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Wind Tunnel: A Tool to Test the Flight Response to Semiochemicals

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1. Introduction

Semiochemicals mediate interactions between organisms (Law and Legnier, 1971), and the term is subdivided into two major groups, pheromones and allelochemicals, depending on whether the interactions are intraspecific or interspecific (Nordlund, 1981). Insect pheromones are the main research target for semiochemicals, because of potentials for practical use in agriculture. A wind tunnel is one olfactometer used as a bioassay method of olfactory stimuli. Wind tunnel tests have been widely used in insect pheromone research (e.g., Baker and Linn, 1984; Kainoh et al., 1984; Hiyori et al., 1986a,b), to study plant volatiles as kairomones (e.g., Kainoh et al., 1980) and to study synomones (e.g., Kainoh et al., 1999; Fukushima et al., 2001, 2002; Ichiki et al., 2008, 2011).

Sabelis and van de Baan (1983) used a Y-tube olfactometer and determined that predacious mites responded to the odors of plants infested with spider mites. This was the first demonstration of a tri-trophic interaction in which predators or parasitoids are attracted by plants infested with herbivore prey or hosts. Studies on the effects of volatile materials (Herbivore Induced Plant Volatiles, HIPVs) on the behaviors of natural enemies were conducted with olfactometers and wind tunnels as indicated by van Driesche and Bellows (1996).

2. Structure of wind tunnel

2.1 Laboratory conditions (temperature, humidity)

When a wind tunnel is set up it is necessary to consider what laboratory is suitable for the wind tunnel. If a laboratory has an exhaust fan on the wall, the downwind end of the tunnel can be connected to the fan (Fig. 1). However, air must be provided from a corridor through a louver on the door. In a closed laboratory, air must be recycled in a wind tunnel and a charcoal filter fixed at the upwind end (Fig. 2). A laboratory with a ventilation system is ideal for setting up a wind tunnel. The downwind end of the tunnel can be connected to the exhaust inlet (Fig. 3).

Temperature can be controlled by adjusting the air-conditioning system, but sometimes it is very difficult to change the temperature of a large system. We used to use an electric heater during the winter to increase the room temperature to 25˚C.

For a humidifier, we fixed an electrode steam humidifier (resN200, presently CP3PRmini, PS Company Ltd., Tokyo, Japan) on the wall of the tunnel (Fig. 6) to maintain a humidity greater than 50-60% R.H., this humidifier is even used in midwinter when the outdoor temperature is below 0˚C. Insects do not respond well below 50% R.H.

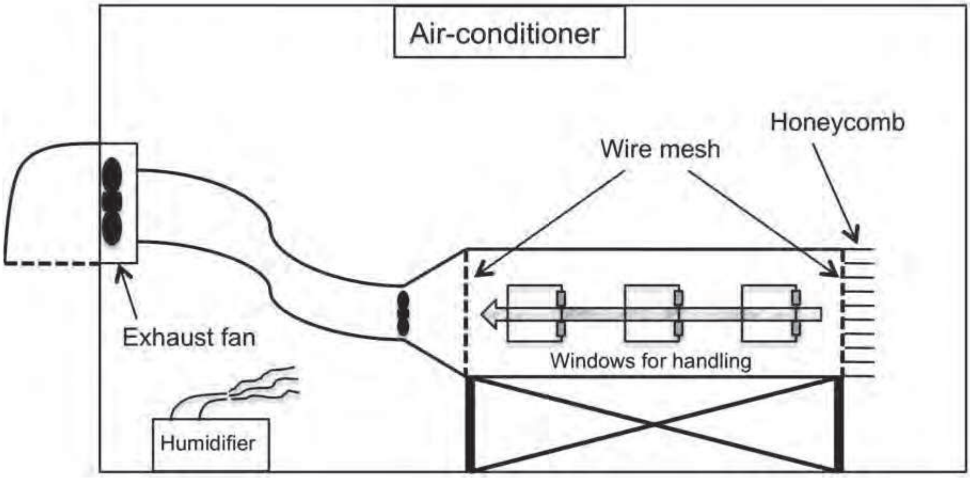

Fig. 1. Pulling-air type wind tunnel.

