

Screening Brassicaceae plants for cabbage stem flea beetle (*Psylliodes chrysocephala*) larval antibiosis

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Abstract

The cabbage stem flea beetle (*Psylliodes chrysocephala*) is a major economic pest of oilseed rape (*Brassica napus*), with host plant resistance now representing a key breeding target. Here, we present a protocol for screening Brassicaceae plants (*Brassica* and *Sinapis* species) for CSFB larval antibiosis (defined as reduced larval survival and development), allowing large-scale antibiosis phenotyping across genetically diverse Brassicaceae populations. This method has been used to demonstrate CSFB larval development across time within *B. napus*, an absence of antibiosis across a large, genetically diverse *B. napus* population, and consistent antibiosis in white mustard (*Sinapis alba*), a relative of *B. napus*. Access to this protocol will allow other research groups and crop breeders to conduct larval screening experiments, with the goal of better understanding genes and traits that confer resistance or susceptibility to CSFB larvae. This protocol should be cited alongside: Brock RE, Courtney C, Penfield S, & Wells R (2026), Larval antibiosis to cabbage stem flea beetle (*Psylliodes chrysocephala*) is absent within oilseed rape (*Brassica napus*), *Pest Manag. Sci.*, <https://doi.org/10.1002/ps.70808>.

Keywords

antibiosis, Brassicaceae, cabbage stem flea beetle, entomology, host plant resistance, integrated pest management, oilseed rape, plant-insect interactions

1. Purpose and scope of protocol

The cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*) represents a major insect pest of oilseed rape (*Brassica napus*) across Europe.^{1,2} Adult CSFB feeding during autumn reduces crop establishment, while larval feeding throughout winter stunts plant growth and increases mortality, ultimately reducing crop yield.²⁻⁶ Therefore, methods for CSFB control are important

from an economic and food security perspective.² Given reductions in insecticidal control methods, identifying Brassicaceae genotypes that exhibit resistance to larval CSFB development (i.e. larval antibiosis) has become increasingly important.^{7,8} This protocol outlines a method for experimental screening of Brassicaceae plants for CSFB larval antibiosis (defined as reduced larval survival and development).

Screening for larval antibiosis involves: (1) horticulture of Brassicaceae species/genotypes of interest (**Section 3**); (2) infestation of plants with <24 hour-old, first instar CSFB larvae (**Section 4**); and either (3) dissection of infested plants (**Section 5**); (4) evacuation of infested plants (**Section 6**); or (5) adult emergence and collection from infested plants (**Section 7**). A timeline and resource list for larval antibiosis screening are given in **Table 1** and **Table S1**, respectively.

Table 1. Experimental schedule for screening Brassicaceae plants for cabbage stem flea beetle (*Psylliodes chrysocephala*) larval antibiosis.

Week	Day	Task
0	Tuesday	Sow seeds for larval screens.
1	Friday	Repot seedlings.
3	Friday	Setup egg-pots, place half at 22°C and other half in fridge (~4°C).
4	Monday	Clear any hatched larvae and place remaining half of egg-pots at 22°C.
	Tuesday	Phenotype plants. Bag up plants for larval infestation. Carry out first larval infestation.
	Wednesday	Carry out second larval infestation.
	Thursday	Carry out third larval infestation.
	Friday	Carry out fourth larval infestation. Phenotype plants for evidence of larval infestation.
6	Friday	If dissecting plants: Harvest plants, phenotype, dissect, and store recovered larvae in 70% ethanol. If evacuating plants: Harvest plants, phenotype, and set up over evacuation boxes. Collect larvae from evacuation boxes every 2-3 days for up to 17 days and store recovered larvae in 70% ethanol.
10	Friday	If waiting for adult eclosion: Begin checking plants for adult emergence every 2-3 days. Collect emerged adults using an insect aspirator and store in 70% ethanol.
11	Friday	Chop down and dispose of plants. If adults are already emerging, this can be carried out sooner. Continue collecting emerging adults weekly until experiment ends.

2. Experimental design

Experimental design for CSFB larval screening should be informed by the following:

- **Treatment number** – total number of genotypes to be screened.
- **Replicate number** – total number of replicates for each genotype to be screened.
- **Block size** – total number of plants that can be screened in one run. This is dependent on CSFB egg production, growth cabinet/CER space limitations (**Figure 1**), and people-hours (i.e. the more plants per block, the more time-intensive the experiment will become).
- **Control inclusion** – whether positive and negative controls are screened alongside the genotypes of interest. In Brock *et al.*,⁹ we used white mustard (*Sinapis alba*) as a positive control (based on results from Doring & Ulber¹⁰) and *Brassica napus* (cv. KWS Campus) as a negative control.

