treatments did not differ from un-injured controls ($F_{2,42}=0.89$,
 3665 $P=0.419$, Table 7 and Figure 10).

3666 Table 7. Average total flower potential of oilseed rape (*Brassica napus*) when exposed to
 3667 early growth leaf area injury and *Psylliodes chrysocephala* larvae introduction. Estimates of
 3668 flower potential taken from final pod and blind stalk counts. * donates significant difference
 3669 (P<0.05).

		Percentage leaf area injury (+/-SED=8.926)		
		0	25	90
No. of <i>P. chrysocephala</i> 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

3670



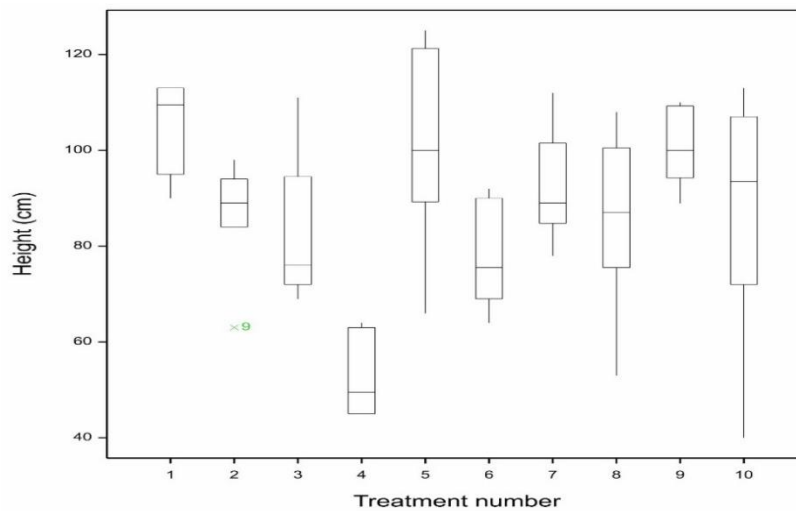
3671

3672 Figure 10 Total flower potential of oilseed rape (*Brassica napus*) plants when exposed at
 3673 early growth stage to leaf area loss (simulated feeding injury by *Psylliodes chrysocephala*
 3674 adults) and infestation with *P. chrysocephala* larvae. Treatments were: (1) 0 larvae/ 0 leaf
 3675 injury, (2) 1 larva/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0
 3676 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larva/ 25% leaf injury, (8) 1 larvae/
 3677 90% leaf injury, (9) 5 larvae/ 25% leaf injury (10) 5 larvae/ 90% leaf injury. Values calculated
 3678 from combining total pod production and blind stalks at harvest.

3679 **5.4.5 Plant height and branching:**

3680 In Experiment 1 (leaf area loss), plant height was unaffected by the amount of leaf area loss
3681 ($F_{3,165}=1.9$, $P=0.132$, mean 116.49cm, range; 80-150cm) for both growth stages ($F_{1,165}=0.09$,
3682 $P=0.765$), with no interaction between the two treatment factors ($F_{3,165}=0.07$, $P=0.976$).
3683 However, in Experiment 2 (leaf area loss x larval infestation), plants in treatment 4 (25
3684 larvae introduced) were significantly shorter compared to all other treatments (whole
3685 treatment mean: 83.8cms, range; 40-125cm, treatment 4: mean: 52.1cm, range: 45-64cm,
3686 $F_{1,40}=27.59$, $P<0.001$, Figure 11), all other treatments did not differ in height from the
3687 control.

3688

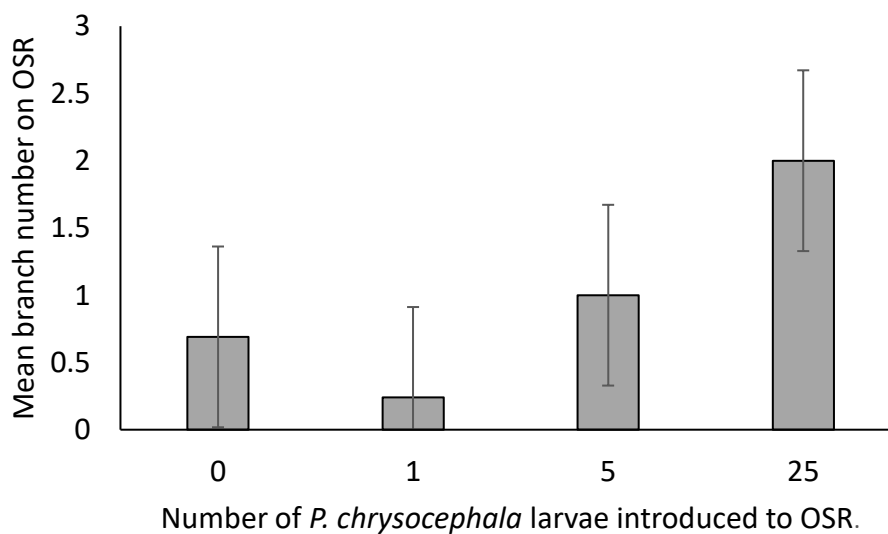


3689

3690 Figure 11. Experiment 2 (2018). Effect of leaf area loss and infestation with *Psylliodes*
3691 *chrysocephala* larvae on plant height (cm) in oilseed rape (*Brassica napus*). Treatment
3692 numbers: (1) 0 larvae/ 0 leaf injury, (2) 1 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4)
3693 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/
3694 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury, and (10) 5 larvae/
3695 90% leaf injury.

3696 Production of secondary branches was low in Experiment 1 (analysis on log scale with
3697 offset) with only 8% of plants producing more than 10 side branches (range: 1-49; overall
3698 mean (log scale): 0.701 (back-transformed mean: 4.023)). No effect of either leaf area loss
3699 ($F_{3,164}=0.70$, $P=0.552$) or growth stage ($F_{1,164}=1.14$, $P=0.288$) was observed and there was no
3700 interaction between these treatment factors ($F_{3,164}=1.61$, $P=0.188$). In Experiment 2 (leaf
3701 area loss x larval infestation), production of secondary branching was also low (16 out of 39
3702 plants produced secondary branches range:1-4, mean: 2.43) but plants in the 25 larvae per
3703 plant treatment produced a significantly higher number of branches than all other
3704 treatments ($F_{1,40}=7.37$; $P=0.010$, see **Error! Reference source not found.12**).

3705



3706

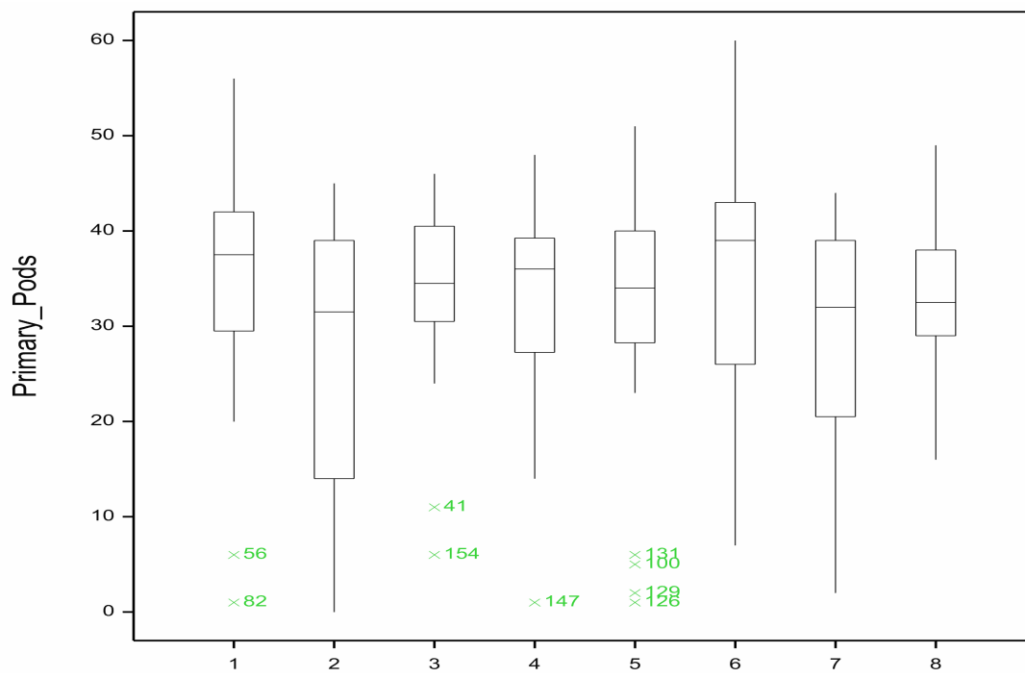
3707 Figure 12. Branching in Oilseed rape (OSR, *Brassica napus*). When inoculated with larvae of
3708 *Psylliodes chrysocephala*.

3709

3710 **5.4.6 Pod production:**

3711 In Experiment 1, primary pod production was not affected by the amount of leaf area loss
 3712 (pod production range: 7-169, overall mean 51.78, $F_{3,157} = 0.79$; $P=0.501$, Figure13) at either
 3713 growth stage ($F_{1,157}=0$; $P=0.956$, Figure13). There was an interaction between growth stage
 3714 and injury level ($F_{3,157}=2.88$, $P=0.038$). The same was found when the total number of pods
 3715 per plant were considered; this was unaffected by injury level ($F_{3,172}=1.49$, $P=0.218$, Table 8)
 3716 at either growth stage ($F_{1,172}=0.02$, $P=0.892$, Table 8) and there was no interaction
 3717 ($F_{3,172}=1.59$, $P=0.194$, Table 8).

3718



3719

3720 Figure 13. Number of primary pods produced by of oilseed rape (*Brassica rape*) when
 3721 exposed to early growth leaf area injury at two Growth Stages (GS, 9 – cotyledon and 10 –
 3722 first true leaf unfurled). Treatments: (1) GS9/0 injury, (2) GS9/25% injury, (3) GS9/50%
 3723 injury, (4) GS9/90% injury, (5) GS10/0 injury, (6) GS10/25% injury, (7) GS10/50% injury, (8)
 3724 GS10/90% injury.

3725 Table 8. Mean total pod production of oilseed rape (*Brassica napus*) exposed to early
 3726 growth leaf area loss Growth Stages (GS, 9 – cotyledon and 10 – first true leaf unfurled). +/-
 3727 SE=5.29.

Growth stage when injury applied	Percentage leaf area removed			
	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

3728

3729 In experiment 2, all surviving plants produced harvestable pods (mean: 18.16, range: 3-51
 3730 pods/plant, Figure 14). Plants in the 25 larva per plant treatment produced significantly
 3731 fewer pods than the other treatments (treatment 4: mean: 9.83, range: 3-16 other
 3732 treatments mean: 21.42, range: 8-51 pods/plant, $F_{1,40}=16.65$, $P<.001$, Table 9 and Figure 14).
 3733 No effect was seen for any other larval level ($F_{2,40}=1.13$, $P=0.332$) or leaf area loss
 3734 ($F_{2,40}=0.69$, $P=0.393$).

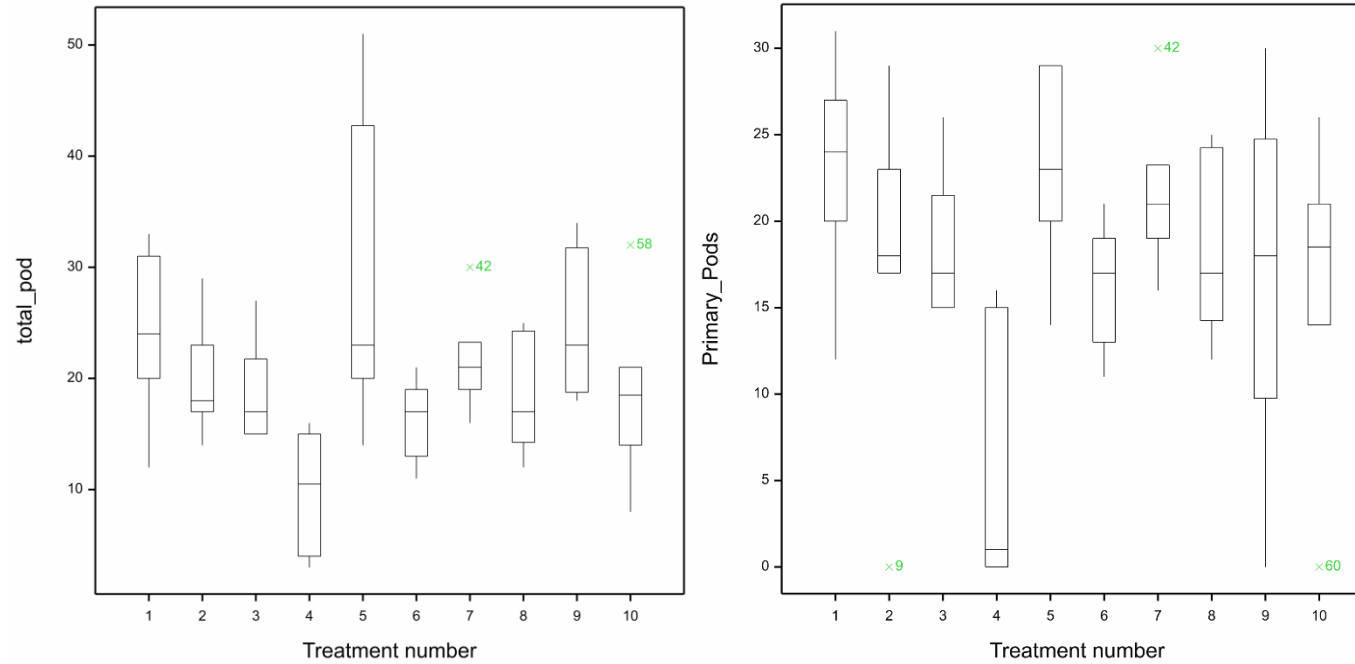
3735

3736 Table 9. Average number of total pods produced from whole plant of winter oilseed rape
 3737 (*Brassica napus*) exposed to leaf area injury (0, 25 and 90% leaf area loss) and *Psylliodes*
 3738 *chrysocephala* larvae introduced (0, 1, 5 and 25). * donates significant difference.

		Percentage leaf area removed (+/-SED=4.308)		
		0	25	90
No. of <i>P. chrysocephala</i> 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

3739

3740



3741

3742 Figure 14. Number of primary pods on the main raceme and total pods from whole plants of oilseed rape (*Brassica napus*) plants when
 3743 exposed at early growth stage to leaf area loss (simulated feeding injury by *Psylliodes chrysocephala* adults) and infestation with *P.*
 3744 *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,
 3745 (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)
 3746 5 larvae/ 90% leaf injury.

3747 **5.4.7 Seeds per pod:**

3748 In Experiment 1 there was no effect of level of leaf area loss ($F_{3, 162}=0.30, P=0.829$) or growth
3749 stage ($F_{1,162}=0.22, P=0.642$) on the average number of seeds per 10 pods (range: 13-275;
3750 overall mean: 198.7) and there was no interaction between the two factors ($F_{3, 162}=0.12,$
3751 $P=0.947$, Table 10).

3752

3753 Table 10. Mean number of seeds/10 pods of oilseed rape (*Brassica napus*) exposed to early
3754 growth leaf area removal at Growth Stages (GS, 9 – cotyledon and 10 – first true leaf
3755 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

3756

3757 In Experiment 2 the average number of seeds per pod was significantly reduced on plants
3758 with 25 *P. chrysocephala* introduced ($F_{1,40}=16.70, P<.001$, Table 11). All other treatments did
3759 not differ from uninjured control plants ($F_{2,40}=0.42, P=0.658$, Table 11).

3760

3761

3762 Table 11. Mean number of seeds in ten pods of oilseed rape (*Brassica napus*) exposed to
 3763 early growth leaf area removal (0,25 and 90% removed) and introduction of *Psylliodes*
 3764 *chrysocephala* larvae (0, 1, 5 and 25 individuals). * donates significant differences.

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

3765

3766 5.4.8 Thousand Grain Weight:

3767 In experiment 1, the thousand grain weight of seed was not affected by leaf area lost
 3768 ($F_{3,161}=0.78$; $P=0.508$, grand mean: 4.552g, range: 1.529 – 8.947g, Table 12) nor by the
 3769 growth stage at which the injury was applied ($F_{1,161}=2.17$; $P=0.143$, Table 12) and there was
 3770 no interaction between the two factors ($F_{3,161}=1.38$, $P=0.251$, Table 12).

3771

3772 Table 12. Mean Thousand Grain Weight (TGW, g) from 10 pods of oilseed rape (*Brassica*
 3773 *napus*) exposed to early growth leaf area removal.

Growth stage when injury applied	Percentage leaf area injury (+/-SED=0.2331)			
	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

3774

3775 In Experiment 2 there was no significant effect on TGW from leaf area injury ($F_{2,38}=0.41$;
 3776 $P=0.67$, Table 13 and Figure 15) or level of larval infestation ($F_{2,38}=0.68$; $P=0.514$, Table 13
 3777 and Figure 15).

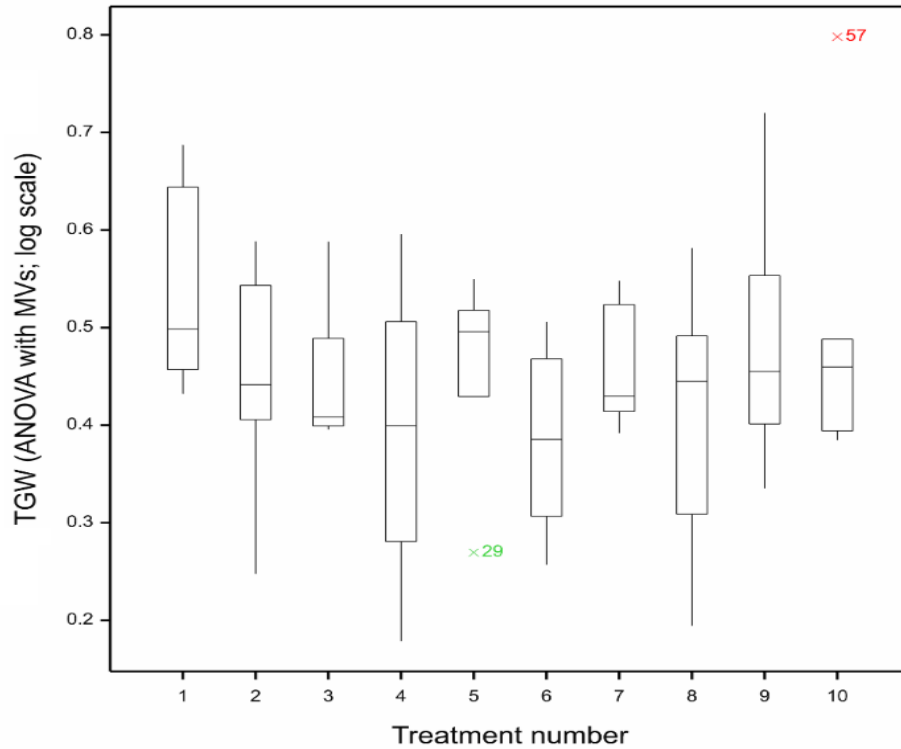
3778

3779 Table 13. Mean Thousand grain weight of seeds (TGW, g) calculated from seed taken from
 3780 10 pods of oilseed rape (*Brassica napus*) exposed to early growth leaf area removal and
 3781 infestation with *Psylliodes chrysocephala* larvae. +/-SED=0.515.

		Percentage leaf area removed		
		0	25	90
No. of <i>P. chrysocephala</i> 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		

3782

3783



3784

3785 Figure 15. Experiment 2 (2018). Thousand grain weight for 10 pods on the primary raceme
 3786 of oilseed rape (*Brassica napus*) when exposed to early growth leaf area injury and
 3787 introductions of *Psylliodes chrysocephala* larvae. Treatment numbers: (1) 0 larvae/ 0 leaf
 3788 injury, (2) 1 larvae/ 0 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0
 3789 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7) 1 larvae/ 25% leaf injury, (8) 1 larvae/
 3790 90% leaf injury, (9) 5 larvae/ 25% leaf injury, and (10) 5 larvae/ 90% leaf injury.

