Mini-Aspirator: A New Device for Collection and Transfer of Small Arthropods to Plants

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MINI-ASPIRATOR: A NEW DEVICE FOR COLLECTION AND TRANSFER OF SMALL ARTHROPODS TO PLANTS

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Abstract

The process of collecting and/or infesting plants with a designated number of small arthropods in biological experiments is tedious and laborious. We developed a modified mini-aspirator, powered with a vacuum pump and fitted with a specially adapted (removable) collection vial to reduce the handling effort. The efficiency of the mini-aspirator was tested with the chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), a predatory mite, *Amblyseius* (= *Neoseiulus) cucumeris* (Oudemans) (Acari: Phytoseiidae), and the insidious flower bug, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae). Using the mini-aspirator, operators collected 10 *A. cucumeris* mites and 10 *S. dorsalis* thrips and transferred them onto pepper plants in 43 s and 37 s, respectively, compared with 639 and 229 s, respectively, using a camel's hair brush as a conventional method. The use of the mini-aspirator for collecting *A. cucumeris* predatory mites and *S. dorsalis* thrips and infesting pepper plants with them represents a 15-fold and 6-fold time saving, respectively. Collection of 10 *O. insidiosus* flower bugs took 20 s with the mini-aspirator compared with 30 s when an unmodified aspirator was used. Proportionally, the amount of time saved with the mini-aspirator for the handling of *O. insidiosus* flower bugs was minimal compared with the timesavings when handling *S. dorsalis* thrips and the *A. cucumeris* predatory mites with the mini-aspirator. Additionally, the mini-aspirator can be fitted with a battery-powered Mini-Vac, which makes it portable for field applications, such as in sampling field populations when screening for pesticide resistant individuals.

Key Words: mini-aspirator, *Neoseiulus cucumeris*, *Scirtothrips dorsalis*, *Orius insidiosus*, manual infestation

Resumen

El proceso de recolectar y/o infestar plantas con un cierto número de artrópodos pequeños en experimentos biológicos es tedioso y laboroso. Desarrollamos una mini-aspiradora modificada, que funciona mediante una bomba de succión especialmente montada con un frasco de recolección especialmente adaptado (desmontable) para reducir el esfuerzo de manejo. La eficiencia de la mini-aspiradora fue probada con el trips de chile, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), un ácaro depredador, *Amblyseius (=Neoseiulus) cucumeris* (Oudemans) (Acari: Phytoseiidae), y el chinche pirata diminuto, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae). Usando la mini-aspiradora, los operadores recolectaron y transfirieron 10 ácaros depredadores y trips de chile a plantas de chile en 43 y 37 segundos, comparado con 638 y 229 segundos usando el metodo convencional con una brocha de pintar. El uso de la mini-aspiradora para mover e infestar plantas con ácaros depredadores y trips representa un ahorro de 15 y 6 veces, respectivamente. La recolección de 10 chinches piratas diminutos tomó 20 segundos con la mini-aspiradora comparada con 30 segundos cuando se uso una aspiradora no modificada. El tiempo ahorrado proporcionalmente fue mínimo comparado con la recolección de trips de chile y los ácaros depredadores con la mini-aspiradora. Además, la mini-aspiradora puede ser mantada con una mini-bomba de succionar de batería, que la hace portable para aplicaciones en el campo, como en la evaluación de resistencia de plaguicidas en poblaciones de campo.

Manual collection and infestation of small arthropods (<2 mm) can be labor intensive and cumbersome and result in injury to the handled arthropods. Thrips and predatory mites are examples of small arthropods used by scientists in numerous experiments (Mound & Palmer 1981;

Chiu et al. 1991; Tatara & Furuhushi 1992; Tschuchiya et al. 1995; Bournier 1999; Seal et al. 2006; Arthurs et al. 2009). *Scirtothrips dorsalis* Hood, chilli thrips, is one of the smallest thrips species with adults ranging from 1.5-2.0 mm. The *S. dorsalis* adult moves rapidly, and may jump or fly when disturbed. Immature stages of *S. dorsalis*, especially first instars, are very small (<1 mm) and have fragile easily injured bodies. Thus, an important requirement for studying small arthropods is to have a reliable method for collecting and releasing designated numbers of individuals without harm.