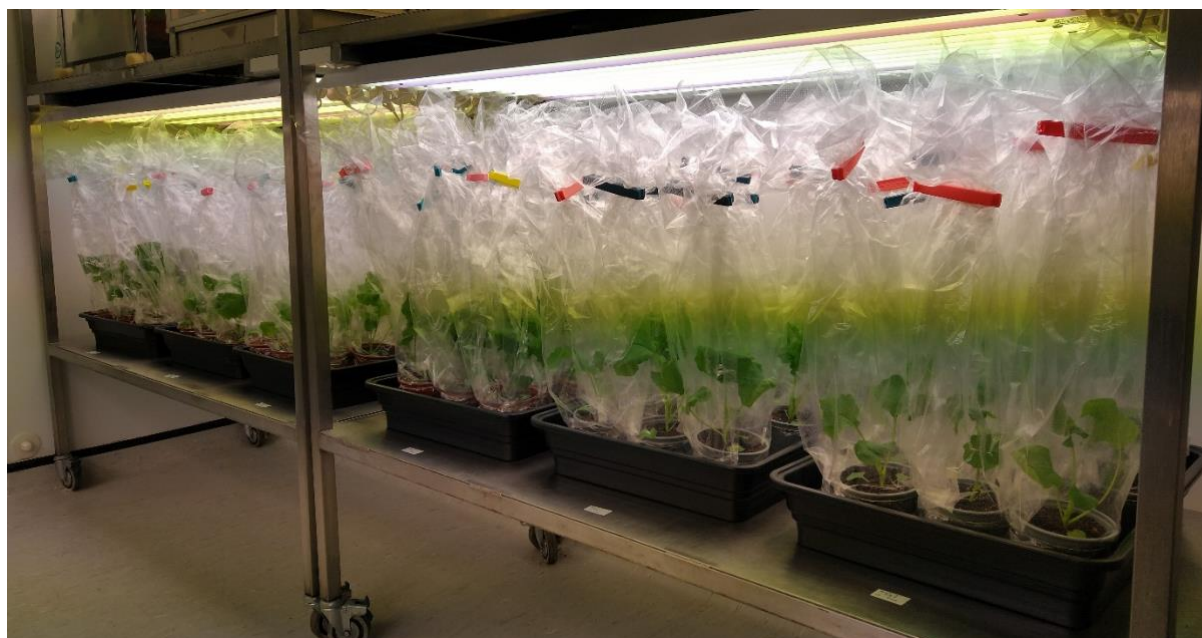


Figure 1. Three experimental blocks of Brassicaceae plants infested with cabbage stem flea beetle (*Psylliodes chrysocephala*; CSFB) larvae setup across a shelving unit in a controlled environment room within the John Innes Centre Entomology facility. Each block contains a total of 26 infested plants, split across two trays each, giving a total of 78 plants being screened. A maximum of 15 plants can fit into a single tray. Hence, in this instance, the maximum block size is 30 plants and the maximum number of plants that can fit onto the shelf is 90. Experimental blocks are staggered, such that block 2 is sown a week after block 1 and block 3 is sown a week after block 2. Due to space constraints, block 4 can only be setup once block 1 has finished. Information on maximum block sizes and space constraints should be used to inform experimental design for CSFB larval screening.

In Brock *et al.*,⁹ we used an alpha block design to screen 96 *B. napus* genotypes (treatments = 96) three times each (replicates = 3) with a block size of 24, yielding 12 experimental blocks in

total. Each block also contained a positive and negative control, taking the final block size to 26 plants per block (24 treatment genotypes + 1 positive control + 1 negative control). Experimental blocks were temporal, with at least a week in between setup of the following block (**Figure 1**). Experimental design software such as the ‘agricolae’ R package¹¹ or CycDesign¹² can be used to generate blocking structures.

3. Horticulture

CSFB larval screening is carried out by infesting 3- or 4-week-old Brassicaceae plants (see **Protocol notes**). This section describes the procedure for growing plants to be infested.

3.1. Sowing seeds

1. To avoid weekend working, seeds should be sown on a Tuesday (**Table 1**).
2. Fill as many 8cm pots as required (one pot per genotype) with Levington Advance F2 compost and place into a gravel tray (**Figure 2A**). Thirty pots can fit inside a single gravel tray.
3. Write genotype names/codes on plant labels and place each label into each pot.
4. Use the forceps to sow 6-12 seeds of each genotype into the corresponding pot. Seeds should be sown roughly 3-4cm deep and evenly spaced. Gently compress the compost to cover the holes left from sowing the seeds.
5. Place the sown seeds in a growth cabinet/CER (10:14 L:D, 18°C, ~60% RH) and use a 2L measuring cylinder to provide a specific amount of water. Record the amount of water added so that each new block of sown seeds can be watered with an equal measure (ensuring experimental consistency across blocks). Plants should be regularly watered throughout the entire experiment. Again, record when watering takes place and how much is provided, such that each new block receives the same watering schedule and amount.

3.2. Repotting

6. Repotting should be carried out on the Friday ten days after sowing (**Table 1**).
7. Fill as many 9 cm pots as required with cereal mix compost and place the pots into new gravel tray(s). Up to 15 pots can fit inside a single gravel tray.
8. Take the first 8 cm pot with germinated seedlings (**Figure 2B**) and gently ease a seedling from the soil, retaining as much of the root system as possible. Create a hole in the soil of a 9 cm pot, place the seedling in the hole, aiming to get as much of the hypocotyl into the soil as possible, and gently compress the soil around the hypocotyl. Transfer the plant label from the 8 cm pot into the 9 cm pot containing the repotted seedling (**Figure 2C**).

9. If screening more than one replicate per genotype within a block, write the genotype name/code and replicate number on extra plant labels and place the labels into the 9 cm pots. Repot as many seedlings from the genotype as required.
10. Dispose the remaining seedlings and soil from the 8 cm pot.
11. Repeat steps 8-10 for all remaining genotypes sown in the 8 cm pots.
12. Once all genotypes have been repotted (**Figure 2C**), transfer the gravel tray(s) into the 18°C growth cabinet/CER, and continue watering regularly.