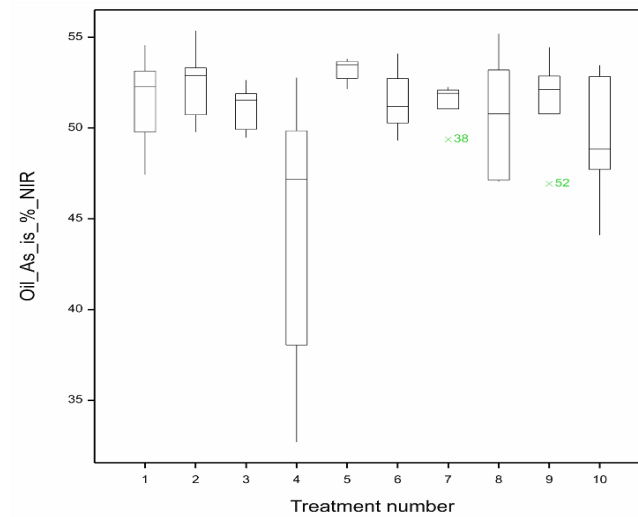
3791

3792 5.4.9 OSR yield quality measures:

3793 Oil content of the seeds was not affected by injury level ($F_{3,163}=0.48$, $P=0.694$) or growth
 3794 stage ($F_{1,163}=0.62$, $P=0.431$, mean: 48.47%, range: 31.61% – 53.87%, Figure 16) in
 3795 experiment 1 (interaction $F_{3,163}=0.20$, $P=0.899$). However, in experiment 2, although there
 3796 was no effect of the leaf area lost ($F_{2,40}=1.12$, $P=0.335$, mean: 50.48%, range: 32.69 –

3797 55.38%), there was a significant reduction in percent oil content of seeds from plants in the
3798 25 larvae per plant treatment (treatment 4, $F_{1,40}=21.84$, $P<0.001$, mean: 44.61, range: 32.69
3799 – 52.78%, Figure 16) , with only sample producing over 50% oil content.

3800



3801

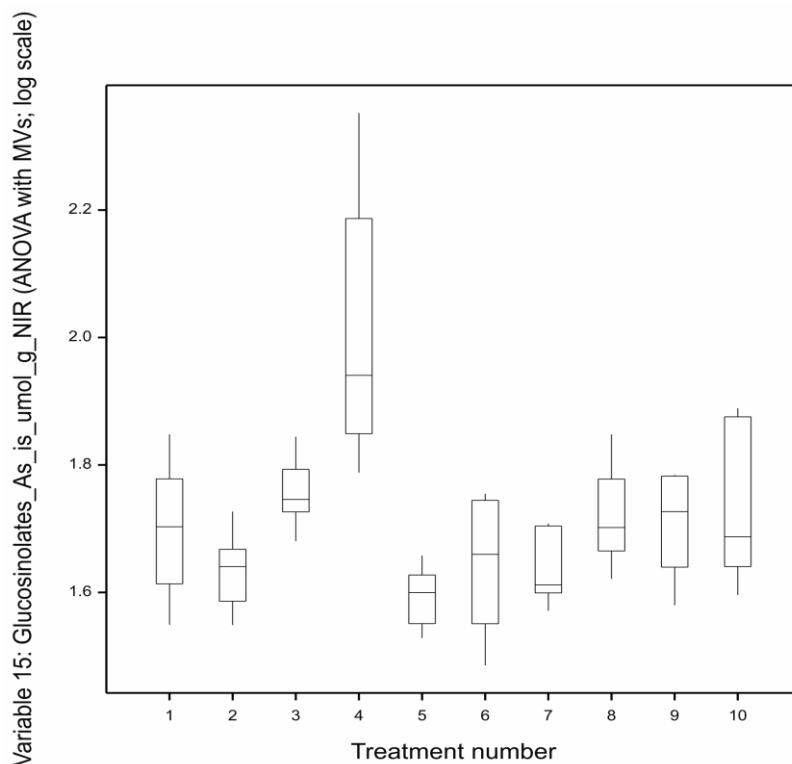
3802 Figure 16. Seed oil percentage content from oilseed rape (*Brassica napus*) seeds exposed to
3803 early growth leaf area injury and infestation with *Psylliodes chrysocephala* larvae
3804 (Experiment 2). Measurements taken from all seeds from 10 pods from the main raceme.
3805 Treatments were: (1) 0 larvae/ 0 leaf injury, (2) 1 larva/ 0 leaf injury, (3) 5 larvae/ 0 leaf
3806 injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6) 0 larvae/ 90% leaf injury, (7)
3807 1 larva/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5 larvae/ 25% leaf injury (10) 5
3808 larvae/ 90% leaf injury.

3809

3810 The levels of glucosinolate in seeds was also shown to be affected when plants were
3811 infected with high numbers of larvae. With levels recorded significantly higher in the

3812 treatment where 25 larvae were introduced ($F_{2,40}=43.59$, $P < 0.001$, Figure17). All other
3813 treatments did not differ from uninjured control plants ($F_{4,40}=0.69$, $P = 0.600$, Figure17)

3814



3815

3816 Figure 17. Total glucosinolates concentration (umol/g), on LOG scale, from oilseed rape

3817 (*Brassica napus*) seeds after exposure to early growth leaf area injury and introduction with

3818 *Psylliodes chrysocephala* larvae. Treatments were: (1) 0 larvae/ 0 leaf injury, (2) 1 larva/ 0

3819 leaf injury, (3) 5 larvae/ 0 leaf injury, (4) 25 larvae/ 0 leaf injury, (5) 0 larvae/ 0 leaf injury, (6)

3820 0 larvae/ 90% leaf injury, (7) 1 larva/ 25% leaf injury, (8) 1 larvae/ 90% leaf injury, (9) 5

3821 larvae/ 25% leaf injury (10) 5 larvae/ 90% leaf injury.

3822

3823

3824 **5.5 Discussion:**

3825

3826 The data reported here suggests that high levels leaf area loss at early growth stage, i.e. leaf
3827 injury as caused by adult *P. chrysocephala* feeding does not impact on final yield of oilseed
3828 rape and low levels of *P. chrysocephala* larvae infestations can also be tolerated. However,
3829 higher larval infestation resulted in negative effects on plant growth which would negatively
3830 affect both farmers and insects which use floral resources, such as bees.

3831 The lack of significant effects on oilseed rape flower production and resource quality, plant
3832 architecture and yield from any of the leaf area loss treatments applied in this study are in
3833 line with studies showing little to no yield penalty from leaf area loss at early growth in both
3834 simulated injury experiments (Kirkegaard *et al.*, 2008b; Antwi, Olson and DeVuyst, 2008;
3835 Ramachandran, Buntin and All, 2000; Sunderland *et al.*, 1995; Syrov, Shirtliffe and
3836 Zarnstorff, 2016) and field observations under natural pest infestations (Gavloski *et al.*,
3837 2000). Susko & Superfisky (2009) demonstrated no significant impact from 50% leaf
3838 (simulated) area injury on early growth in spring OSR.

3839 Actual pest feeding of another leaf flea beetle, *Phyllotreta* sp on spring canola exhibits
3840 reduction in seed production compared to simulated injury (Antwi, Olson and DeVuyst,
3841 2008; Nowatzki. T and Weiss. M, 1997), potentially due to the absence in simulated injury
3842 experiments of biological factors such as pest saliva, which impacts the production of
3843 secondary chemicals, which entails a metabolic cost to the plant (Baldwin, 1990). Without
3844 the extra stimuli to create secondary metabolites the cost of compensation to injury may be
3845 reduced. Therefore, the lack of effect on later growth and yield parameters resulting from
3846 early growth leaf injury observed here may not truly represent the impact of *P.*

3847 *chrysocephala* adult feeding activity in the field; where plants are exposed to all stimuli of
3848 feeding whilst also under potentially sub optimal conditions in terms of water and nutrient
3849 status and potential additional pest injury compared with the potted plants used in this
3850 study. Simulated injury was used here to control exactly and replicate sufficiently the
3851 amount of leaf injury, which is difficult to do with live insects (White *et al.*, 2020). The lack
3852 of cumulative injury may also account for the lack of effect from leaf injury seen here as the
3853 plant may be less able to cope with sustained attack than singular feeding bouts (Crawley,
3854 1983). However, our results concur with field experiments that show that plants can
3855 respond to early growth stage defoliation with strong biomass recovery (Ellis, 2015b;
3856 Kirkegaard *et al.*, 2008a; Ellis, 2015a; Syrový, Shirtliffe and Zarnstorff, 2016).

3857 The results of this study suggest that once the crop is established, larval infestation can pose
3858 a significant effect to yield attainment. Other studies which have attempted to manipulate
3859 *P. chrysocephala* larvae have used complicated and time-consuming methods to obtain
3860 experimental larvae (Döring and Ulber, 2020; Ruck, Cadoux and Robert, 2018). This is the
3861 first attempt to experimentally manipulate numbers of stem boring *P. chrysocephala* larvae
3862 from field populations to quantify their effect on crop yield. The infestation success in
3863 achieving each of the treatment levels was not 100%, particularly for the high infestation
3864 treatment (25 larvae). This was potentially due to unknown factors such as intraspecific
3865 competition or variations in larvae robustness. However, larvae were successfully
3866 recovered from test plants and the capture of adult *P. chrysocephala* towards the end of the
3867 experiment demonstrates that manipulated infestation did not disrupt the development of
3868 the beetle and allowed the empirical test of the impact larval development on OSR growth
3869 and yield with limited equipment and facilities. This method can be used as a simple way of
3870 experimentally transferring *P. chrysocephala* larvae in further research e.g. into the

3871 tolerance of OSR cultivars to prolonged larval feeding or testing effects of exposure to novel
3872 insecticides.

3873 The presence of *P. chrysocephala* larvae have been shown to effect the glucosinolate profile
3874 and concentration in leaf and stem material (Koritsas, Lewis and Fenwick, 1989; Koritsas,
3875 Lewis and Fenwick, 1991; Döring and Ulber, 2020). None of these studies have examined the
3876 chronic effect of larvae as done here and were sampled after 10 -21 days post simulated
3877 injury or larval introduction. All show an increase in GLS concentration as a response to
3878 injury and larvae infection. Here we show that this process is observable in the seeds; these
3879 showed higher levels of glucosinolates from plants when inoculated with high numbers of
3880 larvae.

3881 Inoculating OSR with 25 larvae/plant returned an average of 6.5 larvae per plant when a
3882 subset of plants was dissected at a later stage. This level of actual infestation significantly
3883 reduced flower and pod production and the seeds produced contained lower oil content
3884 and higher concentrations of glucosinolate. There was no effect on these factors when
3885 lower numbers of larvae were introduced. This finding suggests that in this experimental
3886 set up the action threshold level i.e., the number of *P. chrysocephala* above which there is a
3887 danger of yield loss if untreated for *P. chrysocephala* larvae is somewhere above 5.8
3888 larvae/plant. Further study would be required to fully assess exact number in these
3889 conditions and experiments testing a range of OSR cultivars under varying management
3890 conditions are needed to develop truly accurate economic injury thresholds.

3891 The total flower production was not affected by any leaf area injury in either experiment or
3892 by low or medium levels of larval infestation. However, when high levels of *P.*
3893 *chrysocephala* larvae were introduced the plant's capacity to produce flowers was

3894 significantly reduced. Although the high larval infestation treatment plants produced fewer
3895 flowers no effect was seen in either nectar sugar concentration or the amount of pollen
3896 produced. However, as volume and total sugar concentration was not recorded in this
3897 experiment and pollen was not assessed for protein or amino acid content, other flower
3898 resource effects might have been missed. For instance, if the total volumes of nectar
3899 available were substantially lower in injured than in un-injured plants, then pollinator
3900 activity and pollination could be reduced with an impact on seed production and oil content
3901 (Bommarco, Kleijn and Potts, 2013). This requires further investigation as OSR is an
3902 important early flowering crop which is visited by a wide range of insects (Garratt *et al.*,
3903 2014).

3904 The artificial infection method detailed here can facilitate empirical testing of synthetic
3905 insecticides, alternative control methods and economic injury thresholds for *P. chrysocephala*
3906 and will aid the development of biologically relevant action thresholds which would inform
3907 farmers and policy makers to better calculate a cost: benefit of insecticide application based
3908 on empirical data and not economic costs of application.

3909

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4083

4084

4085 **6.0 Feeding preference of *Psylliodes chrysocephala* L. on early true**
4086 **leaves of Oilseed Rape (*Brassica napus* L.):**

4087

4088 **6.1 Abstract:**

4089

4090 With the EU ban on the use of neonicotinoid insecticides in oilseed rape (OSR, *Brassica*
4091 *napus*) protecting the crop from pest levels of damage from the cabbage stem flea beetle
4092 (CSFB, *Psylliodes chrysocephala* L.) has become an increasing issue. For farmers to have
4093 reliable predictors of pest pressure an understanding of how the pest and crop interact
4094 ecologically is crucial for accurate predictions. It has been suggested that *P. chrysocephala* L.
4095 adults selectively feed on true leaves of oilseed rape (OSR, *Brassica napus* L.) over the
4096 cotyledons. In a whole plant choice experiment we support the hypothesis that adult *P.*
4097 *chrysocephala* preferentially feed on unfurled true leaves of OSR over cotyledons. The
4098 implications on crop establishment and pest protection practices for OSR which are
4099 discussed.

4100

4101

4102 **6.2 Introduction:**

4103

4104 Protecting crops from pest pressure is a major aspect of agriculture and understanding how
4105 crops and pest species interact is critical to understanding the risks to the crop and
4106 production. The cabbage stem flea beetle, *Psylliodes chrysocephala* L., is a major pest of
4107 European oilseed rape (OSR, *Brassica napus* L.) production (Williams, 2010; Zhang *et al.*,
4108 2017). The adult beetles enter the crop during early autumn and feed directly on the OSR
4109 leaves. After a period of feeding, the females begin to lay eggs at the base of the OSR stems
4110 and these develop into larvae which then tunnel into the stem and feed internally
4111 throughout the winter before exiting the plant to pupate in the soil in mid spring (Williams,
4112 2010).

4113 The importance of this pest has increased in recent years, particularly in the UK with
4114 populations increasing (Collins, 2017) at least in part, as the result of restrictions on the
4115 major synthetic chemical protection options (EU, 2018) and increasing pyrethroid resistance
4116 reported in France (Bothorel *et al.*, 2018), Germany (Heimbach and Brandes, 2016),
4117 Denmark (Castberg and Kristensen, 2018), Czech Republic (Jitka and Kocourek, 2019) and
4118 the UK (Foster and Williamson, 2015). As pest pressure has increased there have been
4119 reductions in the OSR cropped area in the UK (Scott and Bilsborrow, 2019). This is often
4120 attributed to *P. chrysocephala*, slugs and pigeons (Coston *et al* in prep). It has been shown
4121 that severe feeding damage by *P. chrysocephala* adults during the early growth stages of the
4122 crop can lead to crop failures (Chapter 2 and 3 this thesis).

4123 With reduced pesticides options there is a need for better targeting of those available,
4124 including knowing which growth stages are preferred by the beetles and therefore in most

4125 need of protection. There is currently little data on the feeding preferences of adult *P.*
4126 *chrysocephala*, and information of this kind may be important in determining when the crop
4127 is most vulnerable; facilitating both the targeting of insecticide use and for developing time-
4128 specific trap or nurse crops that coincide with the preferred crop growth stage. Preliminary
4129 work by Diehl (2017) on *P. chrysocephala* feeding on leaf discs cut from leaves of varying
4130 ages of OSR demonstrated low feeding on cotyledons but higher and comparable levels on
4131 the first, second and third leaves after 48hours. This result may be due to experimental bias
4132 where the preparation of leaf discs breaks the leaf cuticle resulting in internal chemical cues
4133 the beetles may be utilising when selecting a suitable feeding site. To confirm the finding of
4134 Diehl (2017) while removing any effect of leaf cuticle damage, a whole plant choice
4135 experiment was used, avoiding any effect of previous leaf injury. Adult *P. chrysocephala* (2
4136 male and 2 female) were introduced into enclosed propagator trays with OSR plants at four
4137 growth stages and the cotyledon/leaf area lost was assessed after 48 hours to compare
4138 feeding location. The experiment was concluded at 48 hours to ensure that all leaf material
4139 was not removed, and the initial feeding can be observed.

4140

4141 **6.2.1 Aims of study:**

4142 This study was designed to confirm the finding of Diehl (2017) on leaf discs feeding
4143 preference in full plants. It is hypothesised that beetles selectively feed on older leaves over
4144 the cotyledons. This will be confirmed if feeding activity is recorded at higher levels on the
4145 true leaves over the cotyledons.

4146 **6.3 Methods:**

4147

4148 **6.3.1 Plants:**

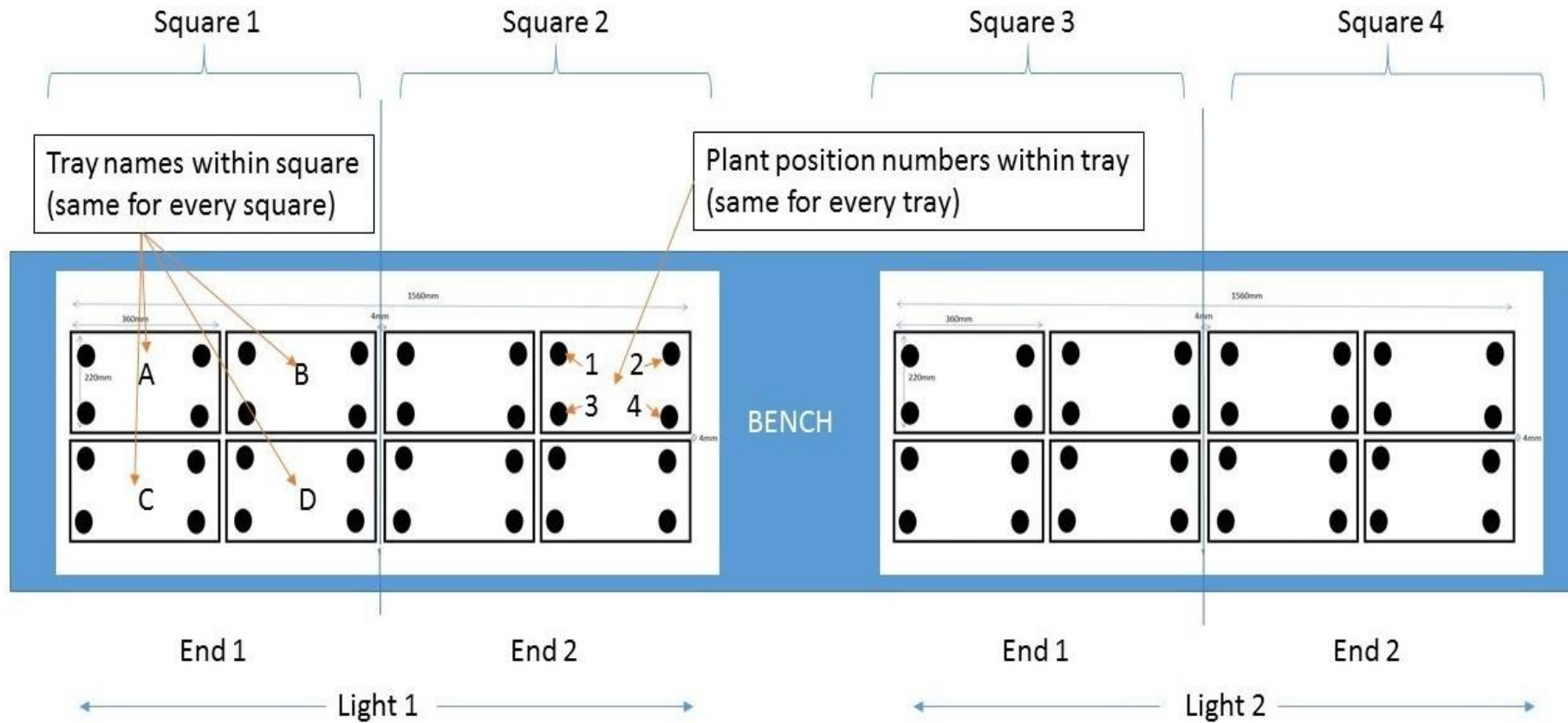
4149 Winter oilseed rape (*Brassica napus*, cv. Falcon) were sown in seed trays using Levington
4150 L2+S compost. Trays of OSR were set up daily (01/11/2016 to 7/12/2016) to produce plants
4151 at Growth stage 10-13 based on the BBCH scale (Lancashire *et al.*, 1991) simultaneously.
4152 Where GS10 is cotyledons extended, GS11 is first true leaf unfolded, GS12 is two true leaves
4153 unfolded and GS 13 is 3 true leaves unfolded. Plants were kept under standard glasshouse
4154 conditions with no additional heat or lighting. Average temperature range was 12-15°C with
4155 9 daylight hours.