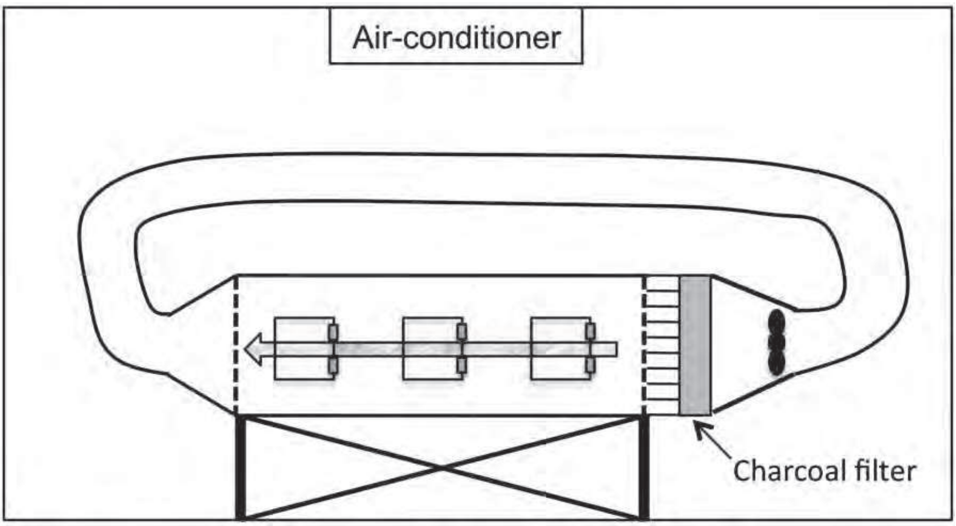

Fig. 2. Pushing-air type wind tunnel to recycle the air.

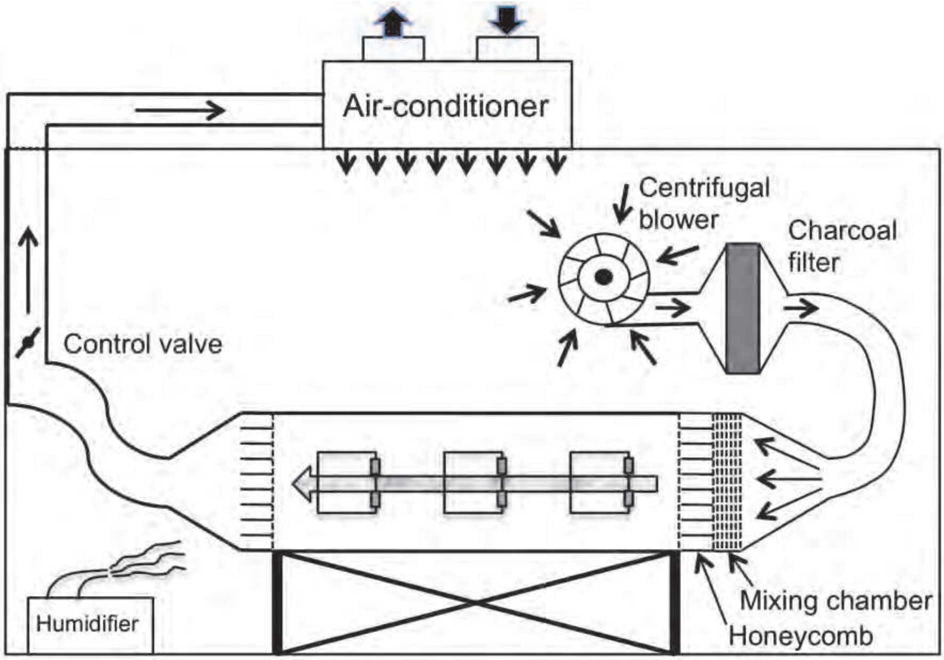

Fig. 3. Pushing- and pulling-air type wind tunnel.

2.2 Cylindrical or rectangular?

Two types of wind tunnels are used in entomological research: cylindrical and rectangular types. In our laboratory, we use a cylindrical tunnel for testing insect sex pheromones because the sex pheromone sample is hung from the ceiling of the tunnel, and a rectangular one to test responses of insect parasitoids to plant volatiles because the flat floor is convenient for placing potted plants. As Baker and Linn (1984) reported, there is no substantial difference between the two types of tunnels. From my point of view, an ideal air current can be produced with a cylindrical wind tunnel rather than a rectangular one because air currents are retarded at the corners of a rectangular tunnel. If insects fly into the corner of a tunnel, they may perceive lower concentrations of the odor coming from the upwind end.

