Please cite this article as: Chen, C. *et al.* (2021). Preference Test of *Plutella xylostella* Larvae upon DMNT Treatment. *Bio-protocol* 11(21): e4208. DOI: 10.21769/BioProtoc.4208.

bio-protocol www.bio-protocol.org/e4208 Bio-protocol 11(21): e4208. DOI:10.21769/BioProtoc.4208

Preference Test of *Plutella xylostella* Larvae upon DMNT Treatment

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[Abstract] We describe a method to test the preference of insects in response to (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT). We use a device that includes a horizontal glass tube, two grooves (with activated carbon), air flow, rubber stoppers/tubes, transparent glass containers (optional), and a holder for the glass tube (optional). Equal amounts of activated carbon in the groove (removable) are placed at both ends to avoid air contamination. The air flow is generated by an air pump. In the closed device, different samples are placed at each end of the glass tube. The air pump at the top of the glass tube forms an air flow that converges to the middle site of the glass tube. In each test, insect larvae are located in the middle of the glass test tube. If the test samples release DMNT that can be sensed by insects, the insects will selectively move to one specific end of the glass tube. The number of insects that move to each end will be recorded for further studies. This method can also be used to test the preference of insects in response to other volatile compounds.

Keywords: Insect preference test, Device, Volatile compound, DMNT, Insect larvae, *P. xylostella*

[Background] Insects have the ability to sense certain volatile compounds and show a differentiated response. However, this response is easily influenced by the surrounding environment. To make the data reliable for scientific research, it is necessary to set up a device to test the response more accurately. Previous research has showed that the preference of insects in response to volatile compounds can be tested by a Y tube (Adhikary *et al.*, 2014; Ndomo-Moualeu *et al.*, 2016). Here, we provide a schematic representation of a choice test system to check the behavior of insects affected by volatile DMNT or other compounds released from different samples.

Materials and Reagents

1. DMNT standard was synthesized according to previous research (Huang and Yang, 2007; Chen *et al.*, 2021).
2. Paraffin oil (Sigma, catalog number: M1180)

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Equipment

1. Air pump (Beijing Municipal Institute of Labour Protection, model: QC-1S)
2. Activated carbon (Sangon Biotech, catalog number: A600287-0001)
3. Glass tube (Length: 35 cm; Inner diameter of glass tube: 3.8 cm)
4. Rubber stoppers/tubes (Tansoole, catalog numbers: 02036109 [8#]; 02025735 [6#]; 02025732 [3#])
5. Holder for glass tube (Height: 15 cm, optional)
6. Glass containers (Length: 4.2 cm; Width: 4.2 cm; Height: 4.2 cm)

Procedure

1. As shown in figure 1, the test samples are located at each end of the glass tube and both ends of the tube are closed with rubber stoppers. The groove (activated carbon included) is connected at both ends of the horizontal glass tube (activated carbon is used to filter the gases in the experimental environment, which may interfere with insect response).

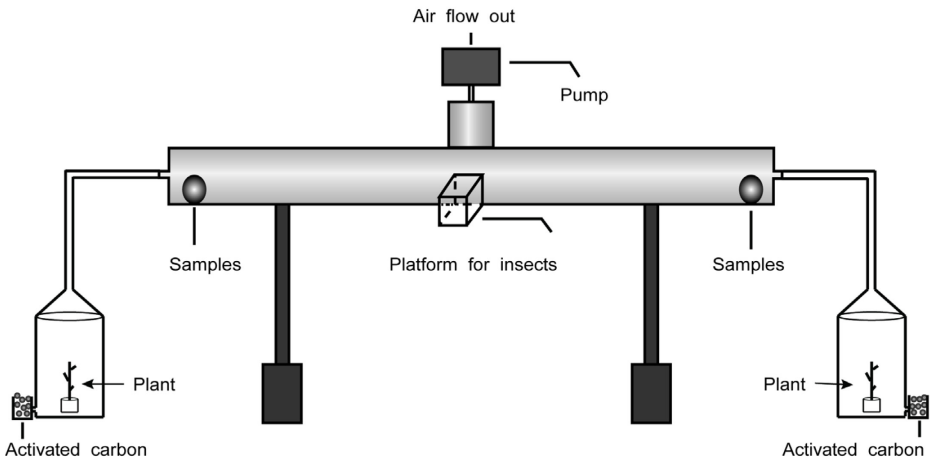

Figure 1. A schematic representation of insect choice test system (modified from Chen *et al.*, 2021)**.**

