Chapter 7

Bioassays to Evaluate the Resistance of Whole Plants to the Herbivorous Insect Thrips

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Abstract

Thrips are tiny, cell-content–feeding insects that are a major pest on crops and ornamentals. Besides causing direct feeding damage, thrips may also cause indirect damage by vectoring tospoviruses. Novel resistance mechanisms to thrips need to be discovered and validated. Induction of jasmonic acid–dependent defenses has been demonstrated to be essential for resistance to thrips, but underlying mechanisms still need to be discovered. For this, it is vital to use robust plant-thrips assays to analyze plant defense responses and thrips performance. In recently developed high-throughput phenotyping platforms, the feeding damage that is visible as silver spots, and the preference of thrips in a two-choice setup is assessed, using leaf discs. Here, we describe whole-plant thrips assays that are essential for (1) validation of findings obtained by the leaf disc assays, (2) assessment of longer-term effects on thrips feeding success and fecundity, (3) determination of spatial-temporal effects induced by primary thrips infestation on a secondary attack by thrips or other insects or pathogens, and (4) assessment of gene expression and metabolite changes. We present detailed methods and tips and tricks for (a) rearing and selection of thrips at different developmental stages, (b) treatment of the whole plant or an individual leaf with thrips, and (c) determination of feeding damage and visualization of thrips oviposition success in leaves.

Key words Cell-content–feeding insect, Thrips, *Arabidopsis thaliana*, Plant immunity, Jasmonate, Clip cage, Aspirator, Oviposition, Rearing

1 Introduction

Thrips are tiny insects (1.5 mm or less) that are cell-content feeders, greatly affecting commercial food crops and ornamentals worldwide. Besides the direct feeding damage that thrips cause, indirect damage can be inflicted by the *tospoviruses* that can be transmitted in a non-ingesting probe. The life cycle of thrips comprises six stages: the egg stage, two larvae stages (first instar L1 and second instar L2), two pupae stages (prepupa and pupa), and the adult stage (Fig. 1; reviewed by Ref. 1). Female thrips lay their bean-shaped eggs in the epidermal layer of various above-ground plant

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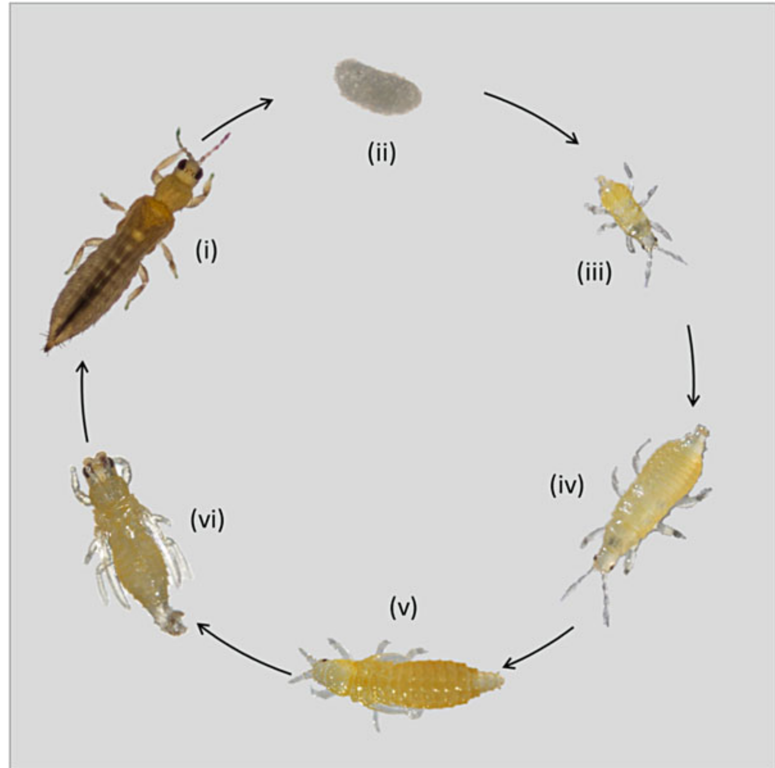