4156

4157 **6.3.3 Experimental Arenas:**

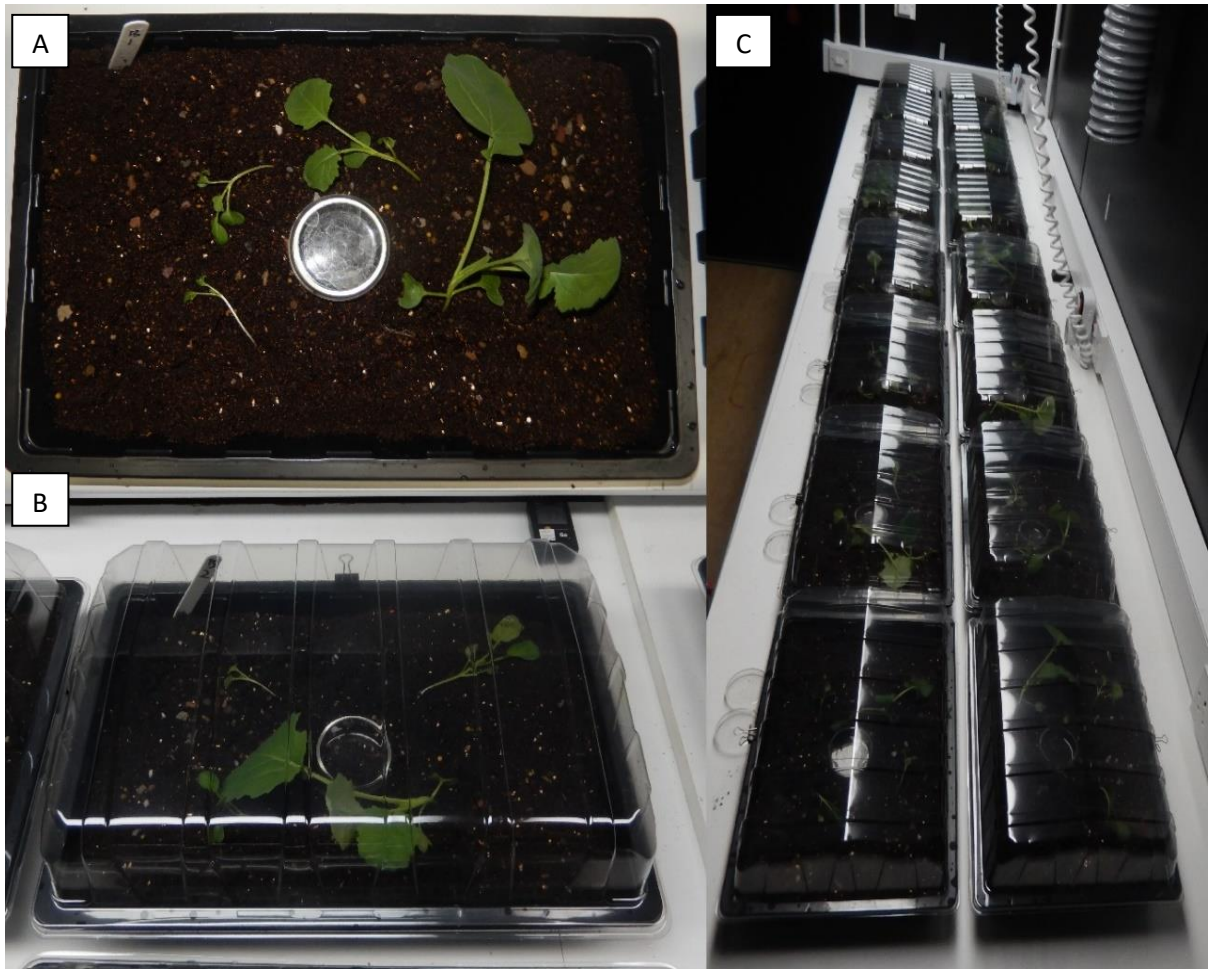
4158 The experimental feeding arena comprised a plastic propagator with a clear lid and a plastic
4159 seed tray base (220 x 350mm) with a Rothamsted standard compost mix (Petersfield Products,
4160 Leicester, UK) and four OSR plants, one at each of BBCH Growth Stages GS10, GS11, GS12
4161 and GS13 (Lancashire *et al.*, 1991), each equidistant from the centre of the tray (see Figure 1
4162 and Figure 2). A total of 16 feeding arenas were established and set in four Latin square
4163 replicates with two replicate squares ('end 1' and 'end 2') placed directly below two lights
4164 blocks (to reduce differences between treatments in lighting), in a controlled environment
4165 room, (18°C +/- 1°C, L-D photoperiod 12-12 with strip fluorescent lights, (see Figure1 and
4166 Figure2). The environment conditions were set to allow activity of the beetle as they are
4167 observed to be highly active at these temperatures. The diel activity of *P. chrysocephala* is

4168 little understood (explored further in Chapter 7 this thesis) therefore, to ensure ample
4169 opportunity for feeding behaviour a 12:12 light: dark split was used. The distribution of
4170 plants within each tray formed a Latin square with each plant growth stage occurring once
4171 in each of the 4 positions within a block of 4 propagators (Figure1). After transplanting, all
4172 trays were watered and given 24hours for plants to recover from wilting stress before the
4173 beetles were introduced.



4174

4175 Figure 1. Schematic diagram of the layout of experimental arenas used to determine feeding behaviour of *Psylliodes chrysocephala* on *Brassica*
 4176 *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
 4177 (rectangles labelled A, B, C D), arenas within Latin square replicates and the distribution of each replicate under two lights suspended above
 4178 the bench in a controlled environment room.



4179

4180 Figure 2. Photographs showing the experimental arena design and layout of the experiment
4181 to test feeding behaviour of *Psylliodes chrysocephala* on *Brassica napus* plants of four
4182 different growth stages. (A) Arrangement of the four plants inside the arena and the release
4183 point (glass petri dish) in the centre. (B) Experimental arena with propagator lid in place. (C)
4184 full layout of all arenas *in situ* during beetle exposure.

4185 **6.3.4 Feeding bioassay:**

4186 Four adult *P chrysocephala* (2 male and 2 female) were introduced into each tray. Little is
4187 known about the behavioural differences of males and females therefore a 50:50 sex ratio
4188 was used throughout to ensure consistency and remove potential unknown variability. The
4189 sex was determined based on the structure of the front tarsal pads, which are flattened and
4190 larger on males than on females (Cook *et al.*, 2006). Beetles were collected from an area of
4191 OSR at Rothamsted Research, Harpenden, UK on the 13th of December 2016, the collection
4192 area had not been exposed to any insecticide treatment and the crop was at GS 17-18.
4193 Collected beetles were starved for a 24-hour period, in insect rearing cages at ambient
4194 laboratory conditions (20-25°C), prior to use. To allow easy introduction of beetles to arenas
4195 they were collected from cages using an electronic aspirator (Watkins and Doncaster Ltd)
4196 and put inside petri-dishes which were set centrally inside the arena equidistant from the
4197 plants (Figure 3). When the trial was started the top of the petri-dish was removed and the
4198 propagator cover set in place, thus allowing the beetles to move freely around the arena
4199 and access all plants equally. The beetles were allowed to feed freely for 48 hours, after
4200 which time all remaining beetles were collected, using an electronic aspirator, and each
4201 leaf was photographed to measure the area which had been removed (see section 6.3.5).

4202

4203 **6.3.5 Assessment of feeding activity:**

4204 Feeding activity was assessed by measuring the area of cotyledon/leaf lost using
4205 photographs of each leaf using ImageJ, an open-access image analysis software package
4206 (Schindelin *et al.*, 2015). In this experiment the cotyledon was considered as 'leaf 0' then

4207 each of the true leaves were leaf 1,2, 3 and 4. Where damage was present the total leaf
4208 area was calculated along with the area lost using the draw area function in ImageJ, where a
4209 polygon is drawn over an image and the internal area calculated (Figure 4). This allowed the
4210 proportion of area removed to be calculated for each leaf. Where the leaf edge was
4211 damaged and the original outline could not be determined, a 'best fit' was used to represent
4212 the outer edge both for the total area and the damaged area measurements (Figure). At the
4213 leaf petiole a straight line across the stem where the leaf green material branches out, was
4214 used to ensure consistency (Figure4).

4215

4216 **6.3.6 Statistical analysis:**

4217 For each leaf, a total area was measured, and any feeding injury was also measured, these
4218 values were used to create a percentage area loss and these values were used for analysis.
4219 Due to high incidences of zero feeding injury all data was log transformed subsequent data
4220 was analysed using REML variant component analysis. All statistics were performed using
4221 GenStat statistical software package v 18 (International, 2016).

4222

4223 **6.4 Results:**

4224

4225 **6.4.1 Beetles recovered at the end of the experiment:**

4226 After the 48h exposure time all the beetles were recovered alive from 12 out of the 16
4227 replicate arenas (Table 1). However, in four of the arena's beetles were missing, in one case
4228 this was due to a male beetle being dead and the remaining were presumed to have
4229 escaped from the arena or to have burrowed deep into compost (see Chapter 7 this thesis).

4230

4231 Table 1 Total numbers of adult *Psylliodes chrysocephala* recovered following a 48h exposure
4232 of *Brassica napus* plants to 2 males and 2 females. Table shows the experimental design of
4233 the arena bioassay details and the sex ratio of beetles recaptured. * - one beetle was found
4234 dead in the arena. ** - missing beetles could not be located (timings unknown).

4235

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

4236 **6.4.2 Numbers of leaves:**

4237 No leaves were completely eaten during the experiment and plant growth during the
 4238 experimental period meant that there were more leaves present at the end of the exposure
 4239 period than at the start (Table 2).

4240

4241 Table 2 Number of Oilseed rape (*Brassica napus*) leaves in each arena at the start and end of
 4242 the experiment and number and proportions that were fed upon by the cabbage stem flea
 4243 beetle (CSRB, *Psylliodes chrysocephala*).

Leaf number	GS10 cotyledons	GS11 1 st true leaf	GS 12 2 nd true leaf	GS 13 3 rd true leaf	GS 14 4 th 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

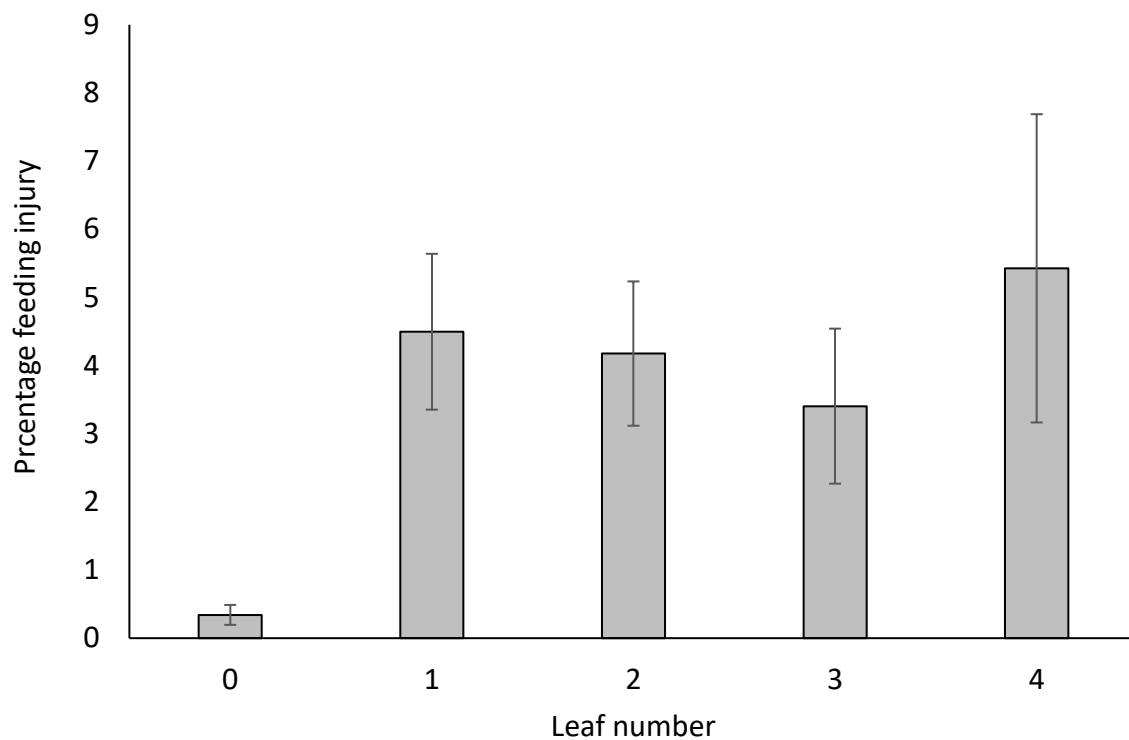
4244

4245 **6.4.3 Leaf area loss:**

4246 Due to the difference in sizes of the differing ages of the leaves ($F_{4,57.9}=10.65$; $P<0.001$) the
 4247 comparison of percentage area of leaf lost was not robust because the same area damaged

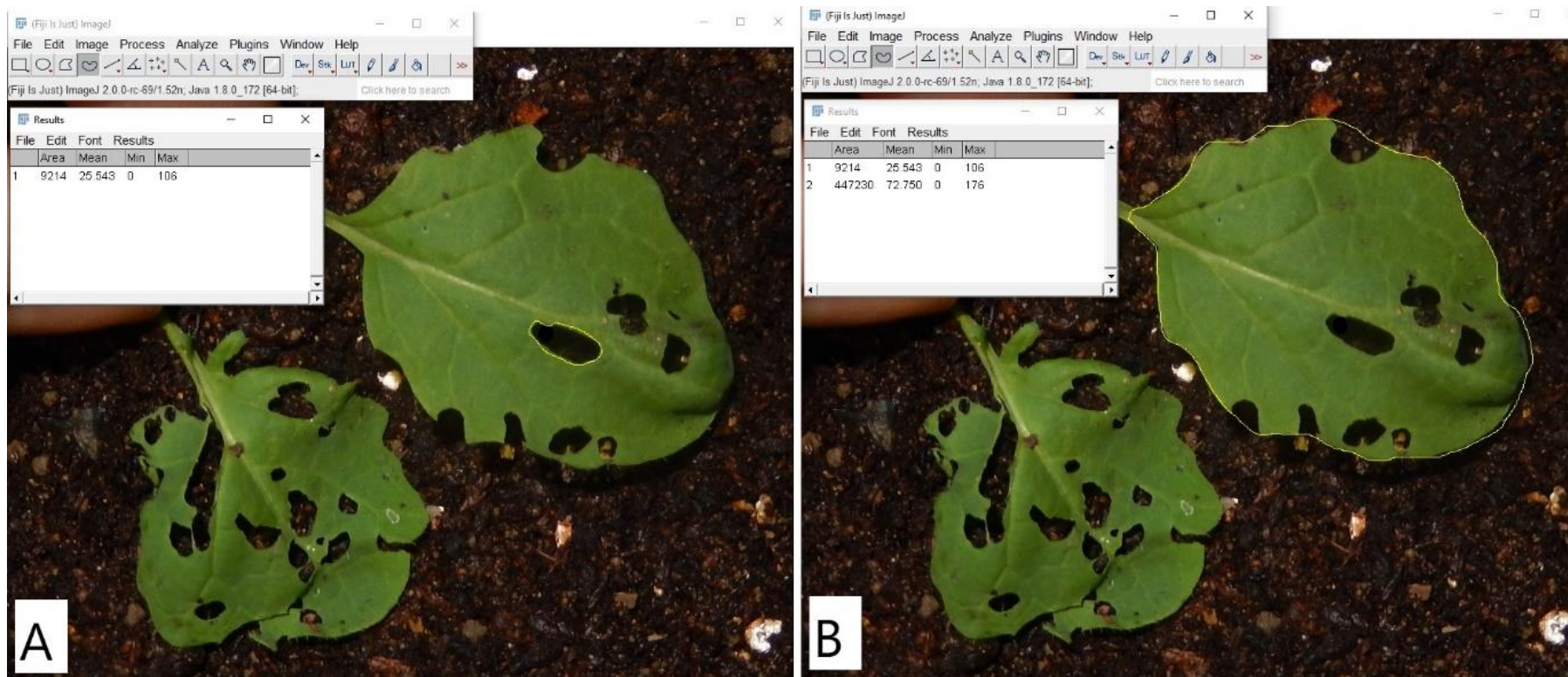
4248 on a small leaf will have a bigger proportion than on a larger leaf. However, this did not
4249 prevent comparisons of the incidences of feeding recorded at different leaf ages and the
4250 percentage of leaf area loss for the cotyledons and four leaves is shown in Figure 3. When
4251 comparing the absolute area loss between the leaf ages a difference was observed
4252 ($F_{3,35,6}=12.9, P<0.001$) with the cotyledons eaten less frequently than true leaves. There was
4253 no difference in area loss on any of the true leaves.

4254



4255

4256 Figure 3. Percentage oilseed rape (*Brassica napus*) leaf area injured by the cabbage stem
4257 flea beetle (*Psylliodes chrysocephala*) for five different ages of leaves. Leaf 0 = cotyledons;
4258 Leaf 1 = first true leaf unfolded; Leaves 2-4 = 2nd -4th true leaves. Values are based on the
4259 total area removed from the leaf in relation to the total area of the leaf. Shown as an
4260 average of all leaves at each growth stage. SE shown.



4261

4262 Figure 4. Photographs of *Psylliodes chrysocephala* feeding damage on *Brassica napus* leaves after 48 hours exposure. Representation of the
 4263 photographic methods of measurements using ImageJ are (A) Here one internal area is marked (yellow border drawn around the edge of the
 4264 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
 4265 (yellow border drawn around the leaf) with the area of the hole shown in the ImageJ programme results box.

4266 **6.5 Discussion:**

4267 The data presented here provide evidence that *Psylliodes chrysocephala* avoid feeding on
4268 the cotyledons of OSR, in favour of feeding on true leaves. This confirms the findings of
4269 Diehl (2017) on leaf discs that *P. chrysocephala* feed primarily on true leaves. As OSR is
4270 known to release glucosinolate when injured by *P. chrysocephala* (Koritsas, Lewis and
4271 Fenwick, 1991) the finding of Diehl (2017) may have been an artifact of preparing the leaf
4272 disc. Here it is shown that the same pattern of feeding is also exhibited on whole plants thus
4273 removing any potential cues from previous injury. The low feeding on cotyledons has been
4274 shown in the under field conditions in Northern Serbia, feeding by *P. chrysocephala* was
4275 shown to be low on cotyledons and the first true leaf and was most intensive in November
4276 when the plants were at GS 16-18 (Sivčev *et al.*, 2016). Overall, levels of feeding were low
4277 with only five leaves having more than 20% leaf area lost. These low levels are to be
4278 expected due to the short exposure time (48 hours) and the low number of beetles (n=4).

4279 The bias-towards true leaves reported here and shown by Diehl (2017) suggest that the
4280 cotyledons are less palatable or do not provide as strong a feeding response as true leaves.
4281 The lack of feeding on the cotyledons might be due to lower levels of glucosinolate, which
4282 may be present at higher levels in the true leaves (Rosa *et al.*, 1996) and have been shown
4283 to act as phagostimulants *P. chrysocephala* when added to agar (Bartlet *et al.*, 1994) but this
4284 was not the case for whole plant choice tests with cultivars of varying glucosinolate level
4285 (Bartlet, Mithen and Clark, 1996). The level of glucosinolate are known to vary depending on
4286 location (Bellostas, Sørensen and Sørensen, 2004) and growth stage (Rosa *et al.*, 1996) and
4287 it would have been interesting to measure the levels in the current experiments.

4288 Although these findings would need to be corroborated with field observations to ensure
4289 the beetles feed in the same way in the field. The data presented here suggests that when
4290 given access to alternative leaves the cotyledons are left alone. Suggesting the efficacy of
4291 seed treatments to early establishment needs to be tested alongside the beetles feeding
4292 activity. Seed dressing insecticides such as Lumiposa (Active Ingredient: Cyantraniliprole
4293 (DuPont, 2017)) are recommended as effective only until the third/ fourth true leaf has
4294 unfurled. In the present study adult *P. chrysocephala* feed on the first four true leaves
4295 equally is in line with the later end of the recommended effective period, suggesting
4296 Lumiposa is active during the crucial period of plant establishment. However, in Northern
4297 Serbia *P. chrysocephala* have been reported feeding most intensely at the 16th – 18th true
4298 leaf (Sivčev *et al.*, 2016) which is after the recommended effective phase of Lumiposa,
4299 however the authors do note that the plant were large and seemed able to tolerate feeding
4300 injury.

4301 This study suggests that it could be a potential benefit to crop production to have suitable
4302 host plants of a range of growth stages in order to distribute the feeding of adults, this could
4303 be in the form of trap crop borders which are drilled prior to the crop. Allowing faster
4304 development and thus drawing *P. chrysocephala* from foraging on the emerging OSR. The
4305 potential of using trap crops for *P. chrysocephala* have been proposed (Barari *et al.*, 2005;
4306 Sivčev *et al.*, 2016) and shown some level of impact (Coston *et al.* in prep). With further
4307 study of *P. chrysocephala* feeding timings and location (both within field and upon plant) the
4308 ultimate mechanism for trap cropping can be understood and utilised more efficiently in
4309 IPM strategies.

4310 Studies which have examined the feeding or impact of leaf area injury on OSR have shown
4311 high levels of compensation (Nowatzki. T and Weiss. M, 1997; Susko and Superfisky, 2009;
4312 Ellis, 2015). Including when 90% leaf area is removed at the cotyledon stage (Coston et al in
4313 prep, Chapter 5 this thesis). Many of these studies simulate the injury mechanically which
4314 was shown to result in different responses from actual beetle feeding (Susko and Superfisky,
4315 2009). To understand how OSR compensated to *P. chrysocephala* attack knowledge on how
4316 the feeding is distributed to various plant parts is crucial. Here we demonstrate little feeding
4317 on cotyledons when true leaves are also available.

4318

4319 **6.6 Acknowledgment:**

4320 Steve Harvey at Rothamsted glasshouses for help growing my plants. Fiona Gilzean for
4321 provision of dataloggers and use of controlled environment space. Susanne Clarke for her
4322 assistance in experimental design and statistical analysis.