2.3 Pulling-air and pushing-air type

One type of tunnel is the pulling-air type (Fig. 1), and another is the pushing-air type (Fig. 2, 3). As Baker and Linn (1984) pointed out, pushing-air type tunnels do not disturb the plume. Opening the window for insect handling does not disturb the air stream in the pushing-air type tunnel (Fig. 2, 3). Therefore, insects on the releasing platform can directly perceive the odor immediately after being released without any disturbance in the air stream. In our experiments, a laminar air stream of incense smoke can be observed even with the windows open. In the case of the pulling-air type wind tunnel (Fig. 1), insects on the releasing platform perceive disturbed air movement when released, but the air current gradually becomes normal after the window is closed. In addition, air should not leak from the tunnel wall and all windows must be tightly closed.

One disadvantage of a pushing-air type wind tunnel is a lack of even laminar flow inside the tunnel (Fig. 3). When an incense smoke plume is observed, the flow is laminar in the central part but not the peripheral part. Care must be taken to maintain a balance of wind pressure in both the pushing-fan side and exhaust-fan side. A stable laminar flow can not be achieved unless there is a good balance in both the inlet and outlet of the tunnel.
From my experience, especially in a pulling-air type wind tunnel, an outdoor hood should be used as the exhaust fan, so strong outdoor winds do not disturb the smooth air flow. The hood in Fig. 4 works very well and the wind speed is not disturbed on windy days.

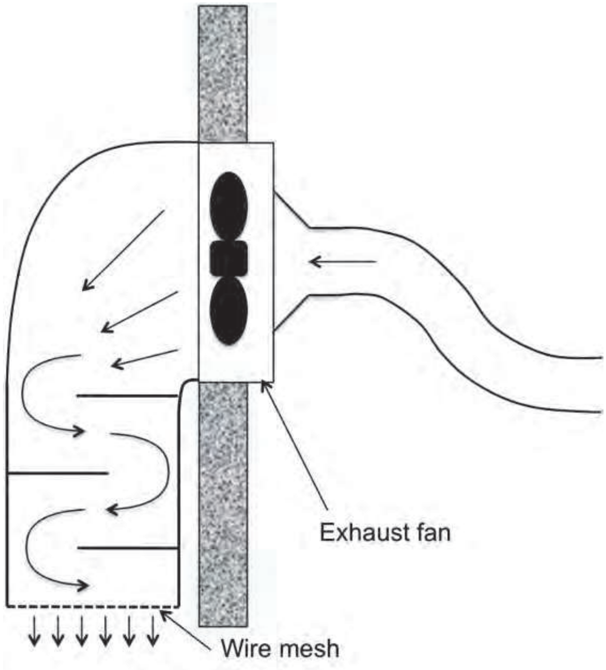

Fig. 4. Outdoor hood for exhaust fan to minimize influence of outdoor wind.

2.4 Air movement (wind speed)

Wind speed is an important factor in wind tunnel experiments. Most studies in the literature use a wind speed of 25 to 30 cm/sec. Kainoh et al. (1984) performed wind tunnel experiments to test the sex pheromone of *Adoxophyes honmai* (Lepidoptera: Tortricidae) and compared the wind speed between 30 and 60 cm cm/sec. There was no significant difference in male moth responses in the 4-component sex pheromone system. However, the flight behavior of male moths, *A. honmai*, seemed to be more stable at a lower wind speed, so we now use 25 to 30 cm/sec.
To measure the wind speed in the tunnel, we inserted an anemometer (ISA-90; probe: P-2, SIBATA Scientific Technology Ltd., Saitama, Japan) with the probe extended to 25 cm in the upper wall of the tunnel (Fig. 5).

Fig. 5. Downwind end of the tunnel. 1: anemometer; 2: VTR camera; 3: thermometer and hygrometer; 4: platform to release insects.