Fig. 1 Thrips life cycle. Adult female thrips (i) can lay fertilized or non-fertilized eggs (ii) from which a female or a male thrips larva will emerge, respectively, after 3–4 days (depending on the temperature, which is optimal at 27 °C). The first instar larva (L1; iii) will develop into a second instar larva (L2; iv) within 2 days. The L2 larva will go through a pre-pupa (v) and pupa (vi) phase after 2–3 days, from which an adult thrips emerges after 4–5 days

parts, like leaves, stems, and flowers, using a saw-like ovipositor [2, 3]. Fertilized (diploid) eggs will develop into female thrips, and unfertilized (haploid) eggs will give rise to male thrips. The larvae that hatch from the eggs will go through two larval stages that actively feed from the plant. During the two pupae stages, thrips are nonfeeding and move to the soil to prepare for the adult stage. Compared to the larvae, the adults are more mobile because of their fringed wings, allowing them to fly off and look for new suitable host plants (reviewed by Ref. 1). Both larval and adult thrips feed from the plant by piercing with their stylet in epidermal, mesophyll, and parenchymal cells and ingesting the contents [4, 5]. Emptied cells collapse or fill with air which, after intensive feeding, results in a localized silvery appearance (reviewed by Ref. 1).

Upon probing and ingestion of the plant cells, different defense responses are initiated in the plant. This activation of defenses requires the perception and recognition of specific and general herbivore- and damage-associated molecular patterns (HAMPs

and DAMPs). Direct and/or indirect defenses against thrips are coordinated by phytohormones such as jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and ethylene (ET). JA has been demonstrated to be essential in the defense against many herbivore species, including thrips (reviewed by Ref. 1), for example, plants insensitive to JA or deficient in JA accumulation are more susceptible to thrips [6]. Subsequent to triggering of the JA pathway by thrips, different direct and indirect defense mechanisms are activated, like formation of leaf trichomes and production of phenolic compounds with toxic characteristics and volatile organic compounds that can attract natural predators of thrips. There is a growing body of evidence for the ability of herbivores to manipulate JA-associated defenses through the action of effector proteins in the arthropod saliva that are secreted into the plant cells, but whether thrips harbor these saliva-borne effectors is still unknown (reviewed by Ref. 1). Another form of defense manipulation that has not been described yet for thrips, but that has been demonstrated for chewing herbivores, is that via egg-associated cues that trigger SA signaling, which suppresses anti-herbivore defenses, thereby benefitting the future progeny of thrips [7].

Thrips are too tiny to measure their weight or length. Therefore, to determine the performance of thrips larvae and adults on different plants, the amount of silver damage inflicted by their feeding is often monitored. Another measure to determine the performance is the reproductive success of thrips on the plants. Moreover, if given a choice between different genotypes or treatments, the preference of thrips for certain plant material, if given a choice, can be assessed. Recently, two high-throughput phenotyping platforms have been developed to quantify host-plant resistance/thrips preference. The first one uses a two-choice setup of half-leaf discs in a 96-well format in which the behavior of the thrips is tracked continuously by a video camera throughout a period of several hours and analyzed with commercial software [8]. The second one is based on a no-choice setup of leaf discs in a petri dish in which the amount of silver damage inflicted by thrips at one endpoint is captured by a photo camera and quantified by freeware [9]. These two screening methods will aid to reduce the quantity of material, and the space and time needed to screen for thrips resistance compared to screening of whole plants. Comparisons between leaf disc and/or detached leaf tests with whole-plant tests revealed that the relatively high amount of damage inflicted to the leaf material prior to the start of a leaf disc assay did not influence the thrips resistance scores of a few selected pepper and *Arabidopsis thaliana* accessions [8–10].