Three methods are used for infesting plants with thrips. One is manual infestation of arthropods with a soft-bristled camel's hair brush (Cloyd & Sadof 1998). This method has been used widely but is labor-intensive and time-consuming. For instance, Cloyd et al. (2001) reported that infesting 50 plants each with 10 adult western flower thrips (WFT), *Frankliniella occidentals* (Pergande), required a technician 3.5 h. In addition, the process of mechanical transfer involved a risk of injury to the specimen. The second method involves placement of plants in a location where thrips are known to occur in order to allow a natural population of the pest to build-up on the test plants. Although this method is less cumbersome than manual infestation, the number of thrips transferred onto test plants cannot be accurately regulated, which introduces variation among test plants. The third method is the use of a commercial mouth operated aspirator purchased from BioQuip, Rancho Dominquez, CA. An improvement to the mouth-operated aspirator was reported by Cloyd et al. (2001), who developed a "Small Insect Aspirator" for collecting WFT. The latter involved the use of an aspirator from BioQuip attached to a battery operated 'Mini-Vac' (MV Instrument, Glendale, CA 91205; http://www.mini-vac.com/index02.html). Use of the small insect aspirator is attended with operational difficulties similar to those encountered with the use of regular aspirators, in particular the collection of extraneous materials. In our studies, we found that the wide suction tubes of the aspirators collect too many extraneous materials along with thrips (e.g., other organisms and plant debris) and sometimes causes physical damage to thrips. Additionally, in our experience the design of the collection vial does not allow the thrips to be released easily following collection.

In order to overcome some of the design limitations of previous small arthropod handling devices, we developed a "mini-aspirator" that can be powered either with a laboratory vacuum pump or with a small portable vacuum pump and fitted with a specially adapted and removable collection vial that allows rapid transfer of the collected arthropods onto plants. We compared the efficiency of the mini-aspirator with a paintbrush and commercial aspirator for collecting and releasing the chilli thrips, *Scirtothrips dorsalis* Hood and 2 of its natural enemies, a predatory mite, *Neoseiulus cucumeris* (Oudemans), and the flower bug, *Orius insidiosus* (Say).

Materials and Methods

Mini-aspirator

The mini-aspirator was built from clear 6.35-mm diam vinyl tubing fitted with a 1-mL filtered pipette tip (VWR International, West Chester, PA). The intake tubing opening was reduced by using an adaptor to attach a 200-µL pipette tip, which facilitated the collection of individual small arthropods (Figs. 1A and 1B). The modified mini-aspirator was powered by an electrical laboratory vacuum pump (Rocker vacuum pump, Rocker Scientific Co., Ltd., Kaohsiung, Taiwan) (Fig. 1A). To collect *S. dorsalis*, an infested leaf was placed under a stereomicroscope (Fig. 1C) and the desired number of thrips was captured in the collection vial for transfer onto plants. The collection vial was removed from the mini- aspirator and attached to a plant with a hair clip to allow the voluntary dispersal of the thrips onto the plant (Fig. 1D). To make the mini-aspirator portable, we integrated the mini-aspirator with a Mini-Vac (MV Instrument, Glendale, CA 91205; http://www.mini-vac.com/index02.html) (Fig. 1E). However, to compensate for the reduced suction power of the Mini-Vac, the pipette tip filter was replaced with fine woven nylon fabric. The assembly of the pipette tip, collection vial, filter and vacuum tube is shown in Fig. 2. Such integration of the "mini-aspirator" with the battery powered Mini-Vac made the system portable for field use.

Arthropods

Scirtothrips dorsalis specimens were obtained from a colony that originated from rose plants in Winter Park, FL. The colony was maintained on cotton plants, *Gossypium hirsutum*, 'Deltapine 493 Conventional'. The health of the colony was maintained by periodically introgressing thrips from naturally infested plants. Commercially available thrips predators (a predatory mite, *N. cucumeris* (Oudemans), and the insidious flower bug, *Orius insidiosus* (Say)) were obtained from Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands.