2.5 Charcoal filter

When we first built a wind tunnel in 1996, no charcoal filter was attached to the wind tunnel (Fig. 1). Our biggest concern was the bad smell from next door, a rearing room for mice and rats. We sealed the door between the two rooms, but the smell remained. A new wind tunnel (Fig. 3) was installed with a charcoal filter (Fig. 6, left) between the centrifugal blower (Fig. 6, right) and the wind tunnel. The filter consists of 6 charcoal filter panels installed in a zigzag pattern, each panel (30×50 cm, 3 cm thick) is filled with charcoal particles. Total area of the filter panels is 9,000 cm^2.

Fig. 6. Centrifugal blower (right), charcoal filter housing (left) and humidifier (center).

2.6 Honey comb structure (or mesh)

Turbulence in the airstream must be controlled for insect flight in a wind tunnel. A honeycomb structure fixed at the upwind end can facilitate laminar flow of the air (Figs. 1, 2, 3). If the honeycomb structure is expensive, a plastic pipe (3-5 cm in diameter) can be cut into lengths of 10 to 15 cm and tightly attach with glue to produce a structure to create laminar flow. Baker and Linn (1984) proposed a 'mixing chamber' at the tunnel opening to dampen the turbulence created by the fan blades and to balance wind velocities inside the tunnel. This mixing chamber consists of several layers of narrow-mesh cloth, screen or both. We do not use the mixing chamber in our wind tunnel but this idea is worth adopting.

We do not fix a structure at the downwind end of the tunnel to produce a laminar air flow, but Prof. K. Nakamuta (personal communication) commented that we should fix the honeycomb structure at the downwind end of the tunnel when air is pulled from the downwind end.

2.7 Light source

In wind tunnel tests with sex pheromones of nocturnal moths, light intensity is a significant parameter. We used *A. honmai* sex pheromones to attract male moths and varied the light intensity. In total darkness, there was no attraction of the moths, but at 0.03, 0.13, 0.77 *lx* attraction was 50 to 60% of the moths released. In a lighter condition at 3.5 *lx* the attraction was 38% and male catches were not stable (Kainoh et al., 1984). With this wind tunnel system, we tested the effect of sex pheromone disruptants on the attraction of male *A. honmai* moths and found that only the 2nd major compound (*Z*)-11-tetradeceny acetate has a disruptive effect on male moth flight, whereas the 1st major compound (*Z*)-9-tetradeceny acetate and other minor compounds have no affect (Hiyori et al., 1986a,b).

For diurnal insects, we established wind tunnel experiments for *Aphidius colemani* in our laboratory and used a light intensity of 150 *lx*, because female *A. colemani* did not show good orientation toward the odor source (herbivore damaged plant) under lighter conditions and flew upward to the ceiling of the tunnel at 2,000 *lx* (Fujinuma et al., unpublished). However, the tachinid fly *Exorista japonica* readily flew to the target plant under full light conditions (>2,000 *lx*) (Kainoh et al., 1999; Ichiki et al., 2008, 2011; Hanyu et al., 2009).

As a light source, we use Vitalite® (40W, 6 tubes) to maintain light conditions similar to sunlight, and the light intensity can be changed with a voltage converter from 0 to 6,000 *lx*. Under the Vitalite® or on a ceiling panel, a plastic light defuser was placed to scatter the light throughout the chamber.

2.8 Visual ground patterns

Flying nocturnal moths watch ground patterns when orientating to female sex pheromones as demonstrated by a moving-floor wind tunnel (Cardé and Hagaman, 1979). Using this type of moving-floor wind tunnel, the flight speed of the moth can be controlled and sustained flight experiments performed. Optomotor anemotaxis is the term used to explain the behavior of male moths orientating to female moths, in which they visually monitor their progress and react to this feedback (Bell et al., 1995). There are several ways to show moving patterns to insects (Baker and Linn, 1984). In our laboratory, we did not add a movable floor pattern to the wind tunnel because the system is too costly. Instead, we place green and ochre color strips (15 cm wide) to represent the soil and plants (Fig. 5). We have not yet compared the flight activity of insects with different colors or widths of the strips, but plan to evaluate these visual effects in the future.