Nonetheless, good setups for whole-plant performance assays are still essential. For example, to study spatial-temporal effects induced by primary thrips infestation on resistance to a secondary attack by thrips or other insects or by pathogens, whole-plant assays

are required. Also, it is difficult to maintain leaf discs in good shape, and therefore, in order to study longer-term effects on the thrips performance in terms of feeding and oviposition success, the use of intact plants is essential. Moreover, findings based on leaf disc assays should be validated under greenhouse or field conditions with whole plants. And lastly, for sensitive gene expression or metabolite accumulation assays, it is preferred to introduce as little variation as possible, which demands for less handling and surely not damaging the tissue, before thrips are introduced. Here, we describe several methods for thrips assays on whole plants. We explain how to rear and select different developmental stages of thrips, for which different tools can be handmade. We present how to treat the whole plant or individual leaves with thrips, for bioassays in which feeding damage or oviposition success can be assessed, or for the study of induced defense responses, locally and systemically. The use of proper thrips–plant assays, as described here, will help to increase our understanding of the defense mechanisms underlying plant resistance to thrips.

2 Materials

2.1 Equipment Used for Multiple Purposes

1. 1.5 mL Eppendorf tubes.
2. Pipet and tips (200 μL, 1000 μL).
3. Fridge.
4. Vacuum autoclave (344 L) and autoclavable plastic bags (40 × 60 cm).
5. River sand.
6. Potting soil.
7. Modified half-strength Hoagland nutrient solution [11]: 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM KH_2PO_4, 2 mM $MgSO_4$, trace elements, pH 7, and 10 mM Fe-ethylenediamine-di[o-hydroxyphenylacetic acid] (Sequestreen).
8. Potting bench.
9. Plant labels.
10. Small trays (150–300 mL; 4 cm high).
11. Big trays (L45 × W30 × H8 cm) with transparent lids.
12. Garden felt.
13. Forceps (curved and straight tip).
14. 15-mL tubes.
15. 1.5-mL safe-lock tubes.
16. Small petri dishes (Ø 6 cm).
17. Scissors.

18. Cork-borer (Ø 4 mm, Ø 15 mm, Ø 20 mm).
19. Bunsen burner.
20. Matches.
21. Heat-resistant gloves.
22. Nylon mesh (80-micron).
23. Hot glue gun.
24. Liquid nitrogen.

2.2 Plant Cultivation

2.2.1 *A. thaliana*

1. Climate-controlled growth chambers with a 10-h day and 14-h night cycle set at 21 °C and 70% relative humidity. Light provided by HPI-T Plus lamps (400W/645 E40) (*see* **Note 1**).
2. Seeds of *A. thaliana*.
3. 0.1% (w/v) agar.
4. Pots (60 mL, Ø 5.5 cm, height 5 cm) with holes in the bottom for plant cultivation after the seedling stage.

2.2.2 Lettuce

1. A controlled greenhouse compartment with natural light conditions at 18 °C and 70% relative humidity.
2. Seeds of *Lactuca sativa var. capitate*.
3. Pots (800 mL, L9 × W9 × H10 cm) with holes in the bottom for plant cultivation after the seedling stage.
4. Plastic plant pot saucers (Ø 18 cm).

2.3 Instruments for Thrips Rearing and Assays

2.3.1 Containers for Synchronized Larval Rearing and Whole-Plant Bioassays

1. 1-L transparent polyethylene terephthalate (PET) jars with screw cap (Ø 10.4 cm).
2. Butyl septa (Ø 20 mm).
3. Hole saw (Ø 6 cm).

2.3.2 Clip Cages for Single Leaf Treatment with Thrips

1. Acrylate rings (Ø inside, 21 mm; thickness, 4 mm; height, 5 mm and 8 mm).
2. Metal hairclips.
3. 4-cm squares of polyether foam (thickness of 3 mm).
4. Two-component glue plastic.

2.3.3 Aspirator for Collecting Thrips of a Specific Life Stage

1. A 15-mL plastic, transparent pot with screw cap (Ø of 25 mm).
2. Polytetrafluorethylene tubes (Ø 4 mm, length 30 cm).

2.4 Thrips Rearing

1. A controlled growth cabinet with a 10-h day and 14-h night cycle at 26 °C, 70% relative humidity, and a light intensity of 100 μmol m^{-2} s^{-1}.
2. BugDorms (L60 × D60 × H60 cm) with 150-micron mesh.
3. Common bean pods (*Phaseolus vulgaris L.*) from the supermarket.
4. Bee pollen.
5. PCR tubes (200 μL) and caps.
6. Paper tissues.
7. Fine paintbrush.
8. White 500 mL polypropylene box (L182 × W135 × H35 mm) with transparent lid.