Plant Material

The infestation methods were tested on sweet pepper plants, *Capsicum annum* L. Pepper seeds were germinated on moist filter papers inside

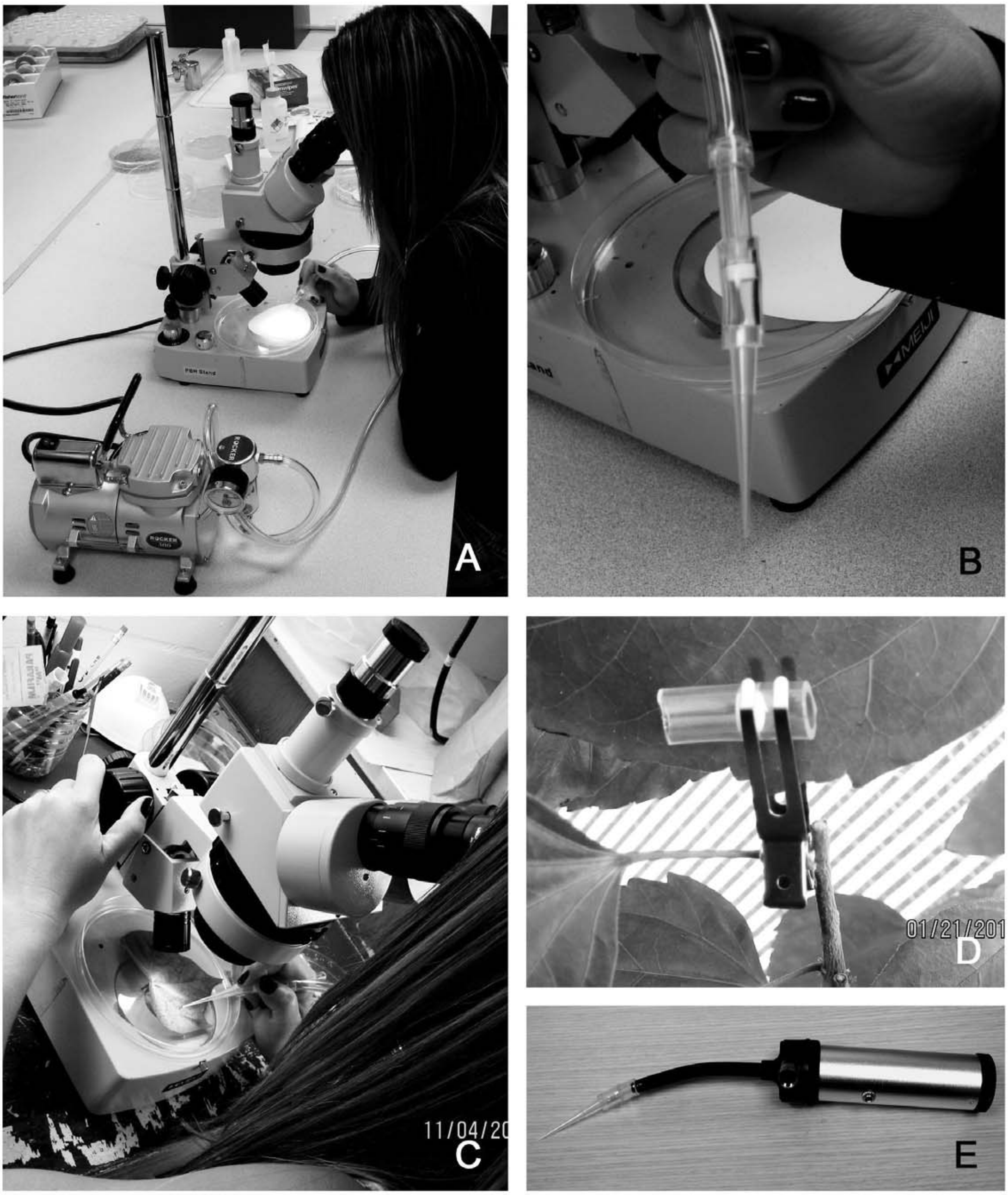

Fig. 1. Novel minute arthropod infestation apparatus and its use. A. The complete system in use; B. The mini-aspirator small arthropod collector; C. Collecting *S. dorsalis* from a leaf; D. Mini-aspirator along with collected arthropods attached to a plant to allow dispersal; E. The portable mini-aspirator consisting of clear 6.35 mm diam vinyl tubing fitted with a 1-mL pipette tip and nylon cloth filter connected with an adaptor to a 200-µL pipette tip; with suction provided by a Mini-Vac.