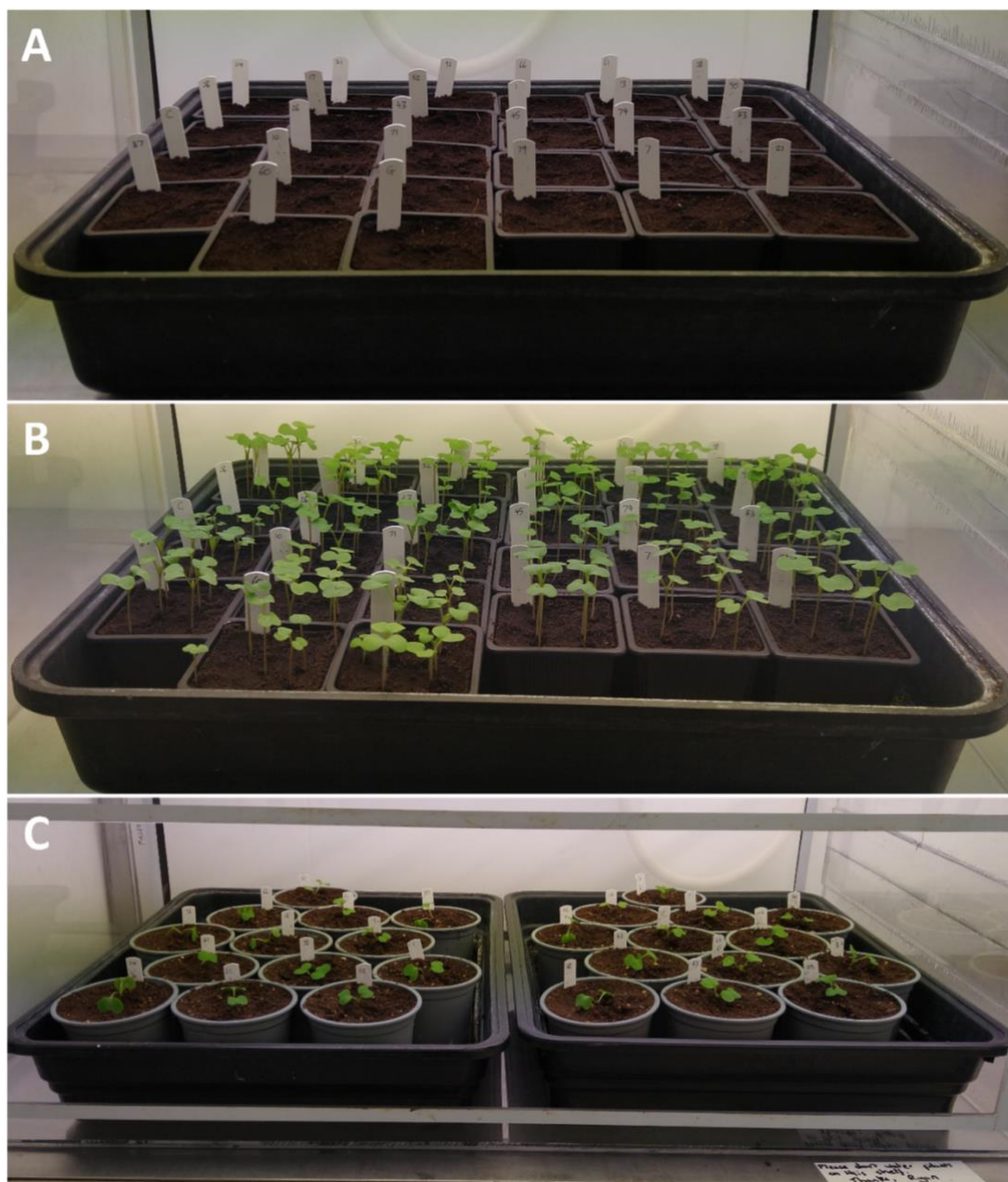


Figure 2. Horticulture of Brassicaceae plants for use in cabbage stem flea beetle (*Psylliodes chrysocephala*) larval screening. **(A)** Gravel tray holding 26 labelled pots, each containing 6 seeds of a different genotype. **(B)** Germinated seedlings, 10 days post-sowing. **(C)** Repotted 10-day-old seedlings (1 replicate per genotype).