2.5 Trypan Blue Staining

1. Trypan blue solution (lactic acid, glycerol, demi-water, and 96% ethanol (1:1:1:3, v:v:v:v) + 0.025% trypan blue (adapted from Ref. 12).
2. Rack for 15-mL tubes.
3. Floating tube rack for Eppendorf tubes.
4. Water bath.
5. Pipette-boy.
6. 25-mL serological pipettes.
7. Empty glass bottle (1L).
8. Lactoglycerol solution (lactic acid, glycerol and demi-water (1:1:1, v:v:v)).
9. Petri dish (Ø 15 cm).
10. Microscope glass slide.
11. 95% ethanol.
12. Stereo zoom optical microscope (10× magnification).

3 Methods

3.1 Plant Cultivation

3.1.1 A. thaliana Cultivation

1. Suspend *A. thaliana* seeds in 1.0 mL of 0.1% agar in 1.5-mL Eppendorf tubes and store at 4 °C for 3 days.
2. Autoclave river sand in double autoclavable bags for 45 min at 120 °C.
3. Autoclave a mix of potting soil and river sand (12:5, v:v) in double autoclavable bags twice for 45 min at 120 °C with a 24-h interval.
4. Add half-strength Hoagland to sand (150 mL/kg sand). Fill up the 4-cm-high small trays with sand until 1 cm below the edge.

5. Distribute seeds (in 0.1% agar) evenly on sand using a 1000-μL pipet. Place the small tray in a big tray (L45 × W30 × H8), add some water to the bottom of the big tray, and cover with the transparent lid to ensure 100% relative humidity. Place in a growth chamber for 10–14 days.
6. Use a potting bench to mix Hoagland solution (50 mL/kg) and an equal amount of water with the sterile potting soil mixture.
7. Cut garden felt in pieces of L45 × W30 cm (the size of big tray), wet with water, and place in big plant trays (*see* **Note 2**).
8. Fill 60-mL pots with the soil mix, pots should weigh approximately 70 g, and make a hole of 1.5 cm deep in the soil of each pot with the conical end of a 15-mL tube. Place the pots in the big trays with felt.
9. To transfer a seedling to the planting hole, flood the small sand trays with water, and use forceps with a curved tip to loosen up the sand and to gently pull out the seedling.
10. After transfer of the seedling into the planting hole, close the hole gently by pushing the soil back around the root.
11. Stick a plant label (colored or with text) in each pot to indicate the genotype or treatment. Randomize the different genotypes/treatments in and between trays (40 pots/tray).
12. Cover the trays with transparent lids for 2 days to ensure 100% relative humidity. Crack the lids for 1 day before full removal to allow the plants to gradually acclimate to a relative humidity of 70% for the following weeks.
13. Every other day, water the plants until the felt is saturated. Once a week, apply 500 mL of Hoagland solution per tray.

3.1.2 Lettuce Cultivation

1. Follow **steps 1–6** of Subheading 3.1.1 for cultivation of lettuce seedlings.
2. Fill 800-mL pots with sterile potting soil (not a mixture with sand) and place them in a big tray (eight pots/tray; *see* **Note 3**). Make a hole of 3 cm deep in the soil of each pot with the conical end of a 15-mL tube.
3. Gently remove a lettuce seedling from sand using your fingers and place it in the planting hole. Close the hole gently by pushing the soil back around the root.
4. From here, follow **steps 10**, **12**, and **13** of Subheading 3.1.1 for cultivation of the lettuce plants after transfer of seedlings.

Fig. 2 Modified PET-container for thrips larval rearing and whole-plant bioassays

3.2 Tools for Rearing and Collecting of Thrips, and for Bioassays

3.2.1 Containers for Larval Rearing and Whole-Plant Bioassays

For both larval rearing and whole-plant bioassays, modified 1-L transparent PET containers (Ø 10.4 cm) with screw caps are used (Fig. 2).