Petri dishes. Germinated seeds were transferred to seedling trays. Seedlings each with 4-6 fully expanded leaves were planted into 15 cm diam pots. Pepper plants at >10 leaf stage were used for the arthropod infestation experiments.

Collection and Infestation of Small Arthropods with the Conventional Camel's Hair Brush

Collecting chilli thrips and infesting plants with them. Either 10 or 20 chilli thrips were cap-

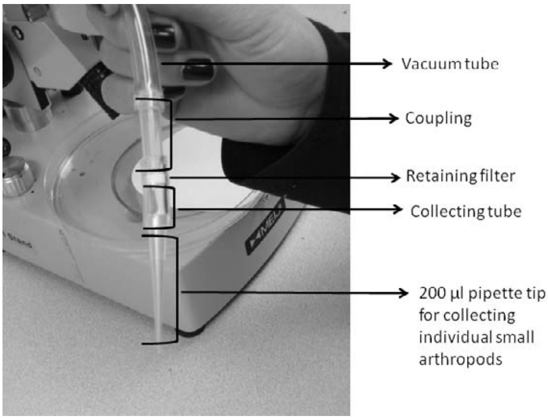

Fig. 2. Assembly of the pipette tip, collection vial, filter and vacuum line of the mini-aspirator.

tured with a moistened camel's hair brush. Each of these thrips was then placed onto a pepper plant leaf. The number of seconds needed to capture and transfer the above designated number of thrips was recorded. This was repeated 8 times each for groups of 10 or 20 chilli thrips.

Sorting and releasing N. cucumeris. The entire content of the package containing *N. cucumeris* and substrate was emptied into a Petri dish (15 cm diam) lined with a filter paper. To separate *N. cucumeris* predatory mites from the substrate, the closed Petri dish was agitated gently several times. Under a stereomicroscope each predatory mite was collected individually from the filter paper and placed onto a pepper plant with a camel's hair brush. Groups of either 10 or 20 *N. cucumeris* were placed on a plant. The number of seconds required to collect and release either 10 or 20 predatory mites was recorded. This was repeated 8 times each for groups of 10 and 20 mites.

Collecting and releasing O. insidiosus flower bugs on pepper plants. *Orius insidiosus* flower bug adults in groups of either 10 or 20 were collected from a purchased colony with a BioQuip aspirator. The contents of the package (vermiculite substrate and bugs) were emptied onto a board and bugs that crawled on the board were captured with the BioQuip aspirator. Each collection vial containing the *O. insidiosus* was placed at the base of a pepper plant that had been infested with thrips to allow the bugs to exit the vial and distribute onto the plant. The number of seconds required to collect and to release either 10 or 20 bugs was recorded. This was repeated 8 times each for groups of 10 or 20 *O. insidiosus*.

Use of the Mini-aspirator to Collect Small Arthropods and Transfer Them onto Plants

Collecting S. dorsalis thrips and infesting plants with them. Ten or 20 *S. dorsalis* were collected with the mini-aspirator as described above. The mini-aspirator along with the collected *S. dorsalis* was attached to a pepper plant leaf with a hair clip in a manner that allowed thrips to distribute themselves on plant leaves (Fig. 1D). The time required for collection and release (the latter being the time required to attach the mini-aspirator along with the collected *S. dorsalis* to a pepper plant leaf) *S. dorsalis* was recorded. This was repeated 15 times each for groups of 10 and 20 *S. dorsalis*.

Collecting N. cucumeris and releasing them onto plants. The entire contents of the package with *N. cucumeris* predatory mites were emptied into a Petri dish (15 cm diam). To separate *N. cucumeris* mites from the packaging material, a filter paper was placed in the Petri dish and the closed Petri dish was agitated gently several times. Under a stereomicroscope, either 10 or 20 *N. cucumeris* mites on the filter paper were collected with the mini-aspirator. The collection tube with the *N. cucumeris* mites was attached to a pepper leaf as described above (Fig. 1D). The seconds required for collection and release (the latter being the time required to attach the collector containing the collected *N. cucumeris* to a pepper plant leaf were recorded. This was repeated 15 times each for groups of 10 and 20 *N. cucumeris*.