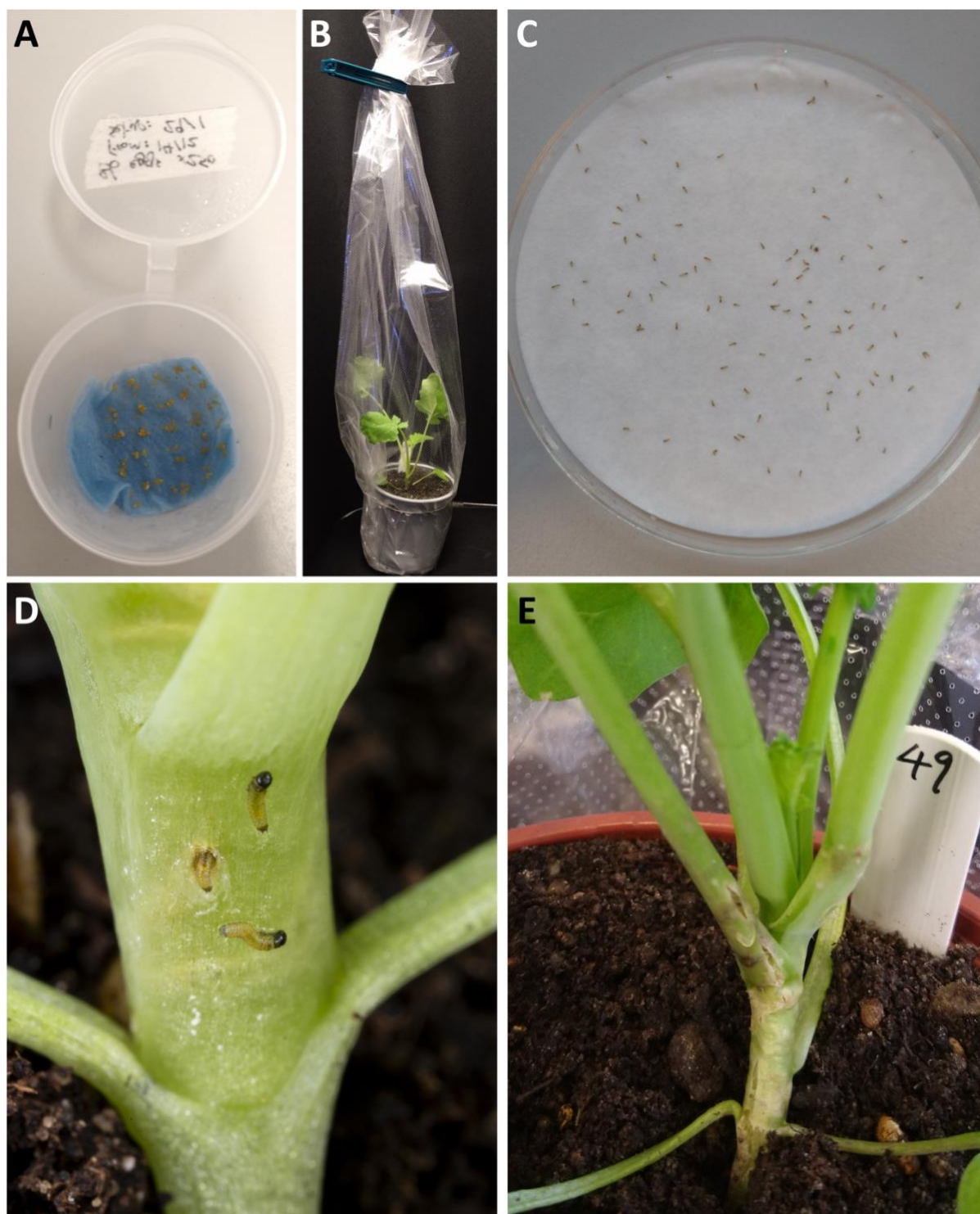


Figure 3. Infestation of *Brassica napus* with <24 hour-old, first instar cabbage stem flea beetle (*Psylliodes chrysocephala*; CSFB) larvae. **(A)** Egg-pot containing approximately 250 CSFB eggs. **(B)** Four-week-old *B. napus* plant placed in a micro-perforated pollination bag ready for larval infestation. **(C)** Petri dish containing moist filter paper and <24 hour-old CSFB larvae that will be transferred to plants. **(D)** *B. napus* plant experimentally infested with three CSFB larvae on the main stem. The plant also shows signs of larval infestation, with an entry hole and mines on the stem caused by larval herbivory. **(E)** *B. napus* plant two-weeks post-larval infestation, demonstrating CSFB larval damage on the stem and petiole bases.

4. Larval infestation

Larval infestation is carried out by adding a total of 12, <24 hour-old, first instar CSFB larvae to each plant across a four-day period (i.e. three larvae per day). Twelve larvae per plant represents a field-realistic infestation level that is likely to have adverse effects on yield.^{5,13,14} Adding larvae across four days rather than all at once ensures that: (1) plants aren't killed by the sudden stress of 12 larvae infesting them at once; (2) the experiment is more field-realistic (whereby infestation occurs over time)⁴; and (3) variability in egg hatching rates is accounted for, making it easier to obtain enough larvae each day. This section describes the procedure for egg setup, plant phenotyping and preparation, and larval infestation.

4.1. Egg-pot setup

1. Egg-pot setup should be carried out on the Friday prior to infestation (**Table 1**). The number of eggs that need to be setup will depend on how many plants are within the experimental block. For instance, infesting 20 plants would require a total of 60 (20×3) larvae each day, and 240 larvae (20×12) overall. Assuming approximately 5-10% of the eggs will hatch each day, this would require setting up 600-1200 eggs in preparation for the larval screen. The number of eggs you can setup is limited by how many CSFB eggs are available. However, it's best to aim towards the upper estimate of eggs required, since this reduces chances of having too few larvae to infest all the experimental plants on a given day.
2. Fold a piece of blue roll into an approximate 3 × 3 cm square and spray with distilled water until moist.
3. Use a moistened paintbrush to carefully transfer CSFB eggs to the square of blue roll. Up to approximately 250 eggs can be placed on a single square of blue roll.
4. Once enough eggs have been placed on the blue roll, place the blue roll at the bottom of a 25ml sauce pot, cap the pot, and label the pot with the number of eggs and the date of egg-pot setup (**Figure 3A**).
5. Repeat steps 2-4 until you have enough eggs setup for larval infestation.
6. Once setup, place half of the egg-pots in a 22°C growth cabinet/CER (to stimulate hatching) and the other half in a refrigerator (~4°C) over the weekend (**Table 1**).
7. On Monday morning (**Table 1**), remove the remaining egg-pots from the refrigerator and 22°C growth cabinet/CER. Uncap all egg-pots and use a moistened paintbrush to remove any hatched larvae. Once all hatched larvae have been cleared from the egg-pots, place all the pots back at 22°C.