1. Using a hot glue gun, apply some glue underneath the bottom of a 6-cm petri dish to attach it on the bottom inside the container.
2. Use a hole saw with a drill to make a Ø 6-cm hole in the white screw cap of the container.
3. Cut circles (Ø 8 cm) from 80-micron mesh and use a glue gun to attach the mesh onto the inside of the screw cap.
4. Heat the tip of a cork-borer (Ø 20 mm) and create a hole on the side of the container at 2 cm from the bottom. Cover the hole with a 20-mm butyl septum.

3.2.2 Clip Cages for Single Leaf Assays on Whole Plants

For assays in which a localized treatment with a defined number of thrips on one leaf is needed, a clip cage can be used, which allows the treated leaf to remain attached to an intact plant (Fig. 3). Clip-cage assays are not only suitable for gene expression or metabolite studies but also to determine systemic effects of thrips feeding on secondary infestation or infection. By selecting leaves of a similar developmental stage, variation between replicates will be reduced.

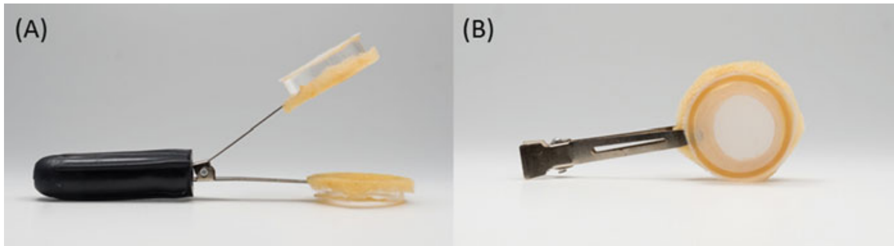

Fig. 3 Clip cage. (**a**) Side view of the clip cage when opened. (**b**) Top/bottom view of the clip cage. Foam is glued on the inside and mesh on the outside of the rings

Clip cages can not only be ordered online but can also be made in the lab as described here.

1. Hold two acrylate rings, one of 5 mm height and one of 8 mm height, between the thumb and the index finger.
2. Use a Bunsen burner to heat the tips of the metal hair clips. Wear heat-resistant gloves when doing this. When sufficiently heated (the tip will start to glow), each tip of the clip (bottom or top) can be gently pressed into the side of the rings (one tip in the top ring, the other in the bottom one).
3. Cut 80-micron mesh into squares of 4 × 4 cm and using a glue gun attach one mesh on the outside of the 5-mm ring and one on the outside of the 8-mm ring.
4. Cut the polyether foam into squares of 4 × 4 cm and make stacks of 10.
5. Wear heat-resistant gloves when heating the tip of the Ø 15-mm cork-borer in the burner. Press it through the middle of the foam stack to create a hole in each patch.
6. Glue one of the foam patches on the inside of the 5-mm ring and another one on the inside of the 8-mm ring (*see* **Note 4**).
7. When the glue is dry, cut the edges of the mesh and the foam.

3.2.3 Aspirator for Adult Thrips Collection

An aspirator is used to collect female thrips. Adult females are used for the whole-plant bioassays because then both feeding damage and oviposition can be assessed as performance parameters. Females are selected from a colony reared in the BugDorm (*see* Subheading 3.3) and can be recognized by their dark abdomen. Male thrips are smaller and have a lighter color (Fig. 4). Aspirators can be bought (online) but can also easily be handmade (Fig. 5), following the protocol below.

1. Heat the tip of a Ø 4-mm cork-borer in a Bunsen burner and pierce two holes in the screw cap of a 15-mL plastic, transparent pot.

Fig. 4 Handmade aspirator to collect thrips

Fig. 5 Adult male (left) and female (right) thrips

2. Cut the silicone tubes into two pieces of 30-cm length. Slide the tubes through the holes of the screw cap (until 1 cm below the cap) and glue-shut the opening between the tube and the hole using a glue gun.
3. Cut 80-micron mesh into small squares of 5×5 mm and glue it to the end of one of the tubes, facing the inside of the pot.
4. Cut 1 cm off the tip of a 200-μL pipette tip and slide it into the other end of the tube that carries the mesh.

3.3 Thrips Rearing and Collecting

3.3.1 Adults

When starting a thrips colony, set up two to three BugDorms. When you start a colony with 100 adult thrips (mix of males and females), you can have 100–200 adult female thrips after approximately 18 days. To prevent inbreeding, mix the colonies between the BugDorms and import thrips from other thrips sources (e.g., from collaborators) every 6 months.