2.9 Data recording

The software 'The Observer (ver. 5)' (Noldus Information Technology, Wageningen, The Netherlands) (Fig. 7) is used to record the behavior of insects in a wind tunnel (Hanyu et al., 2009). We can record each behavioral event (walking, flying, stationary or grooming) and location (release site, floor, wall, ceiling, target), and then calculate the duration, average time on the release site (latency), total time flying in the wind tunnel, total time walking on the floor, wall or ceiling and other parameters from these recordings (Fig. 8). We use a video camera (Fig. 9, Ultra Micro Color Camera, CC431+UN43H, ELMO, Japan) to record the flight of insects. The camera is placed at the downwind end, so we can record all behavioral events from the releasing site to the target. Recordings of tachinid fly (*Exorista japonica*) behavior were easily obtained, but the resolution of the camera was not high enough to see small insects, e.g., the braconid wasp *Cotesia kariyai* (Fukushima et al., 2001, 2002; Hou et al., 2005; Mandour et al., 2011) and aphid parasitoids (Takemoto et al., 2009; Fujinuma et al., 2010). To record the behavior of small insects, two cameras must be set in the tunnel, one near the releasing site and another near the target site.

Fig. 7. Laptop computer with the behavioral software 'The Observer' installed.

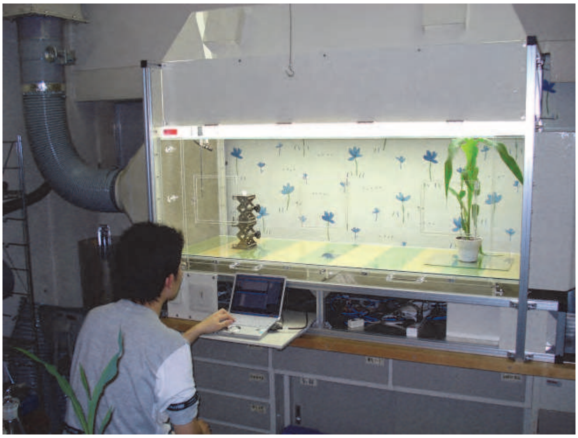

Fig. 8. An observer recording the behavior of the tachinid fly *Exorista japonica.*

Fig. 9. Microcamera fixed on the ceiling of the tunnel at the downwind end.

3. Conclusion

To design a wind tunnel, first choose a pushing-air or pulling-air type tunnel. A pushing-air type is usually recommended, but pulling-air type can work in some situations. A cylindrical tunnel has the ideal air flow, but a rectangular tunnel is useful for arranging potted plants on the floor. Wind speed is best regulated at 25 to 30 cm/sec with a voltage converter or a valve. A charcoal filter is recommended to clean the air before it enters the tunnel inlet. Light intensity can be changed with a voltage converter to maximize the insect flight conditions. Laminar air flow is not always necessary, but can be achieved by inserting a honeycomb structure at the inlet of the tunnel or mixing chamber of screen or mesh. Visual patterns inside the tunnel are not always critical but may sometimes affect insect flight. To record the behavioral events or state of the insect, 'The Observer' or other event recorder software is necessary. Trial-error tests are essential for initially setting up wind tunnel experiments that are optimal for the laboratory conditions and insect species.

4. Acknowledgements

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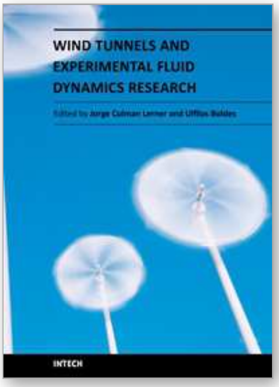

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The book â€œWind Tunnels and Experimental Fluid Dynamics Researchâ€ is comprised of 33 chapters divided in five sections. The first 12 chapters discuss wind tunnel facilities and experiments in incompressible flow, while the next seven chapters deal with building dynamics, flow control and fluid mechanics. Third section of the book is dedicated to chapters discussing aerodynamic field measurements and real full scale analysis (chapters 20-22). Chapters in the last two sections deal with turbulent structure analysis (chapters 23-25) and wind tunnels in compressible flow (chapters 26-33). Contributions from a large number of international experts make this publication a highly valuable resource in wind tunnels and fluid dynamics field of research.