8. **Optional:** At the end of the day, remove the egg-pots from the 22°C CER/growth cabinet and use a moistened paintbrush to transfer all hatched larvae from the egg-pots to a spare pot containing moist blue roll. Place the larvae pot into a refrigerator at 4°C overnight. These collected larvae will be less than 24 hours old the following day and can therefore be used for plant infestation. Placing these larvae in the refrigerator rather than leaving them in the CER increases their survival for use the following day.

4.2. Larval infestation

9. The following day (Tuesday; **Table 1**), count and record the number of true leaves for each plant.
10. Once all plants have been phenotyped, they will need to be bagged (**Figure 3B**), ensuring that larvae cannot move from one plant to another.
11. Take the first plant out of the gravel tray and place it at the bottom of a micro-perforated pollination bag, taking care not to damage any of the leaves or petioles. Secure an elastic band around the pot and roll it up to the pot rim (**Figure 3B**).
12. Seal the top opening of the bag with a bag clip (**Figure 3B**) and place the bagged plant back in the gravel tray.
13. Repeat steps 11-12 for all remaining plants.
14. Remove the egg-pots from the 22°C CER/growth cabinet and, if you carried out step 8, the spare larvae pot from the refrigerator. Uncap each pot and use a moistened paintbrush to transfer all hatched larvae from the egg-pots to a Petri dish containing a moistened piece of filter paper (**Figure 3C**).
15. Take the first bagged plant out of the gravel tray, unclip the top of the bag, and roll the bag down, taking care not to damage any of the leaves or petioles, so that the base of the plant is accessible.
16. Using a moistened paintbrush, carefully transfer three larvae from the Petri dish (**Figure 3C**) to the main stem at the base of the plant in between the cotyledons and the petiole of the first true leaf (**Figure 3D**). Plant defence compounds (e.g. glucosinolates) are differentially distributed across plant tissues^{15,16} and larval penetration success may therefore be influenced by where each larva is introduced. Hence, it is important that all larvae are introduced to the same area of each plant.
17. Once transferred to the plant, observe the larvae for up to 10 seconds for signs of movement. If any larvae do not move during the 10 second observation, remove them from

the plant and replace them with a different larva. Replacement larvae should also be observed for signs of movement.

18. Once infested, roll the bag back up over the plant, taking care not to damage any of the leaves or petioles, reseal the top opening with the bag clip, and place the bagged plant back in the gravel tray.
19. Repeat steps 15-18 for all remaining plants, taking care to **record which plants have been infested as you go**. Failure to record which plants have been infested as you go may lead to you losing track of infestation progress and infesting plants with too many/few larvae.
20. Plant defence compounds are also differentially produced throughout the day, often following circadian rhythms based on light cycling conditions.¹⁷ Larval penetration success may therefore be influenced by the time of day larvae are introduced onto plants. Accordingly, it is important that larval infestations take place at approximately the same time of day within and across experimental blocks.
21. Once all plants have been infested, place the plants back in the 18°C CER/growth cabinet and the egg-pots back in the 22° CER/growth cabinet.
22. **Optional:** As before (step 8), collect any extra hatched larvae at the end of the day and place them in the refrigerator for use the following day.
23. Repeat steps 14-22 for the following three days (Wednesday, Thursday, Friday; **Table 1**), **randomising the order of plant infestation each day** (to limit any potential time of day effects), until all plants have been infested with a total of 12 larvae.
24. On the fourth and final day of infestation, check and record (Y/N) each plant for signs of successful larval infestation. Successfully infested plants should present at least one of the following phenotypes: (1) entry holes on the petioles and main stem caused by larval penetration; (2) mines on the petioles and main stem caused by larval feeding; or (3) the presence of larvae underneath the epidermis (**Figure 3D-E**).
25. Larvae can be left to develop within plants until larval dissection (**Section 5**), larval evacuation (**Section 6**), or adult emergence (**Section 7**).

5. Larval dissection

Larval dissection involves phenotyping each infested plant before dissecting the stem and petioles to recover all surviving larvae. Dead larvae can also be recorded. Larval dissection is carried out two-weeks post-infestation, giving sufficient time for development and mortality to take place,^{9,10} and provides an accurate snapshot of larval development. However, dissection is

labour-intensive and larvae may be damaged during dissection. Hence, larval dissection may be used when sample sizes are small and accurate data on larval size is required.⁹