1. Place the BugDorm in a controlled growth cabinet at 26 °C, which is an ideal temperature for thrips.
2. Place four plastic plant pot saucers in each BugDorm. Cut pieces of garden felt (Ø 18 cm) to put in the saucers.
3. Place a pot with a 5-week-old lettuce plant on each saucer (*see* Subheading 3.1.2 for the cultivation of lettuce).
4. Add a mix of male and female thrips to each BugDorm.
5. Cover the bottom of a small petri dish with bee pollen and place the dish in the BugDorm (*see* **Note 5**). Refresh the bee pollen weekly.
6. Every other day, water the plants until the felt is saturated. Once a week, apply Hoagland solution (*see* Subheading 3.1.2).
7. For collection of female thrips, gently shake a thrips-infested lettuce plant from the BugDorm in the white 500-mL box and close it with a transparent lid.
8. Place the pipette tip of the aspirator made into Subheading 3.2.3 in your mouth; gently lift the corner of the lid of the box and cover the selected thrips with the end of the other tube to suck it up. The thrips is now collected in the pot, and the mesh at the end of the 'sucking' tube prevents the thrips from moving into that tube.
9. If the desired amount of thrips is collected (e.g., 5 for a leaf- or whole-plant assay and 100 for synchronized rearing of thrips larvae), then remove the aspirator cap and screw on a normal cap (without holes).

3.3.2 Larvae

After thrips larvae hatch from the eggs, they develop two larval stages. The transition from the first to the second larval stage is difficult to distinguish. First instar larvae (L1) are small and white and transform into yellow and slightly bigger second instar larvae (L2) after 1 or 2 days, depending on the temperature (Fig. 1). By synchronizing their development, a collection of larvae at a certain developmental stage (L1 or L2) can be obtained.

1. Fold some paper tissues into squares of 10 × 10 cm and place at the bottom of a 1-L container made in Subheading 3.2.1.
2. Rinse the bean pods with water and place five beans in each container.

3. Fill a PCR tube with bee pollen and close with its cap. Make a small hole in the bottom of the tube by cutting it with scissors or by piercing it with a needle, through which the thrips can acquire access to the pollen (*see* **Note 5**). Place one tube in the container.
4. Collect 100 female thrips as described in Subheading 3.3.1 and add them to the container (*see* **Note 6**). Allow them to oviposit on the beans for 48 h.
5. After 48 h, repeat **step 1** and place the oviposited beans in the female-free container. Add the bee pollen and put the females from **step 4** back into the BugDorm.
6. After 4–5 days 100–200 L1 larvae will emerge. They will develop into L2 larvae 2 or 3 days later.
7. Use a fine paintbrush to collect an L1 or L2 larva. Moisturizing the brush will improve sticking of the larva to the brush.
8. Gently place the hairs of the brush on a leaf or in the clip cage and allow the larva to move from the brush. Prevent brushing the larva off as this might damage the thrips.

3.4 Thrips Assays

3.4.1 Feeding Damage Assays

Measuring thrips feeding damage is a good measure to quantify thrips performance. Thrips silver damage can be estimated in mm^2 by eye or quantified from pictures using software [9]. Different thrips developmental stages can be used for this assay, but it should be kept in mind that the duration of the different stages differs (Fig. 1). When starting with L1 larvae, damage can be recorded for 5 days before the pupae phase starts. Damage caused by L2 larvae can therefore be recorded only for 3 days. The damage inflicted by female adults must be recorded within 4 days, before their eggs hatch and L1 larvae start to feed from the plant.

Whole-Plant Damage Assay

1. Test at least ten replicates per treatment and/or genotype. Put 10 mL of water in the petri dish at the bottom of a handmade container (*see* Subheading 3.2.1). Place an *A. thaliana* plant on the petri dish.
2. Collect thrips at the preferred stage as described in Subheading 3.3.
3. Treat the plant with the thrips (*see* **Notes** 7 and **8**).
4. Monitor the amount of feeding damage (see above; Fig. 6a).