4323

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4391

4392

4393 **7.0 Assessing the circadian rhythm of *Psylliodes chrysocephala* L. in**
4394 **UK winter oilseed rape (*Brassica napus* L.):**

4395

4396 **7.1 Abstract:**

4397

4398 With the ban on the use of neonicotinoid insecticides on oilseed rape (OSR, *Brassica napus*
4399 L.), The only alternate synthetic insecticide approved for use belong to the pyrethroid class
4400 and in order to reduce spread of resistance need to be applied as effectively as possible.

4401 One of the main targets for autumn insecticide applications in OSR is the cabbage stem flea
4402 beetle (*Psylliodes chrysocephala* L.). There has been anecdotal evidence that the most
4403 appropriate time to apply spray insecticides to target is *P. chrysocephala* because they are
4404 hypothesised to be primarily nocturnal. In this study we examine the diel activity of *P.*
4405 *chrysocephala*, in an OSR crop at Rothamsted Research, UK by using emergence traps set
4406 before and after sunset. There was not active *P. chrysocephala* seen prior to sunset,
4407 supporting the hypothesis that they are primarily nocturnal in the crop. Adult beetles were
4408 caught in all emergence traps suggesting the *P. chrysocephala* shelters below the soil
4409 surface in the day and emerged from the soil after dusk. If spray insecticide application is
4410 required, then the practice of doing so at dusk would coincide with the highest activity.

4411 **7.2 Introduction:**

4412

4413 Cabbage stem flea beetle (*Psylliodes chrysocephala* L.) is a major pest of winter oilseed rape
4414 (OSR, *Brassica napus* L.) in the UK (Alford, 2003; Williams, 2010), causing severe damage
4415 and, in some cases, complete crop failure (Nicholls, 2015). As discussed, Chapter 1 this
4416 thesis), control of *P. chrysocephala* is now heavily reliant on pyrethroid sprays, although
4417 increasing resistance has been observed across the UK (Højland *et al.*, 2015). Thus, it is
4418 important to time the use of pyrethroids, with regard to the time of the season and even
4419 the time of day, to maximise exposure of the pest whilst reducing non-target exposure and
4420 minimising the number of applications to reduce resistance build up. To determine the most
4421 appropriate time to apply any insecticide sprays, a full understanding of the target pest's
4422 behaviour both in space and time is needed. Since the removal of neonicotinoid seed
4423 dressings in OSR from 2014 (EU, 2018) there has been anecdotal evidence that spraying
4424 pyrethroid at night has more of an effect than spraying during the day (Guide, 2016; Hill,
4425 2016). To understand this principle a better understanding of *P. chrysocephala* is needed.
4426 The life cycle of *P. chrysocephala* has been studied in many countries across Europe
4427 (Thioulouse, 1987; Sivčev *et al.*, 2017; Vig, 2003) and is relatively well understood (Williams,
4428 Buechi and Ulber, 2003): adults migrate into the crop during the autumn (Thioulouse, 1987)
4429 where they feed on leaves, producing characteristic shot holing and necrosis (Alford, 2003)
4430 before reproducing within the crop and laying eggs in the soil and at the base of plants
4431 (Alford, 1979; Bonnemaïson and Jourdeuil, 1954). The larvae then emerge, burrow into
4432 plant stems and feed throughout the winter within the plant petioles and stem before
4433 exiting the plant to pupate within the soil. The timings of each stage are dependent on
4434 environmental conditions and can vary from year to year (Williams, 2010). This level of

4435 detail on the yearly cycle of *P. chrysocephala* is important in predicting risk from the pest
4436 and timing of crop migration which can aid in decision making for insecticide application
4437 (Walters *et al.*, 2003). However, the daily activity cycle of *P. chrysocephala* is much less
4438 understood, and it is these daily cycles which may hold the key to improved pest control
4439 through greater refinement of the timing of application of insecticides and more reliable
4440 monitoring to improve risk and threshold assessments.

4441 Preliminary evidence has suggested that *P. chrysocephala* are mostly active at night and are
4442 observed in far greater numbers after sun set (Diehl, 2017; Hill, 2016). This raises two
4443 questions: 1) Is *P. chrysocephala* a nocturnal species; and 2) if so, where do they shelter
4444 during the day? To answer test the timing of *P. chrysocephala* activity, emergence traps
4445 (pyramidal emergence trap with collection chamber at the upper apex to capture
4446 phototactic insects, which have been used previously to sample *P. chrysocephala* adults
4447 emerging after pupation (Conrad *et al.*, 2018; Sivčev *et al.*, 2017), to enclose area of crop
4448 and trap adult beetles during both daylight and night-time hours and thus determine timing
4449 of adult activity. A better understanding of diel activity could help improve Integrated Pest
4450 Management (IPM) by informing the best time to assess crops to selectively target *P.*
4451 *chrysocephala*, when determining action thresholds and ensuring that any insecticide used
4452 is applied to coincide with the peak in beetle activity and maximise pest exposure.

4453

4454 **7.2.1 Aims of study:**

4455

4456 This study was designed to record the activity of *P. chrysocephala* over time and to
4457 determine if the beetles are more active at day or night. Current evidence suggests they are
4458 nocturnal, but no empirical study has been performed in the UK to support this. From
4459 preliminary quadrat observations it is hypothesised that the *P. chrysocephala* is indeed
4460 nocturnal and shelters under the soil surface during the day. This study will record *P.*
4461 *chrysocephala* activity through direct observations and trap any emerging from below the
4462 soil surface using emergence traps.

4463

4464 **7.3 Methods:**

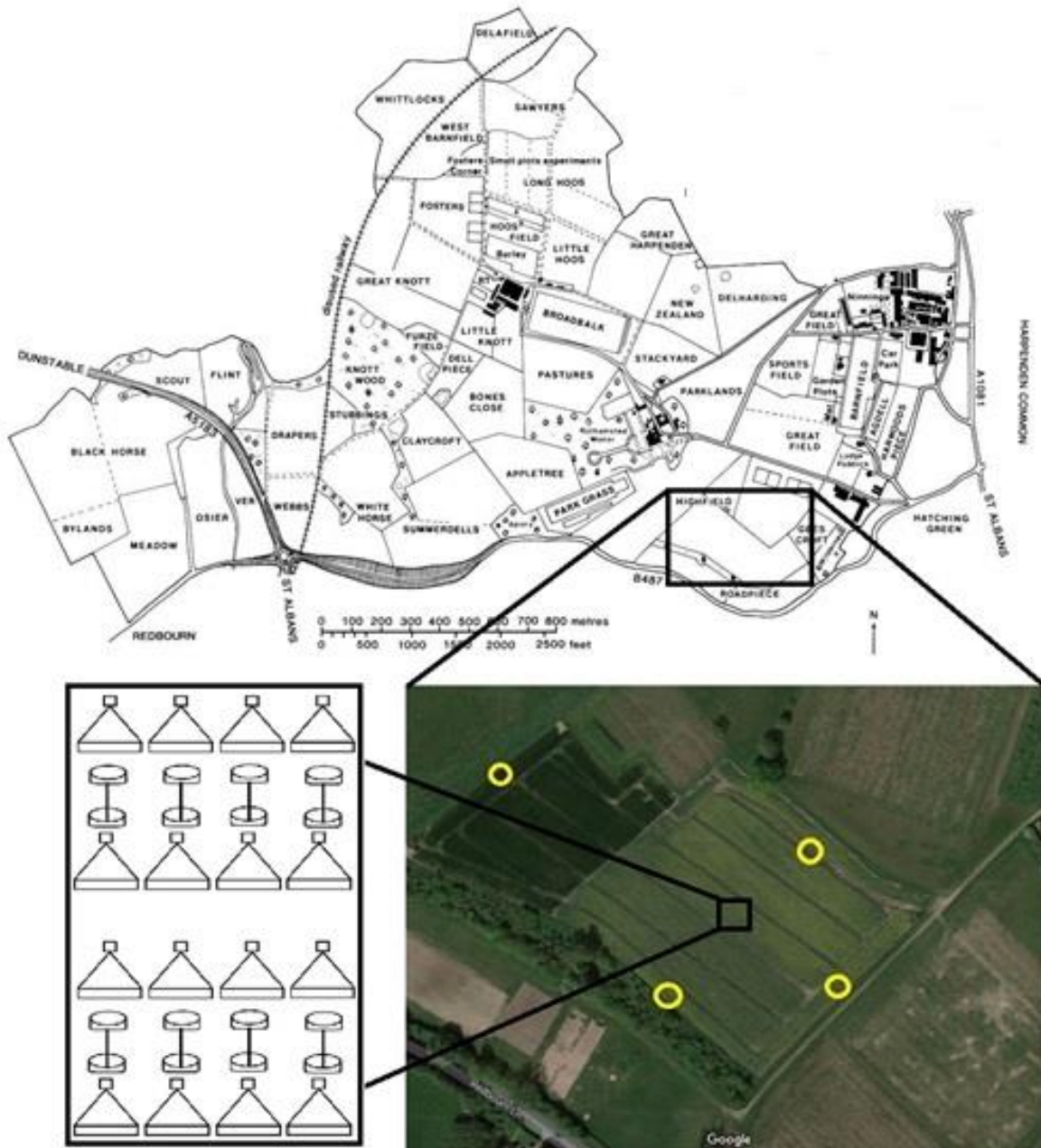
4465

4466 **7.3.1 Experimental site:**

4467 Observations were made in a crop of winter OSR at Rothamsted Farm, Harpenden,

4468 Hertfordshire, UK.

4469



4470

4471 Figure 1. Map of Rothamsted Research showing the location of the experimental oilseed
 4472 rape crop with locations of yellow water traps (yellow circles) and a schematic diagram of
 4473 the layout of emergence traps and associated yellow water traps.

4474

4475

4476 **7.3.2 Sampling of *Psylliodes chrysocephala* adults at the crop edge:**

4477 Prior to any assessment of *P. chrysocephala* activity period it was first confirmed that they
4478 had migrated into the test crop. To measure this Ringot flora (Nickerson Bro, Lincolnshire,
4479 UK) yellow water traps (diameter 265mm) were set up two meters into the headlands on
4480 the 13th of September 2018 at each side of the crop, North, East, South and West (yellow
4481 circles in Figure 1). Each had two traps on a yellow stake; one at ground level and one
4482 suspended at a height of 1.5m and each trap was 1/3 filled with water and a drop of
4483 detergent (Teepol). The traps were designed to measure beetle migration into the crop
4484 (phenology and abundance) and to decipher whether this was by walking/ jumping (ground
4485 level trap) or by flying (above-ground trap). Traps were emptied and reset daily from 13th –
4486 21st September 2018, with the numbers of beetles in traps recorded between 10:00 and
4487 11:00 each day and traps re-set, to ensure a 24-hour exposure.

4488

4489 **7.3.3 Recording of *Psylliodes chrysocephala* adult activity within the**
4490 **crop:**

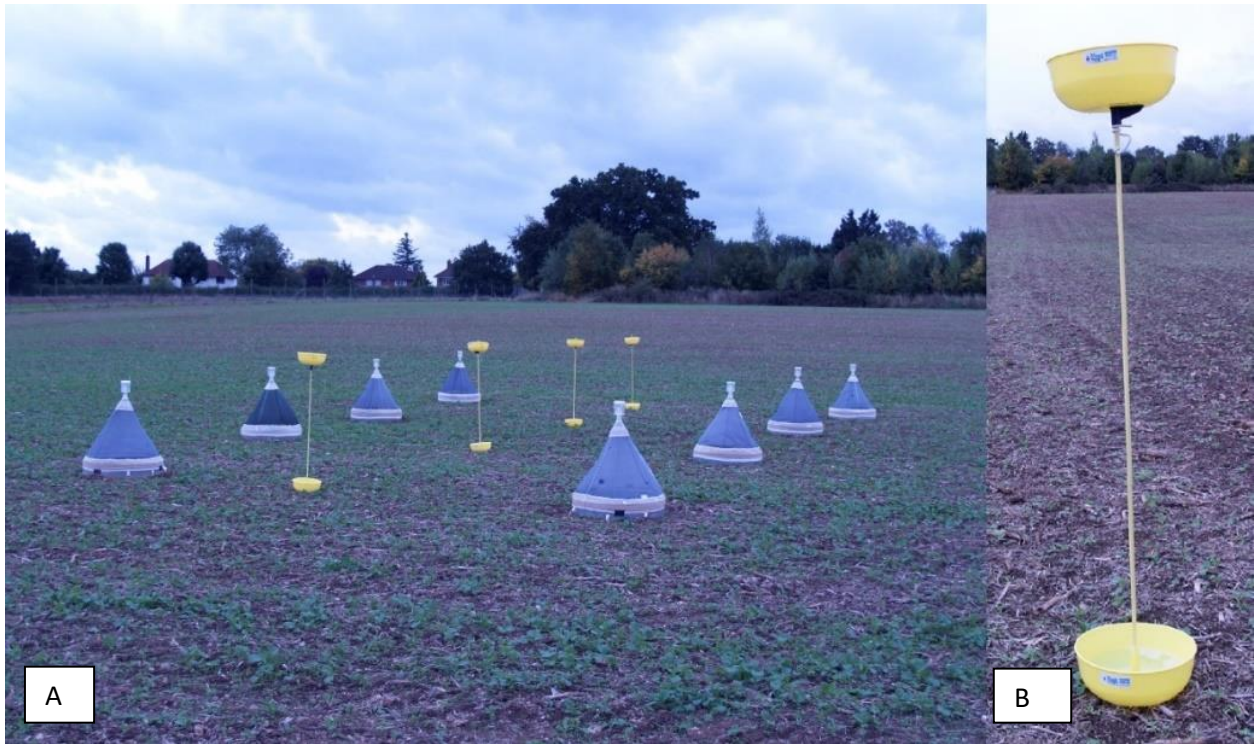
4491 In order to collect *Psylliodes chrysocephala* from enclosed areas emergence traps (Figure)
4492 were used. Emergence traps are designed to capture phototactic-reacting animals, whereby
4493 a metal frame is buried into the ground with a pyramid frame this is then covered with a
4494 fine gage net, at the apical point of the pyramid a collection tub is placed (Figure). The traps
4495 work by enclosing an area of ground with no exit points, thereby, any insects emerging from
4496 the soil will fly to the top of the trap and get trapped in the collection tub. The traps used in
4497 this study were 1m in diameter, 1m height and covered with a 1.5mm gage net, the

4498 collection tub was 1/3 filled with 50% Ethanol to kill and preserve specimens caught. A total
4499 of 16 traps were set up in areas of uniform crop over two days (19th -20th September 2018)
4500 at four times (16:00, 18:00, 20:00 and 22:00 hours). Timing of trap establishment was based
4501 on beetle catches from the crop border yellow water trap. Two emergence traps were set at
4502 each time in two rows, perpendicular to the crop lines, resulting in two blocks of four traps
4503 (eight in total, Figure). Traps were classified as pre-sunset (16:00 and 18:00) and post-sunset
4504 (20:00 and 22:00), as sunset on the days of trap establishment was 19:31 and 19:28,
4505 respectively. These times were selected because of preliminary quadrat assessments
4506 (personal observations) showed no beetle activity prior to sunset thus to confirm nocturnal
4507 activity traps were set before and after sunset. The metal frame of the trap was placed over
4508 the crop and the internal area was carefully inspected for adult *P. chrysocephala*. Any adults
4509 observed were removed after being counted and location recorded as: i) on a plant, ii) on
4510 the substrate surface, or iii) below the substrate surface. Substrate is defined here as any
4511 non-compacted material on the soil surface (e.g., loose stones and soil clumps), which was
4512 lifted to inspect underneath. The number of plants within the frames was also recorded and
4513 the frame was pushed into the soil substrate so that there was no gap under the metal
4514 frame at ground level, through which insects might move into or out of the trap. The trap
4515 was then set by putting the net over the frame and taping the bottom of the net onto the
4516 metal frame to ensure a tight seal. A collection pot 1/3 filled with 70% ethanol to kill and
4517 preserve catches was fitted to the top of the trap and the lid tightened; at this point the trap
4518 was considered active. Traps were left for seven days after which the number of beetles in
4519 each collection pot was recorded.

4520 For time of emergence traps establishment, yellow water traps (as described above, beetle
4521 count taken once at the end of trapping) were set equidistant between the two traps, to

4522 confirm the presence of beetles in the trapping area (Figure). The crop growth stage was
4523 recorded when the traps were dismantled by inspecting 10 randomly selected plants within
4524 the area of each block of traps.

4525



4526

4527 Figure 2. A, In-situ block of emergence traps with accompanying yellow water traps to
4528 assess diel activity of cabbage stem flea beetle (*Psylliodes chrysocephala*) in a crop of winter
4529 oilseed rape (*Brassica napus*). B, Yellow water trap station comprising ground-level and
4530 above-ground traps to measure the proportion of CSFB moving at ground level of aerially.

4531

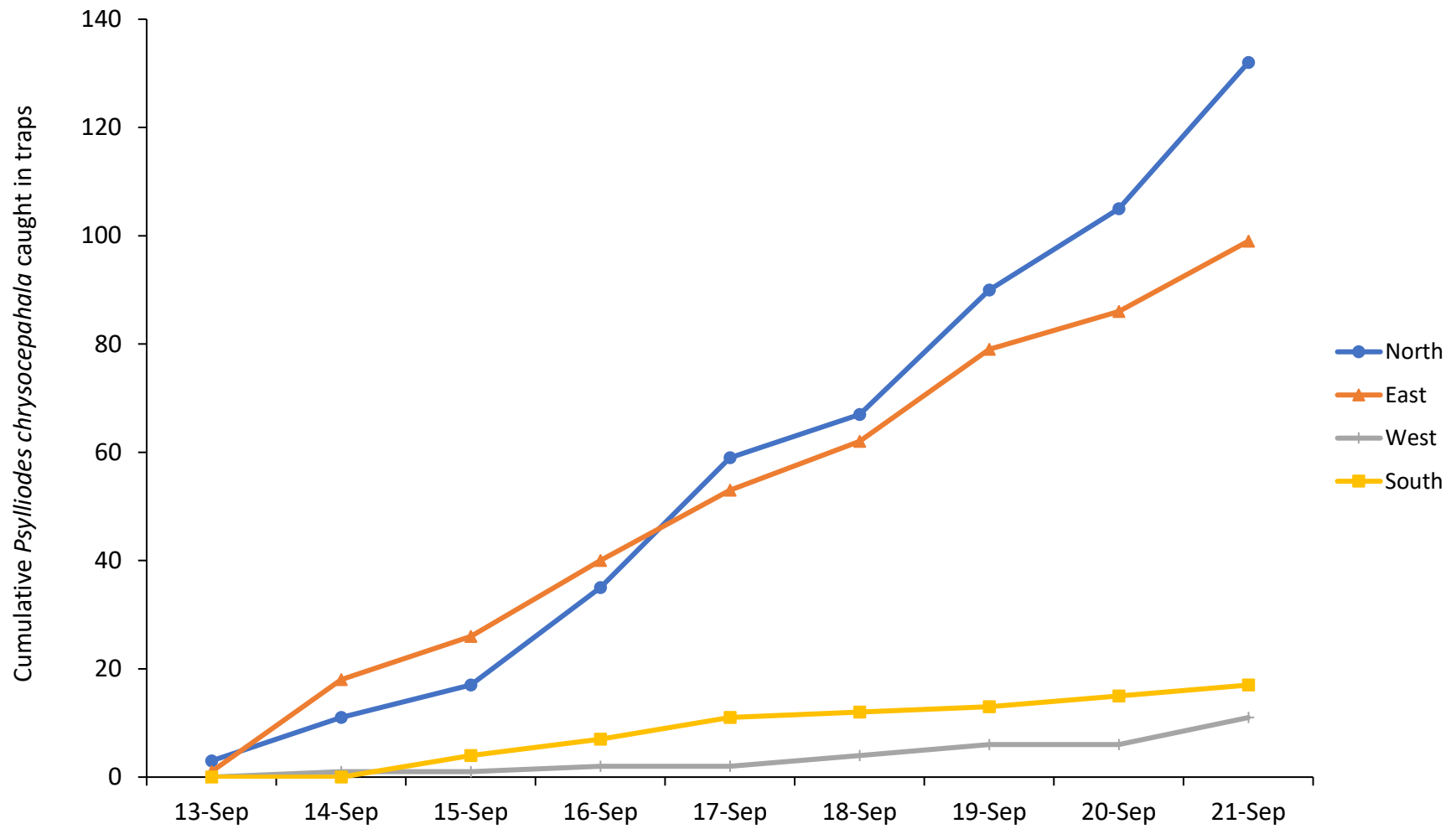
4532 **7.4 Results:**

4533

4534 **7.4.1 Yellow water trap catches:**

4535 Catches in the ground-level yellow water traps set at the crop edge confirmed the presence
4536 of *P. chrysocephala* from the beginning of sampling (Figure 3). Cumulative numbers then
4537 increased over time and there were clear trends for higher numbers in the North and East
4538 edges of the field with relatively few individuals being caught on the South or West sides
4539 (Figure 3). Only two individual *P. chrysocephala* adults were caught in the above-ground
4540 traps at different edges and different dates, so data shown are for ground-level traps only.