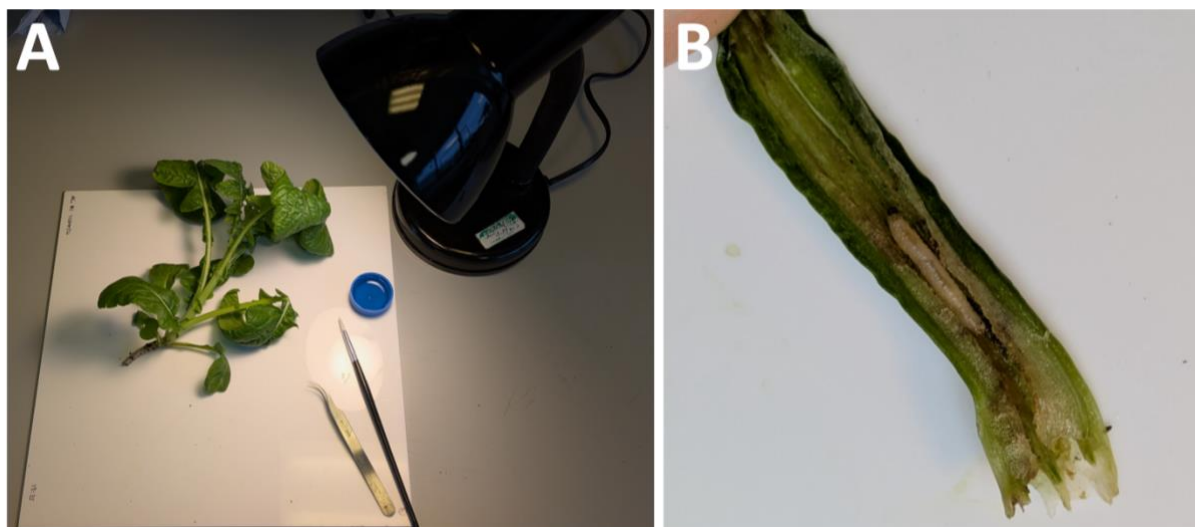


Figure 4. Recovery of cabbage stem flea beetle (*Psylliodes chrysocephala*; CSFB) larvae from experimentally infested *Brassica rapa* via plant dissection. **(A)** Uprooted *B. rapa* ready for dissection. **(B)** Dissected *B. rapa* petiole containing third instar CSFB larva.

5.1. Plant dissection and larval recovery

1. Plant dissection should be carried out on Friday, two weeks after the fourth larval infestation day (**Table 1**).
2. Place 2ml microcentrifuge tubes (one tube per plant to be dissected) into a tube rack, fill with 70% ethanol, and use a permanent marker to write the block, genotype name/code, and replicate number (if screening multiple replicates of a genotype within a single block) on the lid of each tube.
3. Carefully remove the first infested plant from its microperforated bag. Place the microperforated bag to one side (these can be re-used) and dispose of the elastic band (since these become stretched and brittle after exposure to growth lighting).
4. Count and record the number of true leaves on the plant. Uproot the plant from the soil and use secateurs to cut off the main tap root, leaving approximately 4-5cm of the hypocotyl attached to the plant (**Figure 4A**). Dispose of the soil and place the pot and plant label to the side (these can be re-used).
5. Place the plant on weighing scales and record its wet weight in grams.
6. Place the plant in a dissection tray/board (**Figure 4A**). Remove the cotyledons (if remaining) from the plant and gently run dissection forceps up the middle of each one, allowing you to split the cotyledon in half and check for larvae inside. Record the location of any larvae

found and, using a fine paintbrush, transfer the larvae to the microcentrifuge tube corresponding to the plant being dissected.

7. Remove the first true leaf from the plant and gently run the forceps along the petiole midrib and leaf veins. Split the veins and petiole open and check for any larvae inside (**Figure 4B**). Record the location of any larvae found and, using a fine paintbrush, transfer the larvae to the microcentrifuge tube. Continue working your way up to the plant apex, dissecting a single leaf at a time, until you have dissected all true leaves and only the stem remains.
8. Finally, split the stem into quarters and check for any remaining larvae, recording their location before using the paintbrush to transfer them to the Microcentrifuge tube.
9. Dispose all plant tissue once dissection is complete.
10. Repeat steps 3-9 for all remaining plants to be dissected.

6. Larval evacuation

Larval evacuation represents an alternative to dissection for larval recovery and involves individually drying infested plants over a pool of water. As the plant material dries, larvae will leave the plant and fall into the water, providing a highly accurate measure of larval load within the plant.¹³ Larval evacuation is carried out two-weeks post-infestation, giving sufficient time for development and mortality to take place.^{9,10} Evacuation gives a reliable and less labour-intensive estimate of larval survival when compared to plant dissection.¹³ However, it does not necessarily give the best indication of larval development within a plant, since larvae have up to two weeks to evacuate from the plants during drying.^{9,13} Hence, collected larvae will contain a mix of individuals that immediately left the plants alongside other individuals that may have spent an extra week feeding inside the plant before evacuating.

6.1. Evacuation setup

1. Evacuation setup should be carried out on Friday, two weeks after the fourth larval infestation day (**Table 1**).
2. Place a small strip of electrical tape on the front of a 4L ice cream container (one container per plant to be evacuated) and use a permanent marker to label each container with the block, genotype name/code, and replicate number (if screening multiple replicates of a genotype within a single block).
3. Fill each labelled ice cream container with a single pump of washing up liquid and approximately 500 ml of water. The soap breaks the surface tension of the water, ensuring that larvae sink when they fall into the water and cannot escape from the container.