Single-Leaf Damage Assay

1. Test at least ten replicates per treatment and/or genotype. Use clip cages (*see* Subheading 3.2.2).
2. Collect thrips at the preferred stage as described in Subheading 3.3.
3. Transfer the thrips to the clip cage (*see* **Note 9**).

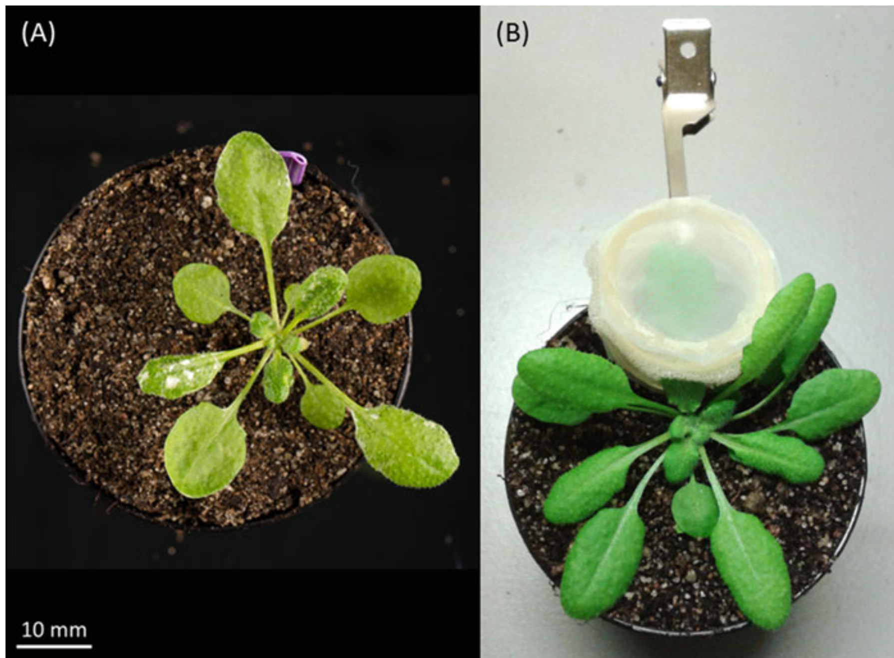

Fig. 6 Silver damage on an *A. thaliana* plant (**a**) and application of a clip cage on an *A. thaliana* leaf (**b**)

4. Gently open the clip and place the leaflet in the opening of the clip cage. Gently release the grip on the clip so that the leaflet is fixed in the clip (Fig. 6b).
5. Let the clip rest on a neighboring pot to prevent damage on the petiole because of the weight of the clip.
6. Monitor the amount of feeding damage (see above; Fig. 6a; *see* **Note 10**).

3.4.2 Oviposition Assays

Feeding damage on the plant often correlates with oviposition by female thrips. As an extra measure of thrips performance, the amount of oviposited eggs can be determined after feeding by adult females. Thrips eggs are kidney shaped and are oviposited in leaf blades and petioles (Fig. 7).

1. After damage assessment of a whole-plant assay (*see* Subheading "Whole-Plant Damage Assay"), rosettes are harvested by cutting the plant at the hypocotyl. Fold the leaves upward like an umbrella and put the rosette in a 15-mL tube. When assaying single leaves (using clip cages), cut the treated leaf and place it in a 1.5-mL safe-lock tube.
2. Heat up a water bath to 100 °C.
3. Add the Trypan blue solution to the leaf tissue (~10 mL for a rosette, ~0.75 mL for a leaf) and close the tubes with their respective caps.