2. When this system is used to test plants that may release DMNT, the plants should be placed in a transparent glass container, which is connected to the glass test tube via plastic tubes. The plants can be kept in the glass containers for a few hours to enrich the environment for DMNT before proceeding to the next steps.
3. Turn on the pump (1 ml/min) for 5 min to create a clean and stable air flow inside the glass tube.
4. Place insect larvae in the port, right in front of the glass tube, and close the tube with a rubber stopper (Video 1).

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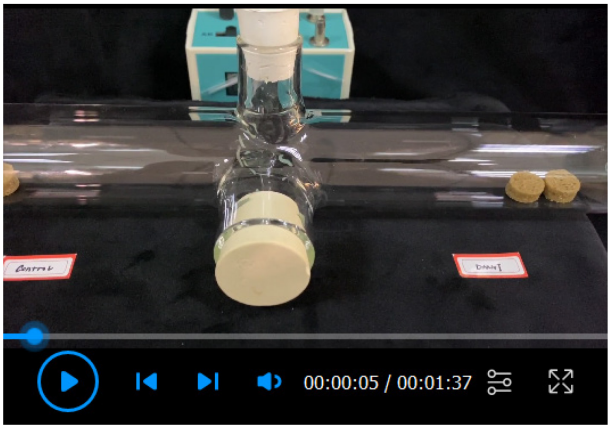
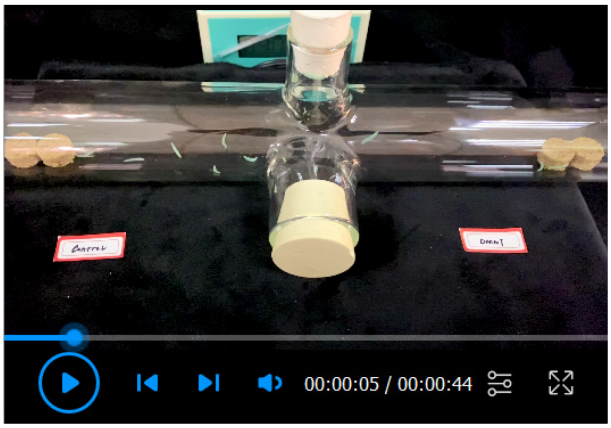


Video 1. Setup of the device to test the preference of *P. xylostella* larvae in response to DMNT.

5. After 3-10 min, the number of insects that moved towards each end of the glass tube is recorded (The standard of choice is for larvae to move 2 cm away from the middle of the glass test tube to each end, Video 2).



Video 2. *P. xylostella* larvae are repelled by DMNT.

6. To avoid interference from the surrounding environment, the test and control samples should swap places during experiments.
7. Clean all the parts of the device and repeat the above procedures several times to obtain reliable results.

Data analysis

We counted the number of larvae that crawled toward each end of the glass test tube and calculated the percentage of larvae at each end of the glass tube.

Notes

1. Before and after different experiments, use a fragrance-free cleaner to clean the whole device, rinse it thoroughly with double distilled water, and dry it in a drying oven to avoid the influence of residual odor or contamination.
2. The air extraction rate, as controlled by the pump, should be slow (approximately 1 ml/min).
3. This device is suitable to test the choice of small insect larvae such as *Plutella xylostella* and *Ostrinia furnacalis*.
4. Activated carbon should be changed regularly between different tests.
5. The device can be extended to test volatile compounds released from plant materials. The plants tested can be placed into closed tanks, which are connected to the two ends of the glass test tube with rubber tubes.
6. All the rubber stoppers and rubber tubes should be made of high standard rubber materials without odor. All the rubber parts should be cleaned and dried thoroughly before experiments.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2017YFD0301301, 2016YFD0101803) and the Natural Science Foundation of China (31670264). This protocol was adapted from the publication by Chen *et al*. (2021; Doi: 10.7554/eLife.63938).

Competing interests

The authors declare no conflicts of interest.

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