Figure 5. Recovery of cabbage stem flea beetle (*Psylliodes chrysocephala*; CSFB) larvae from experimentally infested *Brassica napus* plants via larval evacuation. **(A)** Uprooted six-week-old *B. napus* plant infested with CSFB larvae (as evidenced by the larval scarring on the petioles and main stem). **(B)** Fully set up larval evacuation container with plant material laid across the wire mesh. As the plant dries out, CSFB larvae will evacuate the plant and fall into the soapy water below. **(C)** An experimental block (containing 26 infested plants) set up for larval evacuation inside a growth cabinet. **(D)** CSFB larvae obtained by filtering the water at the bottom of a larval evacuation container through Miracloth. **(E)** Labelled 2 ml microcentrifuge tubes containing 70% ethanol and collected CSFB larvae.

4. Push a wire mesh panel into each container so that the edges of the mesh catch on the sides of the box, forming a concave basket for the infested plant to sit in (**Figure 5B**).
5. Carefully remove the first infested plant from its microperforated bag. Place the microperforated bag to one side (these can be re-used for future blocks) and dispose of the elastic band (since these become stretched and brittle after exposure to growth lighting).
6. Count and record the number of true leaves on the plant. Uproot the plant from the soil and use secateurs to cut off the main tap root, leaving approximately 4-5cm of the hypocotyl attached to the plant (**Figure 5A**). Dispose of the soil and place the pot and plant label to the side (these can be re-used).
7. Place the plant on weighing scales and record its wet weight in grams.
8. Remove the leaf and petiole of each true leaf from the main stem of the plant and place them on top of the wire mesh within the corresponding labelled container (**Figure 5B**).
9. Repeat steps 5-8 for each remaining infested plant.
10. Once all infested plants have been set up over their containers, place the containers in a 22°C CER/growth cabinet (**Figure 5C**) and leave the plants to dry out.

6.2. Larval collection

11. The first larval collection should be carried out on the Monday following evacuation setup (**Table 1**).
12. Place 2ml microcentrifuge tubes (one per plant) in a tube rack, fill with 70% ethanol, and use a permanent marker to write the block, genotype name/code, and replicate number (if screening multiple replicates of a genotype within a single block) on the lid of each tube (**Figure 5E**).
13. Remove the evacuation containers from the growth cabinet.
14. Cut a piece of Miracloth to be larger than the rim of an ice cream container and place the cut Miracloth over a spare, empty container.
15. Take the first evacuation container. Remove the mesh basket containing the drying plant material and place it to one side.
16. Filter the evacuation container by emptying the water into the spare container through the Miracloth.
17. Inspect the Miracloth for larvae (**Figure 5D**) and use a paintbrush to transfer any larvae from the Miracloth to the corresponding labelled microcentrifuge tube (**Figure 5E**).
18. Once all larvae have been collected, tip the water back into the evacuation box and place the wire mesh and plant material above the water (**Figure 5B**).

19. Repeat steps 15-18 for all remaining evacuation containers.
20. Once all containers have been filtered, place them back in the CER/growth cabinet.
21. Repeat larval collection every 2-3 days for a period of two weeks (Monday, Wednesday, Friday; **Table 1**). Longer time periods in between larval collections may lead to the larvae rotting in the water.
22. **Optional:** On the Friday one week after evacuation setup, plant petioles can be split in half, helping to speed up the process of plant drying.
23. Water may evaporate rapidly from the evacuation containers so water levels at the bottom of the containers should be checked each collection day and refilled when necessary.
24. If no further larvae are found on the Friday two weeks after evacuation setup (**Table 1**), then dried plant material can be disposed. If larvae are still found on the Friday two weeks after evacuation setup, then retain plants over the weekend and check on Monday for any additional larvae. If no further larvae are found, then plants can be disposed. Wire mesh and ice cream containers can be cleaned and re-used.

7. Adult emergence

Another method for measuring larval antibiosis is by collecting emerging adults from Brassicaceae plants, with the assumption that more resistant genotypes will yield fewer adults and these adults will take longer to develop. Adult collection therefore involves leaving plants alone after infestation and waiting until adults emerge. Emerging adults can then be collected at regular intervals.

7.1. Prior to adult emergence

1. At 18°C, larvae begin to complete development at four-weeks post-infestation and first adult emergence is observed at seven-weeks post-infestation.⁹ Hence, checks for adult emergence should begin on the Friday six-weeks after the last infestation day (**Table 1**). Checks for adult emergence should be carried out at least once a week. If you need finer scale data on adult emergence timing, then plants can be checked for adult emergence every 2-3 days (Monday, Wednesday, Friday). Signs that adults have begun emerging are the presence of beetles towards the top of the microperforated bag or the presence of adult herbivory on leaf tissue.
2. At seven-weeks post-infestation, unclip the bag for each infested plant and chop down and dispose of the plant, leaving some of the stem above-ground. Chopping down the plant

makes adult collection far easier and stimulates leafy growth from the axillary meristems so that emerging adults have leaf tissue to feed on.

3. **Optional:** If you are concerned that larvae may still be inside plants at seven-weeks post-infestation, you can check for surviving larvae in petioles towards the base of the plant by splitting open the petioles with your thumbs. Given our results,⁹ it is unlikely that any larvae will be found. If larvae are found, plants should be given an extra week before being chopped down.