4541



4542

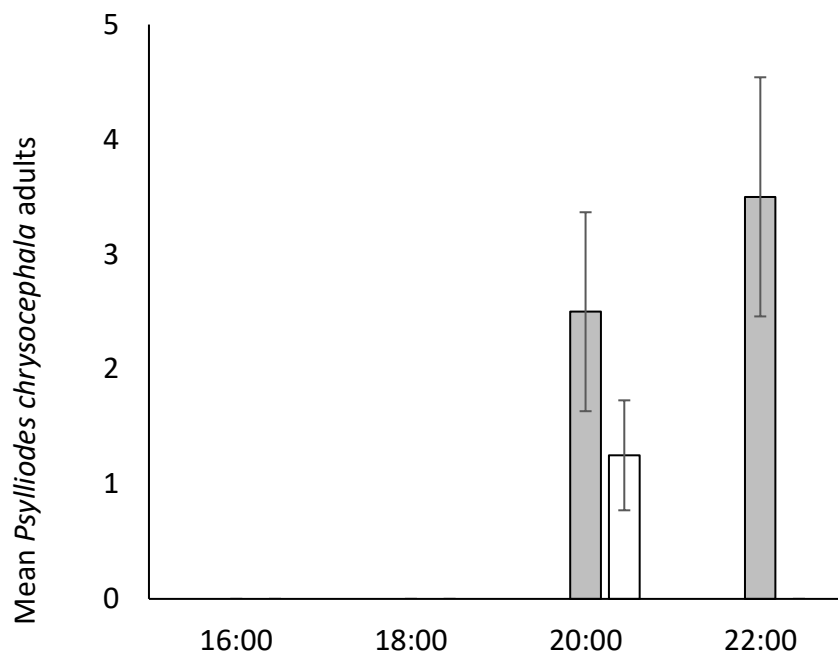
4543 Figure 3. Cumulative numbers of *Psylliodes chrysocephala* in ground level yellow water traps placed at the four edges of a crop of oilseed rape

4544 (*Brassica napus*) in September 2018.

4545 **7.4.2 Recording of *Psylliodes chrysocephala* adult emergence within the crop:**

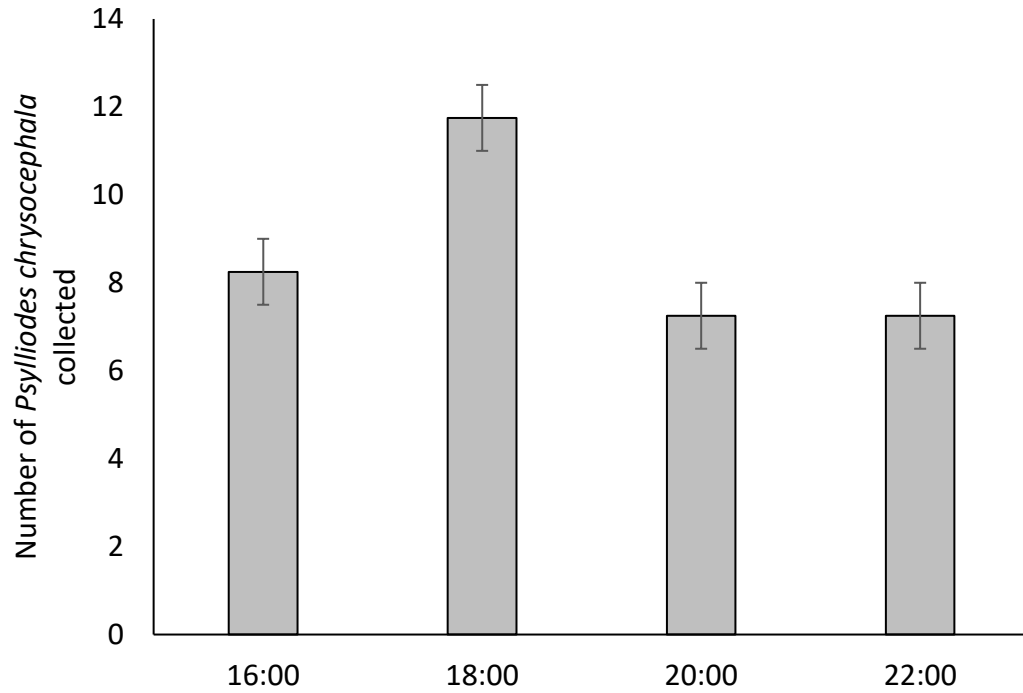
4546 When the traps were set up there were no beetles present before sunset (16:00 and 18:00)
4547 but after sunset active beetles were present on the substrate surface and on the plants
4548 (20:00 and 22:00, Figure 4). After seven days beetles were found in all the traps and the
4549 numbers were comparable regardless of when they were set up. The total numbers of
4550 beetles caught were variable (Figure 5), which could be attributed to an uneven pest
4551 distribution within the crop.

4552



4553

4554 Figure 4. Mean number of *Psylliodes chrysocephala* observed when setting out emergence
4555 traps at four times of day. Grey represents adults on substrate surface and clear is adults on
4556 oilseed rape (*Brassica napus*) leaves. Numbers are from direct observations. Standard error
4557 shown.



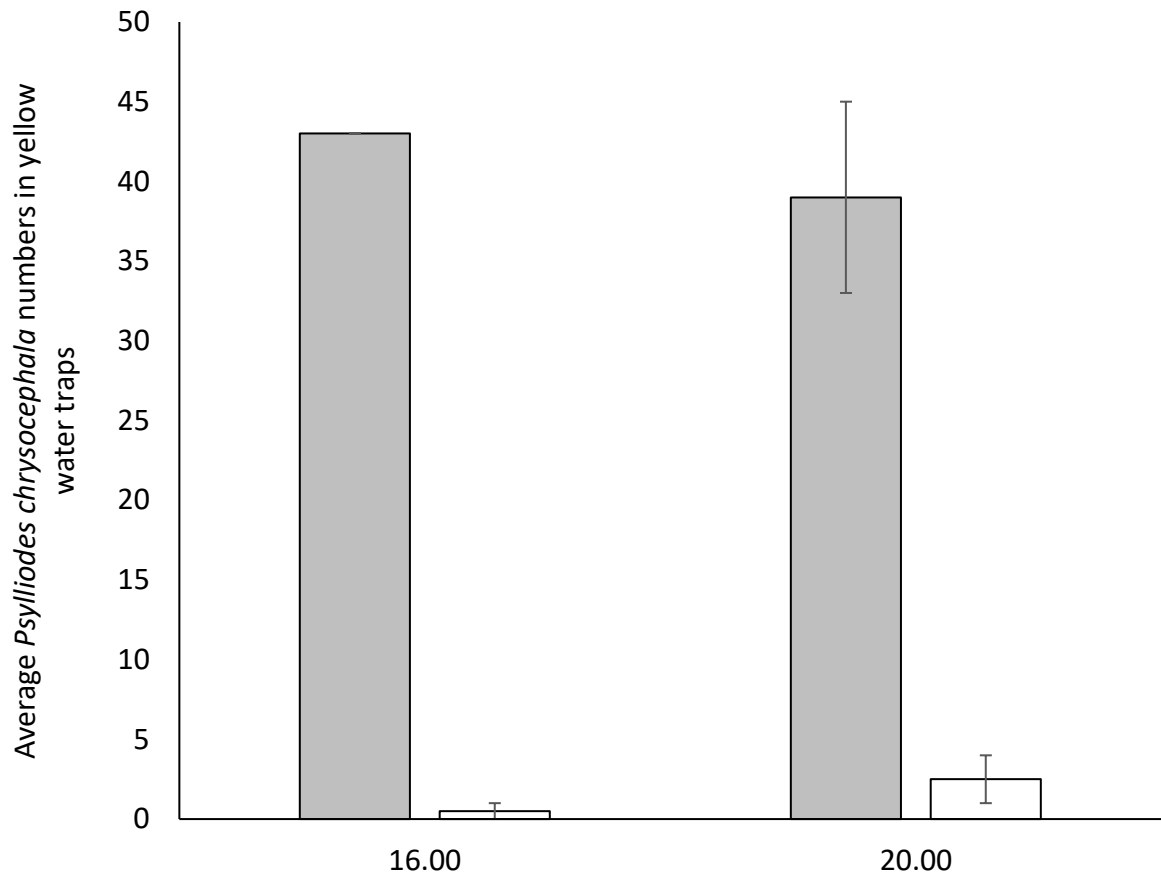
4558

4559 Figure 5. Mean *Psylliodes chrysocephala* adults obtained from emergence traps set out in a
 4560 field of oilseed rape (*Brassica napus*) between 19th and 26th of September 2018. Standard
 4561 error shown.

4562

4563 Data from the yellow water traps confirmed that *P. chrysocephala* were present in the crop
 4564 centre adjacent to the emergence traps, mostly at ground level (Figure 6). At the end of
 4565 emergence traps exposure, the growth stage of the OSR was uniform over the field with 4-5

4566 True leaves, BBCH Growth Stage 14-15 (Lancashire *et al.*, 1991).



4567

4568 Figure 6. Average number of *Psylliodes chrysocephala* caught in yellow water traps set
 4569 adjacent to emergence traps at 16.00 and 20.00 between. Grey bars represent ground-level
 4570 traps and clear represents 1m above ground level traps. Standard error shown.

4571

4572 **7.5 Discussion:**

4573

4574 No active *Phyllodes chrysocephala* were observed before sunset during emergence trap set-
4575 up, supporting the hypothesis that they are predominantly nocturnal when within a crop of
4576 OSR (Figure). All emergence traps caught adult beetles suggesting that once within a crop of
4577 OSR the *P. chrysocephala* shelter under the soil surface during the day (Figure).

4578 Observations of *P. chrysocephala* are known during the day (personal observations)
4579 however, here we demonstrate that once within the crop of OSR they are primarily
4580 nocturnal, with no observations before sun set. Using direct observations of crop area prior
4581 to and post sun set the split in activity observed was clearly associated with sunset. This is a
4582 step towards understanding the circadian rhythm of *P. chrysocephala* which will allow
4583 improved survey and be vital information when assessing action thresholds and application
4584 timings. Several studies in other insect species have demonstrated that circadian rhythms
4585 can vary with season (Meireles-Filho and Kyriacou, 2013; Saunders, 2009). It has been
4586 reported that once in the crop the *P. chrysocephala* wing muscles' atrophy and they no
4587 longer fly (Bonnemaison and Jourdheuil, 1954; Ebbe-Nyman, 1952). this may lead to
4588 nocturnal activity may become more prominent when location of suitable host plants is
4589 achieved, and locomotion is primarily through walking and jumping (personal observation).

4590 The presence of adult beetles in all traps was an interesting observation and should be
4591 explored further to determine the source of the captured beetles. One possible source of
4592 the beetles is that they shelter under the soil surface during the day another is that they are
4593 newly emerged adults which entered aestivation within the crop. Observations from
4594 Hungary (Vig, 2003) and Serbia (Sivčev *et al.*, 2017), suggest that *P. chrysocephala* may

4595 remain within the previous year's stubble and enter aestivation within the stubble area. The
4596 field used in the trial was on its second year of OSR so this cannot be discounted as a source
4597 of the captured beetles. It is, therefore, not possible to confirm that the *P. chrysocephala*
4598 shelters under the soil during the day from the data collected here.

4599 The data presented here suggest that the best time for application of a contact insecticide
4600 targeting adult *P. chrysocephala*, would be after sunset, to coincide with the adult activity
4601 within the crop. It is also suggested that surveying for action thresholds can be carried out
4602 during the day as the majority of activity is at night.

4603 Although post-sunset application would reduce the exposure risk to pollinating insects
4604 (Delaplane and Mayer, 2000), at least diurnal ones (Macgregor and Scott-Brown, 2020), it
4605 might also increase the risk to nocturnal insects including moth pollinators and ground-
4606 dwelling natural enemies such as carabids (Luff, 1978; Lövei and Sunderland, 1996).

4607 The timing of action thresholds survey is important to understand the pressure from the
4608 pest accordingly. Wold-Burkness et al (2006) showed that the common asparagus beetle
4609 (*Crioceris asparagi*) is primarily active at specific times of the day (13:00-17:00). If
4610 appropriate estimations of action thresholds are to be made observations must consider,
4611 the full pest pressure. If the common asparagus aphid were surveyed outside its active
4612 period, the pressure could be underestimated.

4613 To further understand the daily activity pattern of *P. chrysocephala* further observations are
4614 required which cover the full 24hour period and through the autumn period. Prolonged
4615 direct observations or use of time sorting water traps would be a next step in unravelling
4616 the variations in activity for the *P. chrysocephala* and implement IPM strategies accordingly.
4617 The nocturnal activity can be assessed using a combination of dry pitfall traps with direct

4618 observations at distance times. This method is very time consuming and alternatives have
4619 been trailed with Diehl (2017) using automated cat feeders to time sort water traps for *P.*
4620 *chrysocephala*, this proved highly problematic.

4621 *Psylliodes chrysocephala* can even be located easily at night using a torch, in fact for
4622 collection of adults for experiments we now generally search at night with an electric
4623 aspirator (Cook lab group). The authors here suggest that direct observed of the beetles in
4624 the crop would be the most likely strategy, although labour and time intensive, to accurately
4625 assess *P. chrysocephala* activity cycles with a crop of OSR. Similar methods of observations
4626 have been utilised to examine the flight activity of rove beetles (Stylianou, Ashe and Rodney,
4627 2004) and ground beetles (Sunderland *et al.*, 1995).

4628 This combination of detailed life cycle, circadian rhythm coupled with habitat and weather
4629 data would improve the knowledge of *P. chrysocephala* interaction with OSR and inform
4630 decisions on IPM and development of alternative pest control strategies in the future. As
4631 suggested by (Ramsden *et al.*, 2017), for IPM to be an effective crop production strategy, it
4632 is important to have as detailed a knowledge of the pest life cycle and crop exposure levels
4633 as possible.

4634

4635 **7.6 Acknowledgements:**

4636 Susanne Clark helped with experimental design, Sam Cook, Martin Torrance & Jenny Swain
4637 helped with trap collection.

4638

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4708

4709

4710 **8.0 Are farmers really concerned about the neonicotinoid ban?**

4711 **Comparison of UK farmers views on crop protection strategies for**

4712 ***Psylliodes chrysocephala* in winter oilseed rape (*Brassica rape*):**

4713 **past, present, and future:**

4714

4715 **8.1 Abstract:**

4716

4717 Oilseed rape is a major oil crop in the EU and under current regulation neonicotinoid
4718 insecticide use is banned. An online survey of 91 UK winter oilseed rape (WOSR, *Brassica*
4719 *napus*) on the issues surrounding the neonicotinoid ban on the control of the cabbage stem
4720 flea beetle (*Psylliodes chrysocephala*) and the Peach Potato Aphid (*Myzus persicae*) and how
4721 farmers are responding. The survey also aimed to capture data on farmers perceptions on
4722 application thresholds and novel methods of pest control. Respondents reported increased
4723 pest pressure since the restriction, reduced area of OSR grown and expressed concern over
4724 the future pest pressure from *P. chrysocephala*. The majority would use neonicotinoids
4725 again if the ban were lifted.

4726

4727

4728 **8.2 Introduction:**

4729

4730 Oilseed rape (OSR, *Brassica napus*) is a major oil crop in the EU (Ouvrard and Jacquemart,
4731 2019) and globally (FAOSTAT, 2021). It is attacked by several invertebrate pests (Williams,
4732 2010b) which are primarily controlled through the use of synthetic insecticides (Williams,
4733 2010a). In autumn sown OSR the primary pest problem comes from the cabbage stem flea
4734 beetle (*Psylliodes chrysocephala*) and virus vectors such as the peach potato aphid (*Myzus*
4735 *persocae*). Prior to 2013 these pests were controlled by the combination of neonicotinoid
4736 seed treatments and pyrethroid foliar sprays. Since the autumn of 2014, the accessibility of
4737 neonicotinoid seed treatments has been tightly regulated (EU, 2013). With the introduction
4738 of a blanket ban on neonicotinoid use outdoors (EU, 2018), UK growers have voiced
4739 concerns to the future of oilseed rape (OSR, *Brassica napus*) production in the UK (UK,
4740 2020). This is thought to have resulted in a reduction of OSR area as grower's confidence of
4741 a good crop has fallen (Nicholls, 2015).

4742 Surveys of farmers and other pest control experts have highlighted the perceived
4743 importance of neonicotinoids as a pest control strategy, with 72% opposing the ban (Zhang
4744 *et al.*, 2017). In a recent study, the relative efficacy of neonicotinoids and a wide range of
4745 alternatives pest control methods directed at protecting the crop from the cabbage stem
4746 flea beetle (*Psylliodes chrysocephala*) was trialled (Coston et al 2021, chapter 4 this thesis),
4747 demonstrating little impact from neonicotinoid seed dressings (AI: thiamethoxam) and that,
4748 in a field with high percentage of pyrethroid resistance in *P. chrysocephala* (84%), only
4749 minor reductions in larval numbers were reported. This study highlighted the potential of

4750 using cultural methods to reduce pest injury with limiting yield deficit when compared to
4751 control plots of untreated OSR.

4752 Despite this and other evidence to support their effectiveness, given the reported strength
4753 of support for neonicotinoid use, it is important to understand farmer willingness and
4754 barriers to adopting these alternative, non-chemical methods. Linking the responses of
4755 farmers with field trials at Rothamsted (Coston et al 2021 chapter 4 this thesis) on the
4756 efficacy of alternative control methods, a survey an online survey, built in Qualtrics (Provo,
4757 2015), was used to explore UK OSR farmers willingness to adopt alternative control methods
4758 for *P. chrysocephala* and *Myzus persicae* in autumn OSR following the ban on
4759 neonicotinoids, what the barriers towards their use were and the perceived effectiveness of
4760 both neonicotinoid and non-neonicotinoid control methods. The survey also aimed to
4761 collect data on farmers opinions on the neonicotinoid restriction, likelihood of reverting to
4762 them if legislation changes and characteristics of the farm (Location, size) and the farmer
4763 (experience, education).

4764

4765 **8.2.1 Aims of study:**

4766

4767 This survey of UK farmers was aimed to determine which methods of autumn pest control trailed at
4768 Rothamsted in 2016-17 (chapter 4 this thesis) would be acceptable to farmers. The primary aims
4769 were to understand what the current understanding of pest control practices and how effective they
4770 are. The secondary aim was to find out what concerns or barriers to use they have for alternative
4771 practices being trilled in research.

4772

4773 **8.3 Methods:**

4774

4775 **8.3.1 Survey structure:**

4776 The survey was developed on the online survey platform Qualtrics (Provo, 2015). In

4777 February 2019, a pilot of the survey using 19 agriculture students from the University of

4778 Reading was performed. No changes were made from the pilot; therefore, the responses

4779 were retained in the final analysis.

4780 The survey comprised 35 questions (see appendices) divided into five sections,

4781 (i) farm (location and area of OSR between 2012 and 2019) and growers'

4782 characteristics,

4783 (ii) Pest pressure from *P. chrysocephala* and *M. persicae* and view of control efficacy

4784 from neonicotinoids and pyrethroids,

4785 (iii) use of current insecticide application thresholds and view of reliability,

4786 (iv) effectiveness of natural enemies in controlling *P. chrysocephala* and *M. persicae*.

4787 (v) views on alternative control methods for *P. chrysocephala* and *M. persicae* and

4788 willingness to use these methods.

4789 The survey was approved by the ethical committee of the School of Agriculture, Policy and

4790 Development, University of Reading. In line with ethical approval, the collected data can

4791 only be shared in summary and all participants are kept anonymous.

4792

4793 **8.3.2 Survey dissemination:**

4794 The survey was live from March to October 2019. The survey was distributed using social
4795 media, leafleting at Cereals 2019 (see appendices) and by direct contact through. The survey
4796 was also disseminated by the NFU and Farmers Weekly.

4797

4798 **8.3.3 Statistical analysis:**

4799 Analysis of relationships between the probability of a respondent's willingness to use an
4800 option relative was conducted using standard logistic regression modelling in R (R core
4801 team, 2020). Separate analyses were conducted for each option relative to:

- 4802 i) Farmer demographics (size of holdings, AES participation, Organic participation,
4803 years of farming experience, education level and income level),
- 4804 ii) Perceptions of risk from each of the two pests (importance as a pest,
4805 effectiveness of natural enemies as pest control, concern over neonicotinoid and
4806 pyrethroid resistance and change in damage following neonicotinoid
4807 restrictions),
- 4808 iii) Their choice of pest control prior to neonicotinoid restrictions (neonicotinoid
4809 seed treatment, pyrethroid sprays, trap crop borders, intercropping/cover
4810 crops, altered sowing density, topping and other),
- 4811 iv) Their choice of pest control prior to neonicotinoid restrictions (pyrethroid sprays,
4812 other pesticides, trap crop borders, intercropping/cover crops, altered sowing
4813 density, topping and other).