Collecting adult O. insidiosus flower bugs and releasing them onto plants. The mini-aspirator was modified by enlarging the opening of the pipette tip to accommodate the bugs. The bugs were collected from among the vermiculate particles scattered on a board. *Orius insidiosus* in groups of either 10 or 20 were collected with the mini-aspirator. The collection tube containing the bugs was then attached to a leaf with a hair clip as described previously. The number of seconds required to collect and release the bugs was recorded. This was repeated 15 times each for groups of 10 and 20 *O. insidiosus*.

Statistical Analysis

The efficiency (time) of the mini-aspirator was compared with the conventional method for collecting and releasing each of the three arthropods. The study was repeated 8 and 15 times for the conventional and new method, respectively. Data were analyzed by ANOVA procedure (PROC GLM) and means were separated by Fisher's protected LSD test for all the experiments (SAS Institute 1997).

Results

The collection of 10 adult thrips from cotton leaves and their release onto a pepper plant using the mini-aspirator took 37 s compared with 229 s with the camel's hair brush method. This represented a 6-fold reduction in infestation

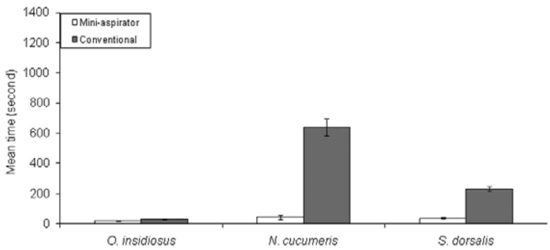

Fig. 3. Comparison of seconds needed for collecting and infesting 10 *O. insidiosus*, *S. dorsalis* and *N. cucumeris* onto plants with conventional methods (camel's hair brush or commercial aspirator) and the developed mini-aspirator.

time when the mini-aspirator method was used (Fig. 3). The difference between the 2 methods was highly significant ($F_{1,21}$ = 325.66; P < 0.0001). The time required to place 20 thrips on a plant with the mini-aspirator was 42 s, compared with 461 s by using the camel's hair brush method, which represented an 11-fold reduction in infestation time when the mini-aspirator was used ($F_{1,21}$ = 974.68; P < 0.0001) (Fig. 4). With the new method, the amount of time to collect and release per thrips decreased as number of increased. Such a reduction was not observed with the paintbrush method. Some thrips adults were also observed to be injured by the commercial aspirator (Fig. 5-A). We also observed some damaged thrips and reduced thrips activity when they were handled with a commercial aspirator (Fig. 5-B).

The collection and release of 10 and 20 predatory mites required 639 and 1154 s with a moistened camel's hair brush compared with 43 and 90 s, respectively, with the mini-aspirator (Figs. 3 and 4). The use of the mini-aspirator saved 15-fold and 13-fold more time in collecting and releasing 10 and 20 predatory mites, respectively, compared with the use of the paintbrush method. The difference between the two methods was highly significant ($F_{1,21}$ = 284.86; P = 0.0001 and $F_{1,21}$ = 379.00; P = 0.0001), respectively. No predatory mite was found to be damaged by the mini-aspirator.

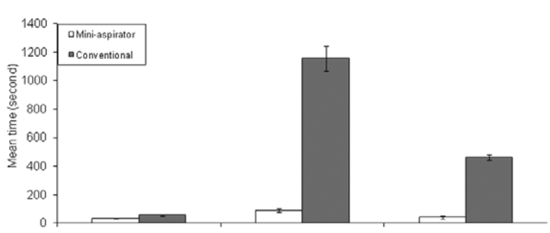

Fig. 4. Comparison of seconds needed for collecting and infesting 20 *O. insidiosus*, *S. dorsalis* and *N. cucumeris* onto plants with conventional methods (camel's hair brush or commercial aspirator) and the developed mini-aspirator.

The time required to collect and release adult *O. insidiosus* with a BioQuip aspirator was less than time needed for collecting and releasing by the paintbrush method. However, collecting and releasing 10 and 20 *O. insidiosus* with the commercial aspirator required 31 and 58 s, but only 20 and 32 s with the mini-aspirator (Figs. 3 and 4). These time differences between the 2 methods of collection and release were also significantly different ($F_{1,21}$ = 43.19; P= 0.0001) ($F_{1,21}$ = 33.52; P = 0.0001), respectively.