7.2. Adult collection

4. Place 2ml microcentrifuge tubes (one per plant) in the tube rack, fill with 70% ethanol, and use a permanent marker to write the block, genotype name/code, and replicate number (if screening multiple replicates of a genotype within a single block) on the lid of each tube.
5. Take the first plant and remove and dispose of the elastic band from around the pot rim. Unclip the bag and use the aspirator to suck up any beetles before transferring them to the corresponding labelled microcentrifuge tube. Firmly tap the sides of the pot to disturb any beetles in the soil, allowing their collection. Record the date and how many beetles were recovered from the plant, reclip the top of the bag, and place back in the gravel tray.
6. Emerging adults should be collected at least once a week, and the experiment can be concluded once beetles have not emerged from plants for two consecutive weeks. Microperforated bags, pots, and plant labels can be re-used, while soil can be disposed.

8. Protocol notes

Having to split large screening experiments into separate blocks means they end up taking a long time. Running a 96 genotype, three replicate larval antibiosis screen (a total of 12 blocks, with 26 plants per block) took 9 months using this protocol.⁹ Some possible ways to speed the experiment up include:

- Using larger gravel trays to increase the number of plants that can fit into a single block.
- Repotting seedlings into smaller pots to increase the number of plants that can fit into a gravel tray, and hence the number of plants in a block. However, smaller pots will lead to smaller plants, which in turn may impact larval screening success (i.e. smaller plants may be unable to deal with 12 larvae).
- Introducing larvae at three-weeks rather than four-weeks post-sowing, speeding up the time it takes to run a single block. The fitting of new lights within our CERs during Brock *et al.*⁹

meant that plants grew faster and were of adequate size to support 12 larvae at three-weeks old during the phenotype validation experiment.

- Booking additional CER/growth cabinet space, allowing more blocks to be run before capacity is full.

Of course, it's also worth considering that block size is limited by your access to CSFB eggs and the people-hours available, since larger blocks will require more eggs for infestations and take extra time.

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Table S1. Equipment and resources required to carry out Brassicaceae screening for cabbage stem flea beetle (*Psylliodes chrysocephala*) larval antibiosis.

Section	Item	Purchasing link
3. Horticulture	Growth cabinet/CER at 10:14 L:D (light intensity ~200 $\mu\text{mol}/\text{m}^2/\text{s}$), 18°C, ~60% RH	
	Rectangular gravel trays (L50 × W40 × H10 cm)	Link
	Seeds of Brassicaceae genotypes to be screened	
	Square 8 cm plastic plant pots	Link
	Levington Advance F2 compost	Link
	Round 9 cm (500 ml) plastic plant pots	Link
	Cereal compost mix plus slow-release fertiliser (65% peat, 25% loam, 10% grit, 3 kg/m ³ dolomitic limestone, 1.3 kg/m ³ PG Mix, 3 kg/m ³ Osmocote)	
	Round-ended steel forceps	Link
	Plastic plant labels	Link
	Fine tip permanent marker	Link
	Water tap and hose	
	2 L measuring cylinder	Link
4. Larval infestation	Growth cabinet/CER at 10:14 L:D (light intensity ~200 $\mu\text{mol}/\text{m}^2/\text{s}$), 18°C, ~60% RH	
	Growth cabinet/CER at 16:8 L:D (light intensity ~200 $\mu\text{mol}/\text{m}^2/\text{s}$), 22°C, ~60% RH	
	Cabbage stem flea beetle eggs	
	Blue roll	Link
	Squeeze bottle with distilled water	Link
	Fine tip permanent marker	Link
	Fine paintbrush	Link
	25 ml sauce pots	Link
	Refrigerator (~4 °C)	
	Brassicaceae plants to be infested (Section 3)	
	Micro-perforated pollination bags (L90 × H38 cm)	Link
	Elastic bands	Link
	Food bag clips	Link
	9 cm Petri dish	Link
	9 cm filter paper	Link
5. Larval dissection	Infested Brassicaceae plants (Section 4)	
	Secateurs	Link
	Electronic weighing scales	Link

	White plastic tray or board	
	Adjustable desk lamp	Link
	Sharp dissection forceps	Link
	Fine paintbrush	Link
	Fine tip permanent marker	Link
	2 ml microcentrifuge tubes	Link
	(one per plant to be dissected)	
	70% ethanol	Link
	Microcentrifuge tube rack	Link
6. Larval evacuation	13 mm wire mesh cut to panels of L17 × H17 cm	Link
	(one per plant to be evacuated)	
	4 L ice cream containers	Link
	(one per plant to be evacuated)	
	White electrical tape	Link
	Fine tip permanent marker	Link
	Washing up liquid	Link
	Water tap and hose	
	Growth cabinet/CER at 22 °C, ~60% RH	
	(photoperiod is not important)	
	Infested Brassicaceae plants (Section 4)	
	Secateurs	Link
	Electronic weighing scales	Link
	Fine paintbrush	Link
	2 ml microcentrifuge tubes	Link
	(one per plant to be dissected)	
	70% ethanol	Link
	Microcentrifuge tube rack	Link
	Miracloth	Link
7. Adult emergence	Infested Brassicaceae plants (Section 4)	
	Secateurs	Link
	Insect aspirator ('pooter')	Link
	Fine tip permanent marker	Link
	2 ml microcentrifuge tubes	Link
	(one per plant to be dissected)	
	70% ethanol	Link
	Microcentrifuge tube rack	Link