4814 v) farmers unwillingness to use each option relative to the perceived disadvantages
4815 (too expensive, too unreliable, ineffective, increase non-insect pests, increase
4816 weeds, unsightly, labour intensive, lower crop yield/quality).

4817

4818 Due to the low sample variation, the majority of these analyses did not return any
4819 significant effects and are not commented upon.

4820

4821 **8.4 Results:**

4822

4823 **8.4.1 Overview of respondents:**

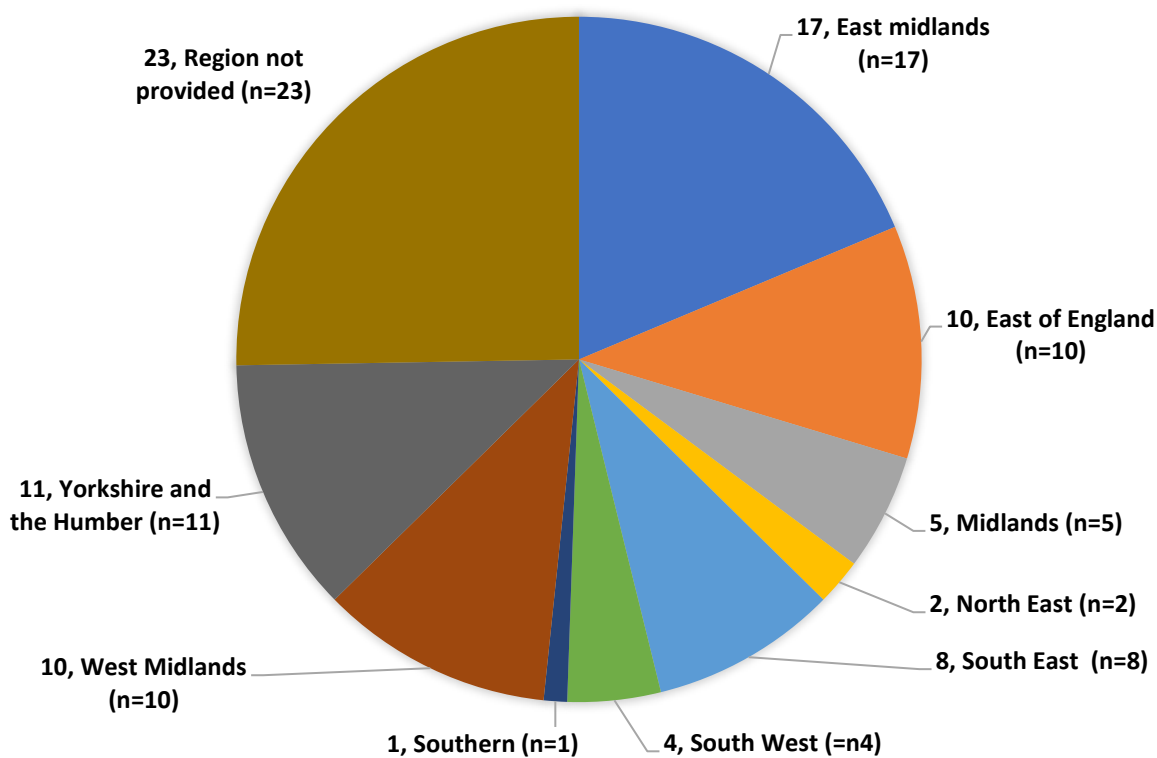
4824 In total 91 useable answers were returned which covered 17,000 to 23,000h over the 2012-
4825 2019 time in the survey (Figure 1 and Figure2). Nationally, OSR area was recorded as
4826 488,000 to 744,000ha in the same period ((DEFRA, 2019, Figure 1). The area under
4827 observation in the study comprises less than 0.05% of the UK OSR production. The low
4828 response rate is typical of online surveys of farmers (e.g., Breeze et al., 2019).

4829 Respondents tended to have large farms (76% had >200 ha farms), be very experienced
4830 (67% had >20 years farm business experience) and many had higher education (only 7% had
4831 O-levels/GCSEs as their highest qualifications, 49% were graduates or postgraduates).

4832 Farmer participation in Agri-Environment schemes (AES) was high at 60% in just AES and a
4833 further 14% in both organic and AES while only 22% were in neither Organic nor AES.

4834 **8.4.2 Regional distribution of respondents:**

4835



4836

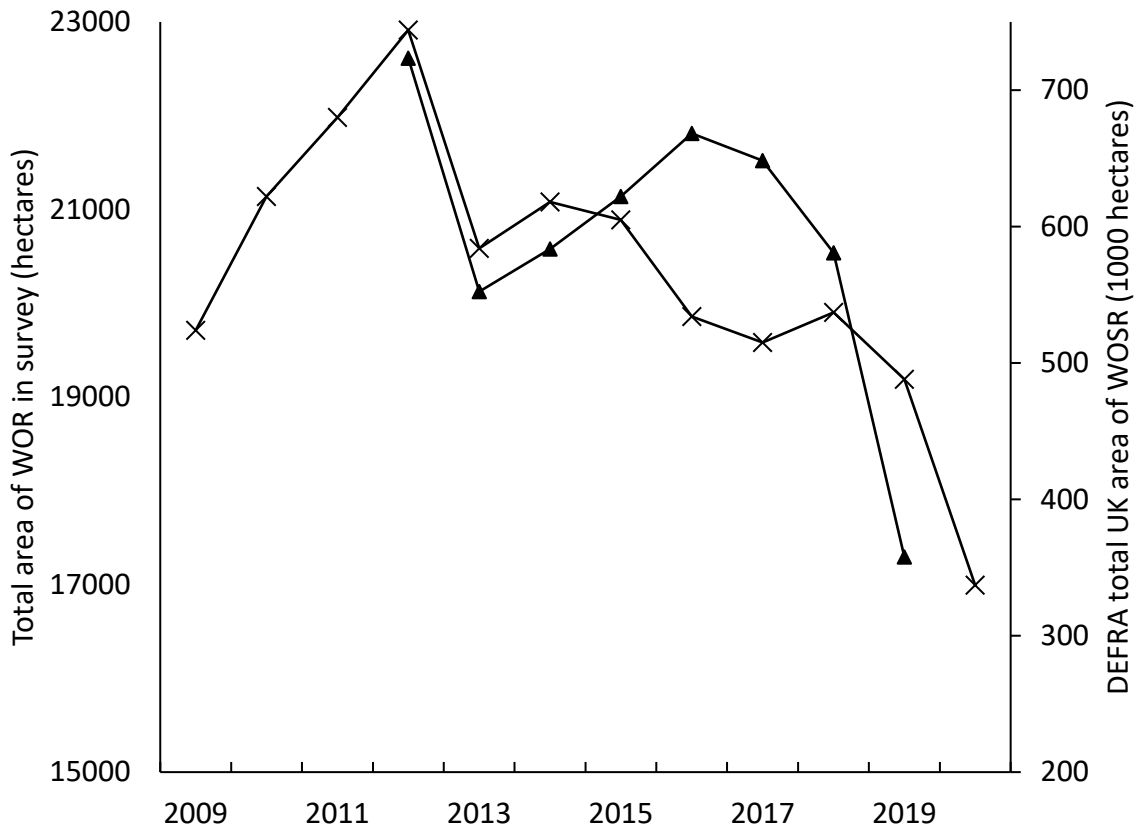
4837 Figure 1. Regional distribution of respondents to survey (n=91). Refined to the nearest level
4838 of accuracy available based on respondents' answers.

4839

4840 **8.4.3 Area of winter oilseed grown:**

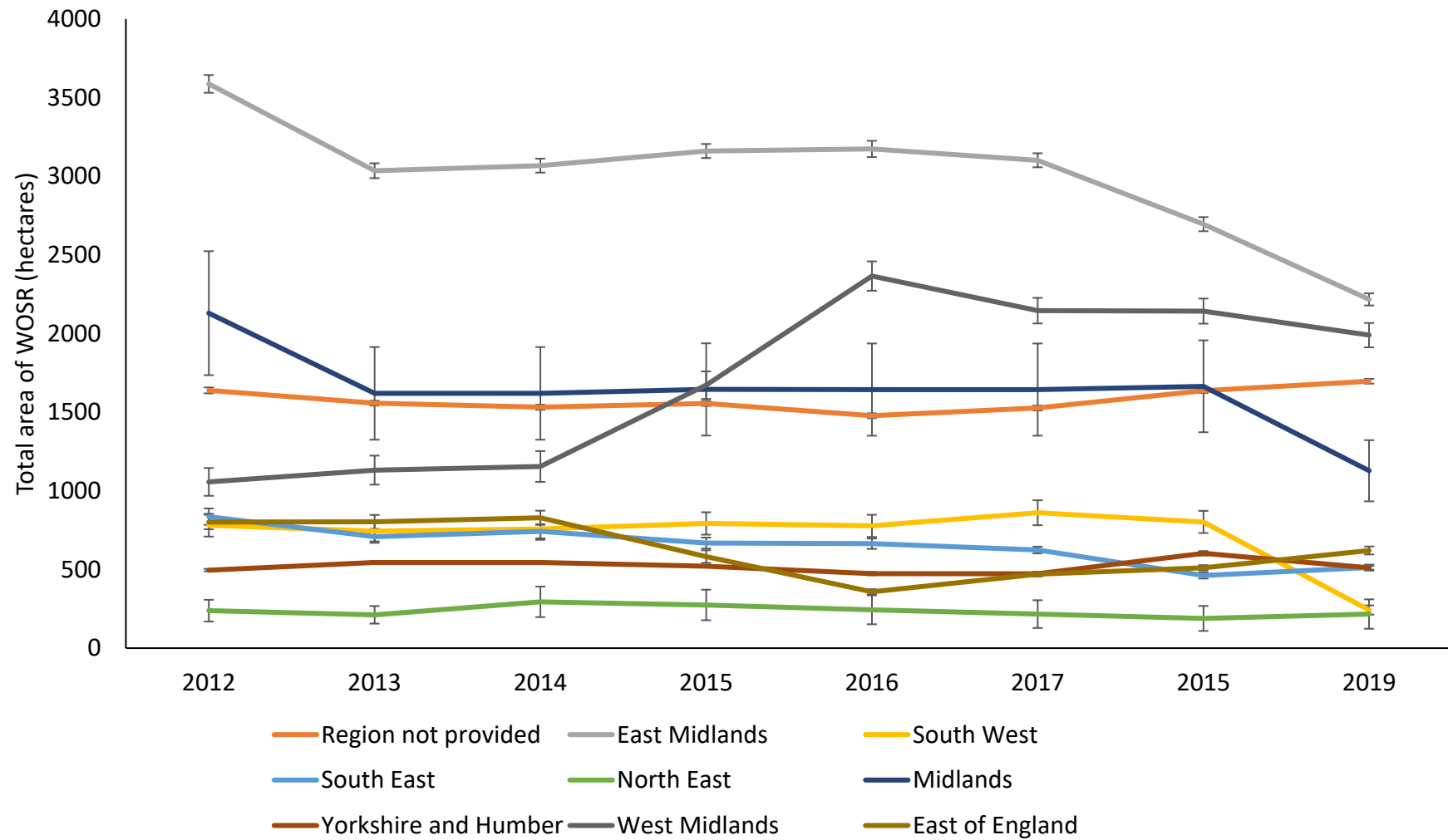
4841 Average area of OSR planted by respondents has declined by 25% between 2012 and 2019
4842 with sharp drops in the last year (Figure 2 and Figure3), mirroring a similar decline in UK OSR
4843 as a whole (Figure 2).

4844



4845

4846 Figure 2 Combined total area, in hectares, of winter oilseed rape (WOSR, *Brassica napus*)
 4847 grown between 2012 and 2019 by survey respondents (closed triangle, left axis). Data
 4848 presented in grand total of all 91 respondents with standard error of means shown. For
 4849 trend comparison the UK winter OSR area is also given (data from DEFRA statistics 2009-
 4850 2020, in thousand hectares, cross, right axis).



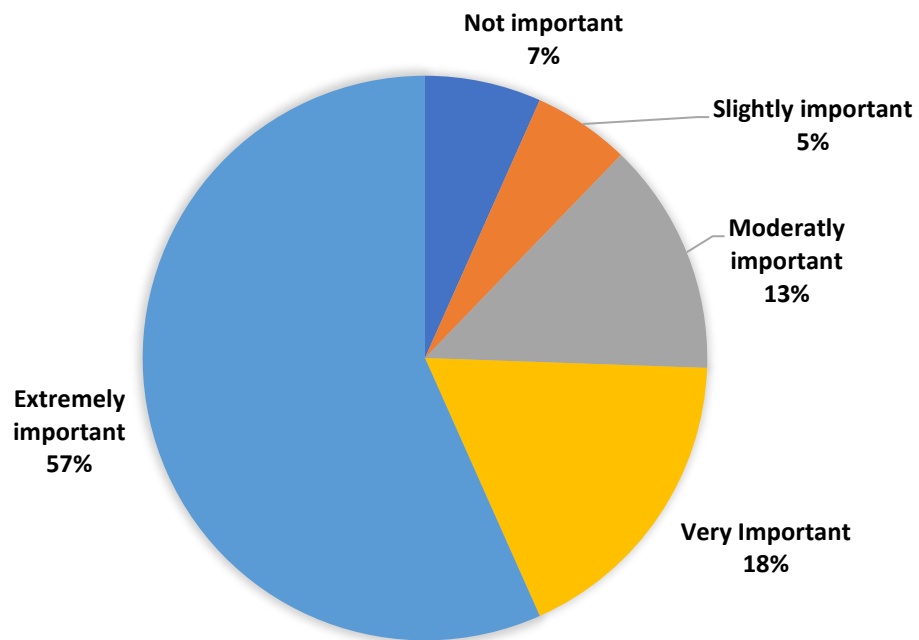
4851

4852 Figure 3. Regional break down of winter oilseed rape (OSR, *Brassica napus*) of survey respondents with standard error of means given.

4853 **8.4.4 Perceptions of pest damage:**

4854 *Psylliodes chrysocephala* was considered “very important” or “extremely important” by 74%
4855 of respondents (Figure4) while *M. perscae* was only considered very important by 3% of
4856 respondents and 65% considered them “not at all important” and “slightly important”. Few
4857 respondents (34%) named other pests, mostly slugs and pigeons, and only 16% rated these
4858 as very or extremely important.

4859



4860

4861 Figure 4. Importance of *Psylliodes chrysocephala* to decision making for growing oilseed
4862 rape in the UK.

4863

4864

4865

4866 **8.4.5 Synthetic insecticide use:**

4867 Prior to the neonicotinoid restrictions 95% of respondents used neonicotinoid seed
4868 dressings to control *P. chrysocephala* while 43% used pyrethroid sprays (41% and 29%
4869 respectively for peach potato aphid). On average, prior to restrictions, farmers used 1.78
4870 measures to control *P. chrysocephala* and 0.85 to control for peach potato aphid. By
4871 contrast, after the restrictions, respondents used an average of 2.08 measures to control for
4872 *P. chrysocephala* and 0.57 measures to control for *M. persacae*, indicating that they have
4873 not increased efforts to control the latter but have had to expand efforts to control the
4874 former.

4875

4876 **8.4.6 Post Restriction:**

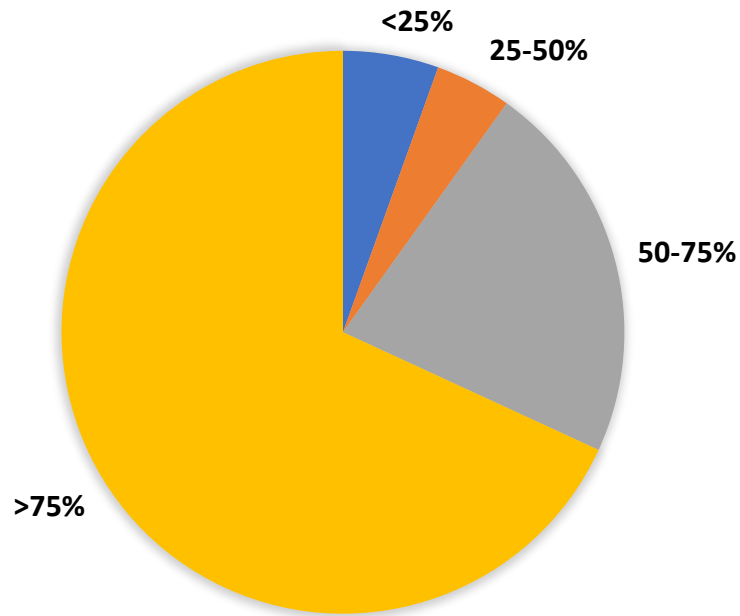
4877 Since the restrictions, to control *P. chrysocephala*, 70% have increased their use of
4878 pyrethroids, 43% altered their sowing density (up from 4% pre-restriction), 35% practice
4879 intercropping (up from 7% pre restrictions) and 31% have adopted new pesticides. For *M.*
4880 *persacae*, 21% have increased pyrethroids and 13% have adopted new pesticides.

4881

4882 **8.4.7 Efficiency of insecticide applications:**

4883 In terms of effectiveness, 90% of respondents felt neonicotinoids were >50% effective at
4884 controlling *P. chrysocephala* (Figure5) while only 38% felt that they were as effective in
4885 controlling *M. persacae*.

4886



4887

4888 Figure 5. Perceived effectiveness of neonicotinoid seed dressings in protection Oilseed rape

4889 (*Brassica napus*) from *Psylliodes chrysocephala* pest damage.

4890

4891 **8.4.8 Resistance:**

4892 Concerns of pyrethroid resistance in *P. chrysocephala* were expressed by 83% of

4893 respondents expressed some degree of concern (Figure6) and 63% to neonicotinoid sprays

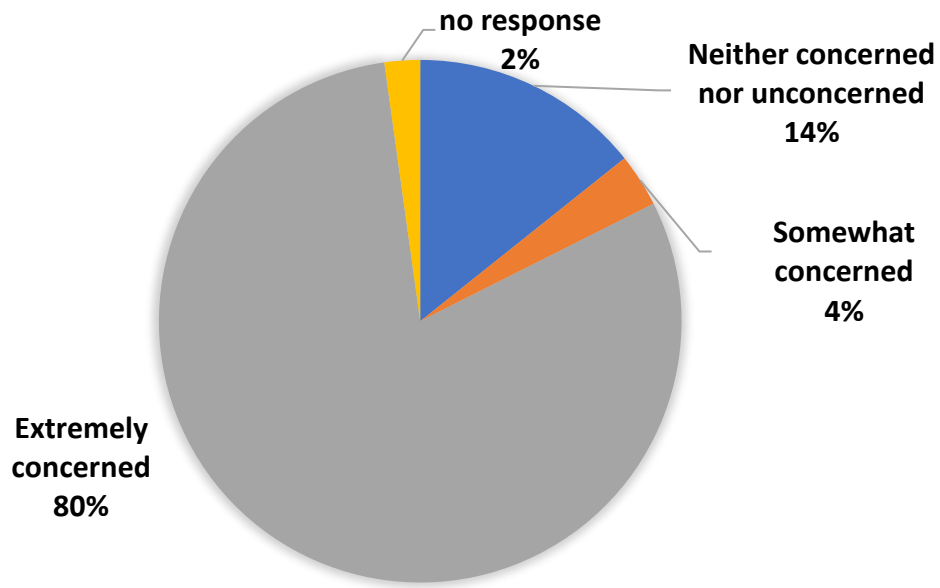
4894 but only 42% were concerned about resistance to neonicotinoid seed dressings. Concern

4895 over each *M. perscae* was more mixed with no definite trends emerging apart from 54% of

4896 respondents expressing concern that they could become resistant to neonicotinoid seed

4897 dressings.

4898



4899

4900 Figure 6. Level of concern of resistance to pyrethroids in *Psylliodes chrysocephala*.

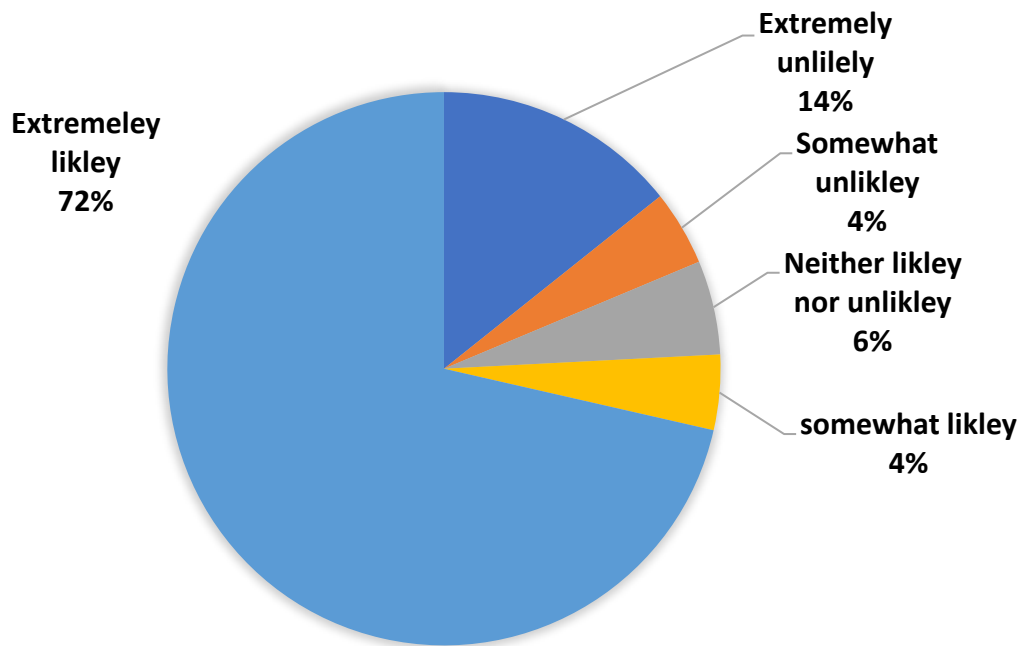
4901

4902 **8.4.9 If the ban were rescinded:**

4903 If they were made available again, 75% of respondents were somewhat likely or extremely
 4904 likely to resume the use of neonicotinoids (Figure7). When asked to explain their response,
 4905 many respondents expressed a preference for neonicotinoids as they were more targeted
 4906 and, they believed, effective at controlling pests without harming beneficial insects.