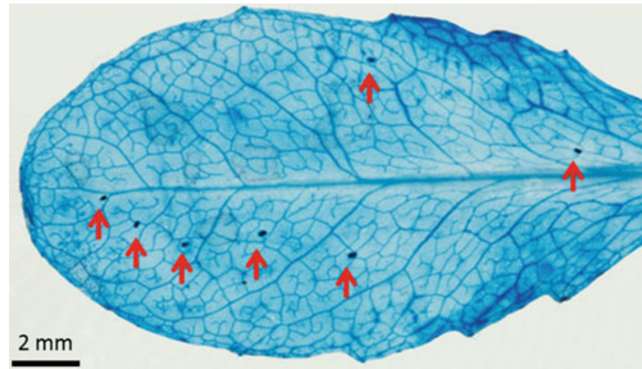

Fig. 7 Trypan blue-stained leaf to visualize thrips eggs. Red arrows point to oviposited eggs on an *A. thaliana* leaf

4. Place the 15-mL tubes for 2 min and the 1–5 mL tubes for 30 s in the water bath. Let the solution cool down for 30 min.
5. Pour off the Trypan Blue solution (use forceps to prevent the leaf material from sliding out of the tube) and replace by the lactoglycerol solution until the leaf tissue is fully submerged (~7 mL for a rosette, ~0.75 mL for a leaf). Samples can be stored at room temperature.
6. Place the rosette in a petri dish (Ø 15 cm) containing 95% ethanol. Single leaves are transferred to a microscope glass slide. Use a stereo zoom microscope and count the detected eggs. A magnification of 10× usually suffices, but a higher magnification may be needed if the level of background staining is high.

3.4.3 Assays for Thrips-Induced Changes in Gene Expression or Metabolite Accumulation

For measuring the plant's molecular responses to thrips, for example, induced changes of gene expression or metabolite accumulation, the use of a single leaf that remains attached to the plant until the point of harvest for the assay is preferred. The variation between replicates will be reduced by selecting leaves of a similar developmental stage. Moreover, by using a clip cage, a defined number of thrips can stay localized on one leaf (Fig. 3).

1. Follow **steps 1–5** of Subheading "Single-Leaf Damage Assay". Instead of ten replicates, this type of assays requires a minimum of five replicates.
2. Harvest the sample after the preferred period of time (*see* **Note 11**) by gently removing the clip and cutting the treated leaf at the petiole. Put the leaf in a 1.5-mL safe-lock tube and snap freeze it in liquid nitrogen, after which it can be stored in the −80 °C freezer.

4 Notes

1. It is important to have separate areas for plant cultivation, thrips rearing, and plant–thrips assays. Thrips are difficult to control, since they can easily maneuver through small openings and consequently contaminate other areas. In our case, we performed all our thrips assays in greenhouse compartments, which were physically separated from our growth chambers (Sylvania Luxline plus lamps F58W/84) where we usually cultivated our *A. thaliana* plants until they were moved to the greenhouse at least 24 h before the start of the thrips assay. Thrips rearing took place in a separate compartment in the greenhouse. In the beginning, we also cultivated *A. thaliana* plants in the greenhouse, but the frequency of contamination with thrips became too high.
2. The garden felt will retain the water longer, creating a moist environment for plants for a longer period.
3. No garden felt was used on the bottom of the trays in which lettuce plants were grown. After a few weeks, the roots of lettuce start to grow out from under the pots and attach to the felt. Removing the pots from the felt will damage the roots. This root outgrowth does not occur with *A. thaliana* plants.
4. Foam prevents the leaf from damaging. Additionally, foam closes openings between the two rings at the side where the petiole is placed between rings. Also, if the cages would be used on leaves of other plant species that might have thicker veins, the foam will close the spaces/openings that are created by the uneven thickness of the leaf.
5. Bee pollen are highly nutritious for thrips and can have a positive effect on, for example, development time, fecundity, and longevity.
6. A 15-mL pot of the aspirator containing the 100 female thrips can be opened and placed inside the 1-L rearing container with beans.
7. When a whole plant is treated with adult (female) thrips, the 15-mL pots of the aspirator can be opened and placed next to the petri dish inside the 1-L container.
8. Taken over several days, in our experiments, the average temperature inside the 1-L containers was 1.06 and 0.32 °C higher than outside the container during the day and night, respectively.
9. To get the female adults in a clip cage, tap against the 15-mL collection pot and hold it upside down above the open clip cage to release the thrips.

10. Gently remove the clip cage from the leaf and keep the leaf attached to the plant when assessing the damage. This will prevent wilting of the leaf, which hinders the scoring.
11. We harvested samples for analyses of gene expression and hormone accumulation every other hour until a maximum duration of two days (leaves will be too heavily infested after that time).

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