Discussion

The mini-aspirator reduces the time required to collect and release a designated number of small arthropods to the plants. The camel's hair brush method in addition to being very slow can cause injury, especially to soft-bodied small arthropods. An operator using the mini-aspirator can collect and transfer small arthropods using controlled air intake velocity, which minimizes injury to the collected arthropods. To avoid injury to thrips, the vacuum was adjusted to the minimum sufficient to collect thrips. Although injury to thrips was not investigated in detail, thrips collected with the mini-aspirator were checked under a stereomicroscope and no serious thrips injury was observed.

The mini-aspirator is different from the commonly available commercial aspirators, which employs larger diameter removable glass or plastic collecting vials. Initially, we used the commercial aspirators but experienced difficulties in transferring the designated numbers of arthropods. The commercial aspirator available to us has a 4-mm diam collection tube that could not be adjusted for the selective collection of individual thrips; and this is a significant limitation when working with mixed colonies of insects. Another advantage of the mini-aspirator is that unlike the traditional collection vials, the smaller removable and disposable collection tubes can easily be attached to small leaves or plant stems without disturbing the insects inside the tube.

The mini-aspirator developed in this study can be powered with a Mini-Vac, which makes the technique portable for field applications. The technique may be used to quickly census wild populations for laboratory testing or for use in insecticide efficacy trials. The mini-aspirator could also be adapted for quick pesticide resistance or efficacy trials in the field (Rueda and Shelton 2003). The inside of the collection tube of the mini-aspirator could be treated with pesticides of interest or a treated leaf disk could be placed in the mini-aspirator before collecting small arthro-

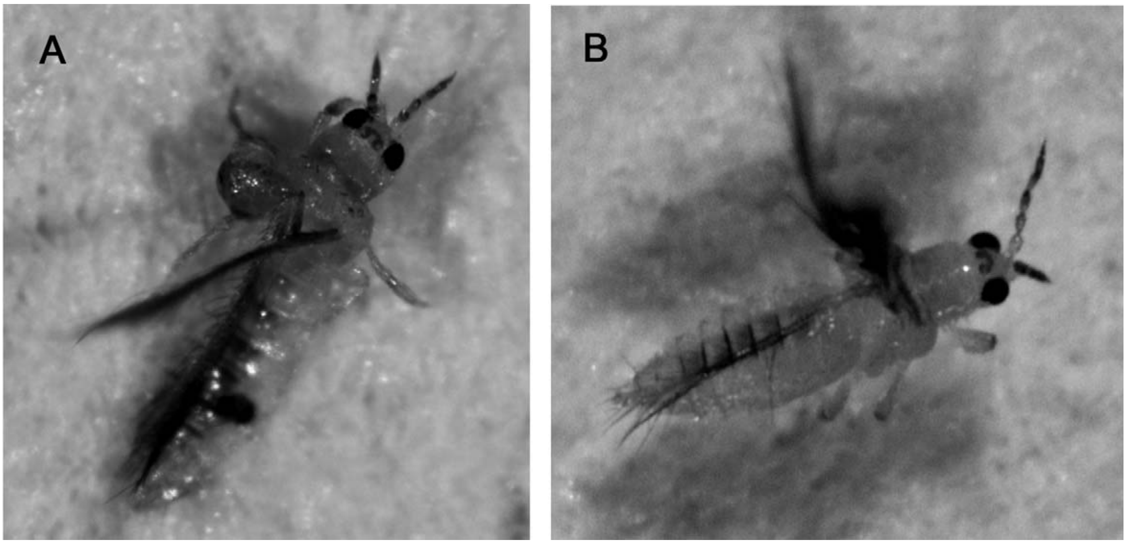

Fig. 5. Illustration of an injured *S. dorsalis* when collected with a regular made aspirator. A, Injury to abdomen of *S. dorsalis*. B, Injury to wing of *S. dorsalis*.

pods, and then collected arthropods would be held for a fixed exposure period to quantify pesticide efficacy. This kind of monitoring would be helpful to confirm pest susceptibility to pesticides before their wide area applications.

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