4907 However, several farmers expressed that they would not readopt to protect wildlife and
 4908 beneficial insects such as bees and natural enemies.

4909



4910

4911 Figure 7. Likelihood respondents would use neonicotinoids if the current ban were
 4912 rescinded. Based on 91 respondents.

4913

4914 **8.4.10 Application thresholds and natural enemies:**

4915 A high proportion of respondents used spray thresholds, 91%, with 82% indicating that they
 4916 carried out such spraying at threshold levels of pests observed either by themselves or at
 4917 the advice of an agronomist. However only 11% of respondents felt that this strategy was
 4918 very or extremely effective while 53% felt it had little or no effect.

4919

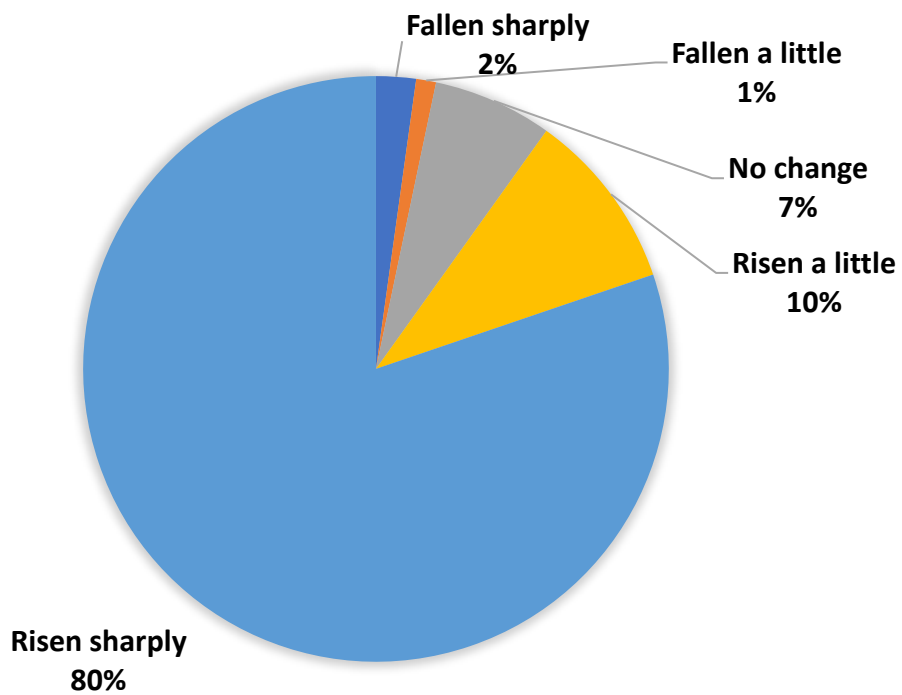
4920 **8.4.11 Pest control by natural enemies:**

4921 Similarly, 76% of respondents felt that natural enemies alone had <50% effectiveness in
 4922 controlling *P. chrysocephala* and 56% that they had <50% effectiveness in controlling *M.*
 4923 *persacae*.

4924 **8.4.12 Post restriction changes in pest pressure:**

4925 When asked to consider how the damage inflicted by pests has changed since the ban on
4926 neonicotinoids, 80% of respondents felt that damage by *P. chrysocephala* had risen sharply
4927 since the ban (Figure8) while only 20% felt damage from *M. perscae* had risen at all
4928 (Figure8).

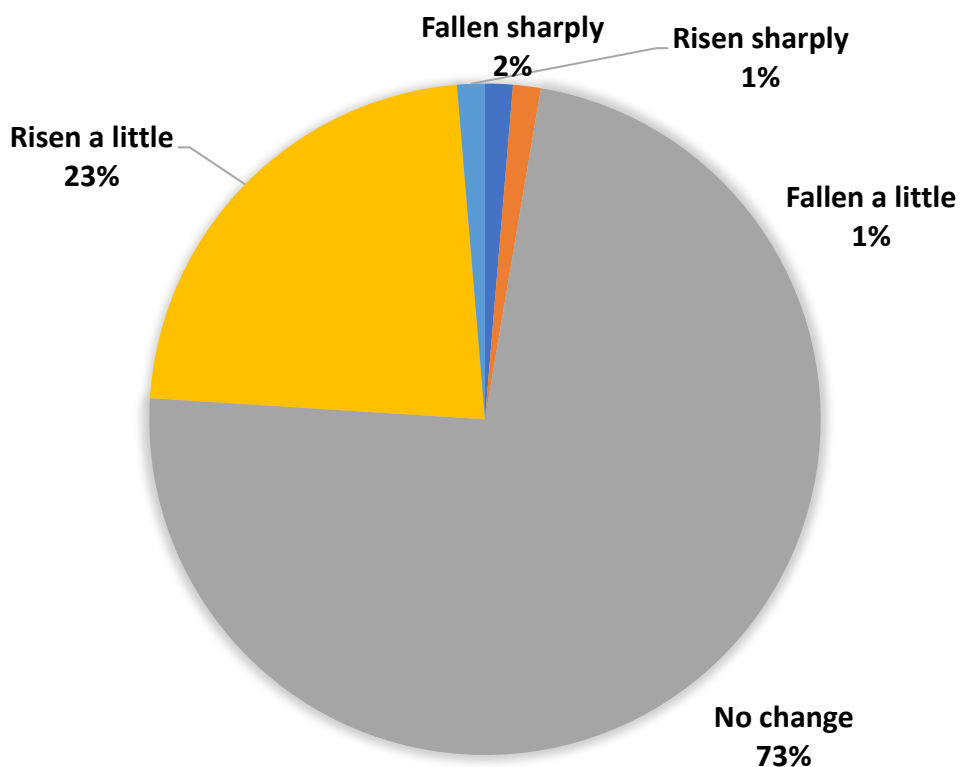
4929



4930

4931 Figure 8. Perceived change in crop damage from *Psylliodes chrysocephala* since the
4932 restriction on the use of neonicotinoid seed dressings came into effect in in the UK in 2014.

4933



4934

4935 Figure 9. Perceived change in *Myzus persicae* damage to oilseed rape crop since the
 4936 restriction on neonicotinoid seed dressing came into force in the UK.

4937

4938 **8.4.13 Adopting new methods:**

4939 Respondents were asked if they would adopt any of a number of alternatives to chemical
 4940 control if sufficient evidence was supplied. Of the options presented for *P. chrysocephala*,
 4941 88% would adopt insect resistant varieties 81% would adopt intercropping or cover crops,
 4942 71% would alter sowing density and 63% would use trap crop borders. For *M. persicae*,
 4943 there was substantially less interest in adopting new measures, only 42% would adopt
 4944 resistant cultivars, 34% would adopt intercropping, 21% would adopt trap cropping and 20%
 4945 would alter sowing density. When asked why they would not adopt any of the measures
 4946 given, no measure was a common concern (all <30% of responses). However, topping the

4947 crop before stem elongation was always the measure that most respondents indicated they
4948 would be unwilling to use, notably for lowering yield and quality (26% of respondents) being
4949 ineffective (23% of respondents) and unreliable (21% of respondents).

4950

4951 **8.4.14 Open questions:**

4952 Of the questions where open answers boxes were available there was relatively little
4953 responses for Q 8, 13, 17, 21, 24 and 25 (Appendix) with each being utilised in 3, 5, 3, 5, 2
4954 and 1 times, respectively. Questions 5, 11 and 12 were filled out more frequently with 19,
4955 47 and 21 responses, respectively. For question 15 on other pests causing a problem,
4956 respondents stated slugs (n=15), pigeons (n=3) and cabbage root fly (n=2) out of 19 total
4957 responses. Question 11 elicited the most engagement with 47/91 respondents leaving a
4958 comment, highlighting the importance of the topic to the participants. The main concern
4959 raised was the inability to grow OSR without the use of neonicotinoids (n=12) with the
4960 increase in *P. chrysocephala* as a problem since the restriction in 2014 and ban in 2018
4961 being stated in 9 responses. Neonicotinoids were stated to not work by 6 participants with
4962 an equal amount saying they are more targeted and better for the environment than a
4963 broad spray application. The impact on non-target species was highlighted by 8 responses
4964 with impact on beneficials mentioned 6 times and impact on soil biodiversity thrice. The
4965 two main comments for question 28 were related to needing robust evidence alternative
4966 techniques work (n=5) and a lack of knowledge of potential techniques (4). Barriers to
4967 botanical diversification (trap crop or cover crop) were stated as the increase in labour
4968 would not be attainable during the busy time of year when OSR seed bed is prepared and
4969 seed drilled (n=3). In other words, alternative methods are too labour intensive to establish

4970 and won't be used. In specific relation to the topping of crop prior to stem elongation 3
4971 respondents made the comment that topping the field at that time of year (February-April),
4972 when the soil is wet and cannot easily support heavy machinery, would be too damaging to
4973 the crop and leave rut ways in the field, this was countermarked by 2 respondents that
4974 using sheep to graze rather than topping would be preferred with one saying the sheep
4975 would be preferred to prevent the damage caused by heavy machinery on wet soil.

4976

4977 **8.4 Discussion:**

4978

4979 **8.4.1 Overview:**

4980 The key research interest of the survey was to explore trends in farmer preferences towards
4981 different alternatives and barriers towards the adoption of options they would like to adopt.
4982 This will be conducted using logistic regression analysis (due to the large number of binary
4983 response variables assessed) or similar statistical methods. However, due to the bias
4984 towards agri-environmental farmers, there is some caution to be exercised in interpreting
4985 these results, however as agri-environmental farmers are more likely to be early adopters of
4986 alternative methods this is not expected to hinder the strength of the outputs. Otherwise,
4987 much of the remaining analysis will be descriptive, looking into the unique insights that this
4988 survey provides regarding farmers rationales for using different methods of pest control and
4989 their answers to open questions where they could state their opinions freely.

4990

4991

4992

4993 **8.4.2 Area change:**

4994 The participants of this survey have reported significant reductions of the area of OSR they
4995 grow since the neonicotinoid restriction came into effect. The reduction in area's grown in
4996 in line with other reports on reductions in OSR post the neonicotinoid restriction (Nicholls,
4997 2015; DEFRA, 2019). This is evidence that the farmers are highly risk adverse and were
4998 heavily reliant on the insurance of seed treatments in OSR. This was born out by the
4999 proportion of respondents who would use neonicotinoids if the ban were rescinded.

5000

5001 **8.4.3 Crop damage levels:**

5002 The levels of damage from *P. chrysocephala* to crop plants is perceived to have risen sharply
5003 since the neonicotinoid ban highlighting, the importance farmers put on neonicotinoid seed
5004 treatments in reducing *P. chrysocephala* feeding damage. In a recent study the effect of
5005 neonicotinoid seed dressings was brought into doubt with little to no reduction in leaf area
5006 loss or larvae numbers from *P. chrysocephala* when compared to untreated OSR (Coston et
5007 al 2021 – chapter 4 this thesis). The findings in plot-based experiments are in contrast to the
5008 level of effectiveness perceived from neonicotinoid seed dressings expressed by
5009 respondents.

5010

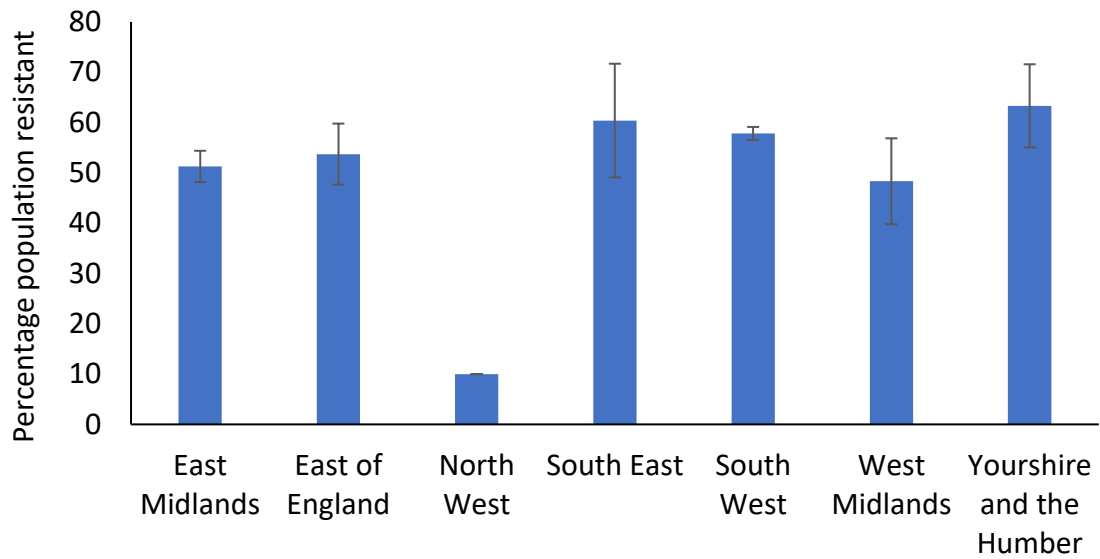
5011

5012

5013 **8.4.4 Pest resistance to insecticide:**

5014 Farmers are fairly confident that *P. chrysocephala* won't become resistant to neonicotinoid
5015 seed dressing but will become resistant to sprays – however despite some concern from
5016 nearly half the respondents regarding *P. chrysocephala* resistance to neonicotinoid sprays
5017 they are very likely to resume using them. The high levels of concern raised by respondents
5018 to pyrethroid resistance developing in their region is in line with a recent survey of *P.*
5019 *chrysocephala* resistance in the UK reporting widespread resistance (Willis *et al.*, 2020), see
5020 Figure 10. The levels of resistance reported by Will *et al.*, (2020) were from adult beetles
5021 and in a recent study (Coston *et al* 2020 chapter 4 this thesis) showed up to 84.9%
5022 resistance. However, in this field trial the application of pyrethroids significantly reduced the
5023 number of *P. chrysocephala* larvae in plants. Suggesting that even when adult populations
5024 exhibit high levels of resistance there is still a potential protection against eggs and larvae of
5025 *P. chrysocephala*. Further investigation into the susceptibility of *P. chrysocephala* to
5026 pyrethroids at different life stages should be carried out soon.

5027



5028

5029 Figure 10. Percentage population of *Psylliodes chrysocephala* exhibiting resistance to
 5030 pyrethroid insecticide (data from Willis *et al.*, (2020)).

5031

5032

5033 **8.4.5 Adopting new methods:**

5034 The majority of respondents were open to novel methods of OSR cultivation in terms of new
 5035 methods for *P. chrysocephala* protection. They were less inclined to adopt methods for
 5036 protecting the crop from *M. persicae*, which was perceived as of little importance by the
 5037 majority of respondents. One method which shows promise in reducing *P. chrysocephala*
 5038 larvae is the topping or grazing of the crop. This was trialled in the linked field experiment and
 5039 showed significant reductions in larvae numbers (Coston et al 2021 – chapter 4 this thesis).
 5040 However, the subsequent delay in flowering brought on by topping left these plants at greater
 5041 risk to pollen beetle (*Brassicogethes aeneus*). The respondents were concerned about such
 5042 factors but were more concerned on the practicalities of using machinery on the crop before
 5043 OSR stem elongation. An issue which can be removed by grazing the crop instead of topping,

5044 which was noted by respondents. Recent work at AHDB is examining the potential of sheep
5045 grazing as a means of reducing *P. chrysocephala* in plants (White *et al.*, 2020). There is also
5046 evidence in spring OSR from Canada and Australia that if grazing is timed correctly little
5047 reduction in the grain yield is seen (Kirkegaard *et al.*, 2008; Seymour *et al.*, 2015). This survey
5048 shows that farmers are willing to use these methods and are awaiting evidence of
5049 effectiveness. The primary issue respondents had to alternative methods was a lack of
5050 evidence of effectiveness. Some of this lack of evidence may be brought on by the limited
5051 access to scientific journals in the farming community.

5052

5053 **8.4.6 Conclusion:**

5054 The levels of concern about loss of sufficient crop protection in OSR with the removal of
5055 neonicotinoid seed dressings highlights, the level of importance they were regarded by
5056 growers. This being said one respondent made special note on the issues of ease of use and
5057 I quote “Neonics have allowed poor establishment techniques to remain viable. Planted
5058 correctly, there is no risk of insect attack-Insects only attack stressed plants.” This and the
5059 willingness to adopt new methods by growers such as intercropping or elevated seed rate
5060 shows there is a willingness to adapt to a new non neonicotinoid cropping system. It is
5061 highlighted the uptake of novel sustainable crop protection practices are in need of furthers
5062 robust evidence to convince farmers of their effectiveness.

5063

5064 **8.5 Acknowledgments:**

5065 I would like to thank farmers weekly, the NFU and Rothamsted research for publicising the
5066 survey and aiding in its distribution to appropriate growers. The university of Reading
5067 agricultural students who participated in the testing of the survey.

5068

5069 **8.6 Appendices:**

5070

5071 Q00 Thank you for participating in this study on British Farmer's perceptions of pest issues
5072 and management in winter oilseed rape. We hope the findings of this survey will help
5073 identify how British farmers feel about pest management strategies to common oilseed
5074 rape pests. This study is part of a PhD project conducted by The University of Reading and
5075 Rothamsted Research and should take no more than 20mins to complete. This study is
5076 funded by Lawes Agricultural Trust, and has been designed, administered and all data
5077 collected will be analyzed by The University of Reading (UK). As part of this survey, we will
5078 ask a few basic demographic questions (e.g. your age, location of your farm etc.) in order to
5079 identify trends in perceptions between farmers. Under data protection law, we are required
5080 to inform you that the use of the personal data we may hold about you is on the lawful basis
5081 of being a public task in the public interest and where it is necessary for scientific or
5082 historical research purposes. All of the information collected will be held by the University of
5083 Reading (Data controller) and by Qualtrics, the online platform that this survey uses. Both
5084 the University of Reading and Qualtrics are fully complicit with EU and UK data protection
5085 laws. The data collected in this survey is only intended for use as part of the PhD research
5086 project "Quantifying the impacts of the neonicotinoid restriction on oilseed rape pest

5087 control, pollination and productivity", although summarised versions of this data may be
5088 made available for later analysis. Should you wish to withdraw your answers from this
5089 survey at any time prior to the publication of results please call me on 0118 378 6419 or e-
5090 mail t.d.breeze@reading.ac.uk and quote the questionnaire ID below – this number is linked
5091 to your responses. If you withdraw from a research study, which processes your personal
5092 data, dependent on the stage of withdrawal, we may still rely on this lawful basis to
5093 continue using your data if your withdrawal would be of significant detriment to the
5094 research study aims. The survey does not ask for your name, or the address of either you or
5095 your farm operations. Your individual responses will be held by the University of Reading for
5096 the duration of the project and then destroyed. Anonymised summaries of responses from
5097 all participants will be retained by the University of Reading indefinitely for use in future
5098 work. The data will be stored on a secure drive only accessible to the University of Reading
5099 (Dr Tom Breeze, Duncan Coston) and will not be passed on to any third parties. By
5100 participating you are consenting to these terms of data storage and use which have been
5101 approved by the University of Reading's Ethics.

5102

5103 **Q1:** Please enter a unique identifier text (e.g. the present date and time) so we can retrieve
5104 your questionnaire upon request.

5105

5106 **Q2:** Which region are your farming activities based in?

5107

5108 **Q3:** Approximately how large is your farming operation currently?

5109 Less than 20 Hectares

5110 20-50 Hectares

5111 51-100 Hectares

5112 101-200 Hectares

5113 More than 200 Hectares

5114

5115

5116 **Q4:** Approximately how many hectares of winter oilseed rape did you/ do you plan to plant
5117 in each of the following years? This does not have to be exact. Please put NA if you were not
5118 farming or 0 if you did not grow winter oilseed rape that year.

5119 2012 2013 2014 2015 2016 2017 2018 2019

5120

5121 **Q5:** How important are levels of autumn pests in determining the amount of winter oilseed
5122 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)					

5123

5124 **Q6:** Following the restrictions on neonicotinoid insecticide use, how much do you believe
5125 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					

5126

5127 **Q7:** How effective do you believe neonicotinoid seed treatments were/ are at controlling
5128 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				

5129

5130 **Q8:** How concerned are you about the cabbage stem flea beetle becoming resistant to
5131 common synthetic insecticides?

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

5132

5133 **Q9:** How concerned are you about the peach potato aphid becoming resistant to common
5134 synthetic insecticides?

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

5135

5136 **Q10:** If restrictions on neonicotinoid seed treatment use in winter oilseed rape were lifted,
5137 but you had the option of using untreated seed, how likely are you to use neonicotinoid
5138 seed treatment again?

5139 Extremely likely, Somewhat likely, Neither likely nor unlikely, Somewhat unlikely, Extremely
5140 unlikely

5141

5142 **Q11:** Please use this space if you wish to explain your answer further

5143

5144 **Q12:** At any point since 2012, have you used any pest spray threshold strategies (only
5145 spraying when a certain number of pests are detected)?

5146 Yes or No

5147

5148 **Q13:** If you do not use pest spray thresholds which of the following most accurately
5149 describes your spraying strategy?

5150 Spray by date, Spray by crop growth stage, Other (please state)

5151

5152 **Q14:** Which of the following most accurately describes your inspection and spraying
5153 strategy?

5154 Inspect one field, spray all fields if any are found to be above the threshold.

5155 Only spray fields where threshold levels of pests have been observed.

5156 A mix of the above.

5157 I don't know – this was carried out by/under advice from agronomists.

5158

5159 **Q15:** How reliable do you think current pest threshold strategies are at protecting your
5160 winter oilseed rape crops?

5161 Extremely effective, Very effective, Moderately effective, Slightly effective, or Not effective
5162 at all.

5163

5164 **Q16:** Without insecticides, how much do you think arthropod natural enemies (e.g. ground
5165 beetles, wasps, ladybirds etc) alone could control cabbage stem flea beetle and peach
5166 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					

5167

5168 **Q17:** Before the restrictions on neonicotinoid seed treatments, did you use any of the
 5169 following measures to control autumn pests in your winter oilseed rape crop? (please tick all
 5170 that apply)

	Neonicotinoi d seed treatment	Pyretheroid spray application s	Trap crop borde r	Intercro p or cover crops	Altere d sowing density	Topping the crop before stem elongatio n	Othe r
Cabbag e stem flea beetle							
Peach potato aphid							
Other (please state)							

5171

5172 **Q18:** Which crops did you use as trap crops or intercrops before the restrictions on
 5173 neonicotinoid seed treatments?

5174

5175 **Q19:** How did you alter your sowing density of winter oilseed rape before the restrictions on
 5176 neonicotinoid seed treatments?

5177

5178 **Q20:** Which other methods did you use to control these pests before the restrictions on
 5179 neonicotinoid seed treatments?

5180

5181 **Q21:** Following the restrictions on neonicotinoid seed treatments what measures have you
 5182 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)

5183

5184 **Q22:** Which crops did you use as trap crops or intercrops following the restrictions on
5185 neonicotinoid seed treatments?

5186

5187 **Q23:** How did you alter your sowing density of winter oilseed rape following the restrictions
5188 on neonicotinoid seed treatments?

5189

5190 **Q24:** Which other methods do you use to control these pests following the restrictions on
5191 neonicotinoid seed treatments?

5192

5193 **Q25:** If there were further evidence that they could potentially control autumn pests, would
5194 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)

5195

5196 **Q26:** Which other measures would you want to use to control autumn pests in your winter
5197 oilseed rape crops? Please use this space to tell us about them and why you would like to
5198 use them.

5199

5200 **Q27:** If you are not willing to use any of the following management options, please indicate
5201 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					

5202 **Q28:** Please use this space to tell us about other reasons why you would not want to use
5203 these methods on your winter oilseed rape crops

5204

5205 **Q29:** How long have you been involved in farming at a business level?

5206 Under 5 years, 5-10 years, 11-15 years, 16-20 years, or more than 20 years.

5207

5208 **Q30:** What is the highest level of formal education in subjects relevant to farming you
5209 possess?

5210 GCSE/O-Level, A-level, Undergraduate degree, Postgraduate degree, or Other (including
5211 BASIS).

5212

5213 **Q31:** Do you participate in the Countryside Stewardship or any other agri-environment
5214 scheme (government or private)?

5215 Yes or No

5216

5217 **Q32:** Which agri-environment scheme do you participate in?

5218

5219 **Q33:** Do you participate in any organic or low input farming schemes (government or
5220 private)?

5221 Yes or No

5222

5223 **Q34:** Which organic or low input farming scheme do you participate in?

5224

5225 **Q35:** Finally, please indicate your average net farm income in your last two financial years
5226 using the categories below.

5227 Less than zero (loss), 0 to less than £5,000, £5,000 to less than £10,000, £10,000 to less than
5228 £20,000, £20,000 to less than £30,000, £30,000 to less than £50,000, More than £50,000 or I
5229 would rather not say.

5230



5231

5232 Figure 11. Promotional flyer distributed at events and to farmers as a means of distributing
 5233 the survey.

5234

5235

5236 8.6 References:

5237

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5275

5276

5277 **9.0 Concluding discussion:**

5278

5279 The restriction of neonicotinoids across the EU in 2013 (EU, 2013) and its subsequent re-
5280 enforcement and expansion to a total ban in 2018 (EU, 2018). This has manifested in
5281 reductions in cropping area in the UK (DEFRA, 2019; Scott and Bilsborrow, 2019) and wide
5282 publication of farmers concerns of growing OSR without neonicotinoids (Guardian, 2020).
5283 The development of alternatives is vital to maintain OSR in the UK cropping system (Impey,
5284 2020). The continued reduction in OSR production may be reducing the complexity of field
5285 rotations removing the benefits of complex systems in limiting disease and weed prevalence
5286 (Hilton *et al.*, 2018; Malik, 2010; Weiser *et al.*, 2018).

5287

5288 **9.1. Methods of pest control targeted at *Psylliodes chrysocephala*:**

5289

5290 **9.1.1 Seed treatments:**

5291 In the work presented here the effectiveness of novel cropping systems to protect OSR from
5292 *P. chrysocephala* were directly compared to OSR treated with a neonicotinoid seed
5293 treatment (Cruiser®, AI: thiamethoxam). In no assessments in any of the field trails
5294 undertaken in this thesis did neonicotinoid seed dressings reduce pest injury, mortality or
5295 affect yield in comparison to un-treated seed. The lack of any detectable difference
5296 between treated and un-treated seed puts doubt into the level of protection provided. The
5297 data presented here would support the ban on neonicotinoid use in the fact no appreciable
5298 benefit to the crop was detected.

5299 At the time of the experiment, the seed treatment Lumiposa™ (AI: cyantraniliprole) was
5300 registered for use in Poland, Hungary and Romania to protect OSR from *P. chrysocephala*
5301 attack, but not in the UK yet (NFU, 2018). No effect on the level of *P. chrysocephala* feeding
5302 or larval infection in relation to un-treated OSR was observed in this thesis (chapter 4).
5303 Lumiposa™ is recommended to protect early growth OSR until BBCH 13-14 from *P.*
5304 *chrysocephala* and is marketed to “protect seedlings producing uniform and healthy stands”
5305 (DuPont, 2017). The data shown here would suggest that the time of Lumiposa
5306 recommended active period is outside the preferred feeding of adult *P. chrysocephala*,
5307 reducing the efficacy as an aid to crop establishment.

5308

5309 **9.1.2 Spray application:**

5310 High levels of pyrethroid resistance have been reported across the UK (Willis *et al.*, 2020).
5311 The fields in this study were no different with resistance exhibited in up 84.9% resistance in
5312 field populations (chapter 4). When examining the reductions in *P. chrysocephala* larvae a
5313 significant reduction was seen when pyrethroids were applied (chapter 4), suggesting that
5314 the eggs or larvae are more susceptible. This variation in toxicity to differing life stages may
5315 be in part due to the form of resistance being expressed (Panini *et al.*, 2016). where
5316 metabolic resistance is concerned, where the toxic compounds are metabolically broken
5317 down, a cost to resistance applies (Kliot and Ghanim, 2012; French-Constant and Bass,
5318 2017). This mode of action exhibiting resistance may be limited or impossible at the egg or
5319 larval stages (Zimmer, 2015). One further hypothesis is that the cost of resistance exhibited
5320 by *P. chrysocephala* also has negative effects on the adult fecundity. The sub-lethal effects
5321 of insecticide have included a reduction in fecundity (Lee, 2000; Rivero *et al.*, 2011). Further

5322 investigation into the sub-lethal effects of pyrethroids on *P. chrysocephala* may aid in
5323 interpreting the reduction in larvae seen here from its application.

5324 The reduction in larvae reported here suggests that even at high adult resistance
5325 pyrethroids do express a level of pest control. The timing of application to avoid adults and
5326 target eggs or larvae should be examined along with the variations in toxicity at different
5327 developmental stages.

5328

5329 **9.1.3 Seed application rate:**

5330 Responses of pest pressure from *P. chrysocephala* were shown to fluctuate depending on the
5331 time of observation (chapter 4), lower larvae numbers at lower seed rate in November but
5332 significantly higher in February (60seeds/m² against 100 and 120seeds/m²). Slight increases
5333 in yield were apparent as the seed rate applied reduced, recording declining yield return
5334 with increasing seed rate, although not significant. The level of adult feeding was in line with
5335 the findings of Alves et al. (2015) that reported crops sown using low seed rates suffer from
5336 greater adult *P. chrysocephala* feeding activity per plant than crops sown using higher seed
5337 rate. The increase in seed rate did trend towards lower yield returns but these were not
5338 significantly different (chapter 4). The increase in plant density is a likely cause for reduced
5339 yield as a response to increased competition between plants (Roques and Berry, 2016; Berry
5340 and Spink, 2006). The limited yield penalties reported here suggest that current advice to
5341 increase seed application to reduce *P. chrysocephala* problems (AHDB, 2019) is worth
5342 further investigation.

5343

5344 **9.1.4 Trap cropping:**

5345 The pollen beetle has been shown to migrate into the crop from the edge (Cook *et al.*,
5346 2007). Therefore, aligning a trap crop as a boarder will aid in disrupting the pollen beetle's
5347 migration into the crop centre (Mauchline *et al.*, 2017; Juhel *et al.*, 2017). The migration
5348 pattern of *Psylliodes chrysocephala* do not appear to colonise the field edges equally
5349 (chapter 4 and 7). This raises the potential to design an area of migration-based trap crop
5350 area rather than a full field boarder. This could be set as an area along with a flower margin,
5351 shown to benefit pollination services (Haaland, Naisbit and Bersier, 2011). Thereby the area
5352 of flower margin contains brassicas as a dead-end trap crop (Veromann *et al.*, 2014).
5353 Therefore, combining attributes of both a trap crop and pollinator reserve, increasing the
5354 ecological benefit to the crop.

5355 Future survey work into farmers willingness to adopt measures more specifically could
5356 explore whether farmers who are willing to use a flower boarder would add brassicas to this
5357 mix, as if seen in many flower mixes for birds. In addition to the potential of OSR seed to
5358 incorporate a trap crop element for both *P. chrysocephala* and pollen beetle if left to flower
5359 in the margin, a benefit for pollinators as well.

5360

5361 **9.1.5 Nurse cropping:**

5362 The use of brassica nurse crops may be considered for further research combined with the
5363 grazing or topping of the crop prior to stem elongation as shown in Chapter 4 to reduce *P.*
5364 *chrysocephala* numbers. The lack of major frosts and limited action from the herbicide
5365 application led to the nurse cropping being cleared along with the OSR, so no measurement

5366 on yield could be attained. The issues involved in small plot experiment here would put
5367 more doubt into the full field scale use of the mix tested here.

5368

5369 **9.1.6 Defoliation:**

5370 When OSR is defoliated prior to stem elongation it exhibits a remarkable ability to
5371 compensate from the injury (Syrový, Shirtliffe and Zarnstorff, 2016; Ramachandran, Buntin
5372 and All, 2000; ADAS, 2013). The development of dual use canola in Australia (Kirkegaard *et*
5373 *al.*, 2008a) shows that this can be possible without significant yield penalties. The use as a
5374 pest control method for *P. chrysocephala* has been receiving attention more recently in the
5375 UK in response to the neonicotinoid ban. The use of this method a component of field trials
5376 in this thesis (chapter 4). Where the crop was mown to 5cm and shown to significantly
5377 reduce the number of *P. chrysocephala* larvae (Chapter 4), whilst the plant density at
5378 harvest was not affected by defoliation (chapter 4). This has been reported in other similar
5379 studies using different modes of defoliation e.g. Kirkegaard *et al* (2008b) have shown sheep
5380 grazed OSR can compensate from the injury with little to no yield penalty if controlled and
5381 timed correctly and this was reflected here (chapter 4). Due to the delay in flowering,
5382 induced by the defoliation, the effected plants were severely damaged by pollen beetle
5383 (*Brassicogethes aeneus*). Which are known to be more damage to less developed plants
5384 (Dosdall and Mason, 2010). This highlights the importance of timing of defoliations is not
5385 only crucial to reduce direct damage to the plant but to avoid leaving defoliated plants more
5386 open to secondary pest attack. The method of defoliation was a major concern of farmers
5387 when asked their views on alternative pest control methods (chapter 8). The concept of
5388 defoliation or sheep grazing was generally supported (chapter 8). However, concerns were

5389 raised on the use of machinery to accomplish the defoliation at the appropriate time of
5390 season (chapter 8). They did indicate a preference to using sheep to avoid soil bed damage
5391 (chapter 8). Further work into sheep grazing as a means of pest control is currently under
5392 way at AHDB (White *et al.*, 2020). There is a potential to combine the technique with nurse
5393 cropping to combat the issues around removal of non-crop plants (chapter 4). By developing
5394 a nurse crop mix which is a suitable host for *P. chrysocephala* and a forage plant of sheep
5395 this may then be an effective means of removing the cover and gaining the benefits of soil
5396 cover of the nurse over winter which in this study showed reducing *P. chrysocephala* adult
5397 feeding injury on the OSR crop and the reduction in larvae number reported here and in
5398 other studies (White *et al.*, 2020; White, Ellis and Kendall, 2018; White, 2019).

5399

5400 **9.2. Assessment of *Psylliodes chrysocephala* in the field:**

5401

5402 **9.2.1 Feeding location on plant:**

5403 Data shown in Chapter 6 demonstrated a feeding preference for true leaves over cotyledon.
5404 If this is to be confirmed in the field future research surveying *P. chrysocephala* injury would
5405 be wise to also record the leaf location to build a bigger data set to determine if the *P.*
5406 *chrysocephala* does have any preferential feeding location on the plant.

5407

5408

5409

5410 **9.2.2 Nocturnal feeding diurnal sheltering:**

5411 The data presented in chapter 7 suggests that *P. chrysocephala* are primarily active at night
5412 making direct observations during this time critical. Using emergence traps the hypothesis
5413 that *P. chrysocephala* are primarily nocturnal was supported and that during daylight hours
5414 they shelter under the soil surface. This information supports current advice for synthetic
5415 insecticide application being carried out at night. This idea was partly brought forward to
5416 avoid exposure to day-flying bees to pest protection products. Data collect here supports
5417 evidence that this is also a benefit for application in order to maximise likelihood of
5418 contacting the desired pest. It also shows that to accurately measure the abundance and
5419 therefore pest load to crop during night.

5420 However, this suggests that examining the interactions with *P. chrysocephala* and below soil
5421 nematodes could be fruitful. Levels of entomopathogenic nematodes have been shown to
5422 be at low density in OSR fields in Europe but are being tested as a pest control option
5423 against *P. chrysocephala* by inundation/inoculation of fields (Hokkanen *et al.*, 2006) and the
5424 survival of these nematodes can be boosted by providing ground cover and restricting UV
5425 radiation at soil level. Reduction of *P. chrysocephala* of up to 73% was reported (Hokkanen
5426 *et al.*, 2006) although as the authors state the target pest during that trial was pollen beetle.
5427 This especially linked to the diurnal resting period of the *P. chrysocephala* being under the
5428 soil surface and therefore near potential entomopathogenic nematodes (Tangtrakulwanich
5429 *et al.*, 2014). Distributions of *P. chrysocephala* have been shown to be variable across a field
5430 (Thioulouse, Debouzie and Ballanger, 1984; Ferguson *et al.*, 2006). Although it is not known
5431 if this variability is consistent over seasons. With the strong directional bias reported here

5432 coupled with the knowledge that *P. chrysocephala* shelters below soil during the day the
5433 potential exists to target the inundation/inoculation proposed by Hokkanen *et al* (2006).

5434

5435 **9.2.3 Migration into the field:**

5436 Migration into fields from the surrounding landscape of *P. chrysocephala* was measured in
5437 the study with water traps along crop edges in two fields in 2016 and one field in 2018. Each
5438 sowed a distinct bias in catch numbers to certain edges (in 2016 South and South-East and
5439 in 2018 North-East). Further investigation would be needed to determine if this bias is due
5440 to factors such as wind direction, known to influence aphid migration into fields (Mauchline
5441 *et al.*, 2017), or location of aestivation sites for the beetles. The data may already exists in
5442 farmers records for application thresholds.

5443

5444 **9.3. Direct impact of *Psylliodes chrysocephala* on Oilseed rape:**

5445 When examining the direct interactions of pest injury on crop growth OSR was shown to
5446 compensate well to leaf area loss but less well to low larvae introduction (chapter 5). When
5447 larval numbers are higher (25 introduced to plant, 6 estimated taking up residency) levels of
5448 flower production are reduced, and subsequent yield matrix are equally reduced (chapter
5449 5). This expands on work on the physiological response of OSR to flea beetle feeding, with
5450 Nowatzki and Weiss (Nowatzki. T and Weiss. M, 1997) reporting reduction in biomass but
5451 high level of survival when attacked by *Phyllotreta crucigerae*, and increased glucosinolate
5452 production when infected with *P. chrysocephala* larvae (Koritsas, Lewis and Fenwick, 1991;
5453 Koritsas, Lewis and Fenwick, 1989). The higher levels of glucosinolate reported in seeds in

5454 this study when plants were infected with high numbers of *P. chrysocephala* larvae (chapter
5455 5) is evidence that the increase in glucosinolate reported by Döring and Ulber (2020) in early
5456 response to larval infection are still evident when measuring the end seeds (chapter 5). The
5457 effect of which was reductions in floral potential, a primary concern for both the farmer and
5458 pollinators (Westphal, Steffan-Dewenter and Tschardt, 2009; Holzschuh *et al.*, 2012), and
5459 reductions in yield amount and quality (chapter 5). This confirms the concerns raised by
5460 farmers that *P. chrysocephala* is a major and potential increasing problem to OSR
5461 production (UK, 2020). Further work is needed to understand in detail the interactions
5462 between *P. chrysocephala* larvae and OSR and the methods outlined here (chapter 5) allow
5463 simple and quick establishment of experiments without expensive equipment or long-term
5464 husbandry of *P. chrysocephala*. These high numbers of larvae do not occur every season
5465 (personal observation). With the abundance in 2015 being particularly high resulting in
5466 complete crop failure (chapters 2 and 3), even when treated with neonicotinoids, chapter
5467 3). There is some evidence suggesting that *P. chrysocephala* populations abundance peaks
5468 every 7 years in Germany and Sweden (Nilsson, 2002). There is potential to monitor such
5469 trends using the Rothamsted insect survey data (<https://insectsurvey.com/>), although this
5470 has not been explored yet (Shortall, C. personal communication). Further investigation of
5471 long running data sets may be useful to examine the cycles in the UK and predict years of
5472 high abundance. The addition of this data into any farmer support, such as proPlant (Johnen
5473 *et al.*, 2010), tools would be a great benefit.

5474

5475 **9.4. Conclusion:**

5476 This thesis has attempted to quantify the impacts of the neonicotinoid restriction on OSR
5477 pest control, and production through field trials testing the relative effect of neonicotinoid
5478 seed treatments to alternative options available to farmers. The findings demonstrate that
5479 neonicotinoid insecticides are likely to be less effective than perceived (Chapters 4 and 8)
5480 and that alternative measures can be effective at mitigating the risks of common oilseed
5481 rape pests (Chapters 2-4). Key to the effective implementation of these measures will be
5482 deeper understanding of a) the ecology of pests (Chapters 5-7), the economic impacts of the
5483 damage caused (Chapters 5 and 6) and farmer acceptance of these methods in relation to
5484 their perceived drawbacks (Chapter 8). These showing that the effect of neonicotinoid seed
5485 treatment can be achieved with ecological based methods. The crop failure in two of the
5486 field experiments demonstrate the problems facing farmers, who cannot afford a total crop
5487 failure. No method tested was enough to protect OSR and produce a viable crop, including
5488 neonicotinoid treated seed. The data collected here suggests that control of *P.*
5489 *chrysocephala* can be achieved through non-chemical based methods and further research
5490 is needed to develop these.

5491

5492

5493 **9.5 References:**

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