Computer-Monitored, 16-Channel Flight Mill for Recording the Flight of Leafhoppers (Homoptera: Auchenorrhyncha)

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Computer-Monitored, 16-Channel Flight Mill for Recording the Flight of Leafhoppers (Homoptera: Auchenorrhyncha)

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ABSTRACT A multichannel, computer-monitored flight mill suitable for studying flight performance of small, weakly flying insects such as leafhoppers was developed. The flight mill uses a magnetic bearing and light-weight arm to minimize friction and drag. Sixteen mills can be monitored simultaneously by a PC. The construction of the mills and monitor, and data from a small number of test experiments, are presented. The problems associated with interpreting flight mill data are also considered.

KEY WORDS *Dalbulus* spp., flight mill, computer monitoring

THE MEASUREMENT OF insect flight performance requires estimates of a number of variables: periodicity and thresholds (e.g., temperature and light) for flight, frequency and duration of bouts of flight, total duration and speed of flight, and rate of fuel utilization and size of fuel reserves. The simultaneous estimation of these variables presents a number of problems. The experimental conditions required to measure the quantitative factors may not be appropriate for reliable observations of qualitative behavioral factors and vice versa.

The first measurements of flight performance were made by Hollick (1940), who used *Muscina stabulans* (Fallén) tethered in a stream of air to investigate the dynamics of insect flight. Later, Krogh & Weis-Fogh (1952) flew *Schistocerca gregaria* in a "roundabout" and Hocking (1953) used a slightly different apparatus to investigate the flight performance of the honey bee and seven flies from four families. Since the early 1950s, dozens of tethered flight studies have been reported.

Hocking (1953) designed and built a number of mills ranging from 16 to 64 cm in diameter. These used thin steel arms and a glass bearing. His recording system permitted the simultaneous operation of two mills. Since then, several materials have been tried to reduce weight and friction, and the number of mills in simultaneous operation has increased. Working with *Aedes aegypti* (L.), Rowley and colleagues devised flight mills (similar to Hocking's) and monitoring devices for 4 (Rowley et al. 1968), and then 16 mills (Clarke et al. 1984). The latter system was the first to use a personal computer (a Commodore PET) to record the data. About the same time, McKibben (1985) used another first-generation PC to monitor a single mill flying the boll weevil, *Anthonomus grandis grandis* Boheman. Glass arms had been used before (Atkins 1961, Smith & Furniss 1966), but McKibben (1985) used lighter silica capillaries in his mill. One of the most inventive developments in materials is Dybovskiy's (1970) flight mill with a horseshoe magnetic bearing to monitor flight in "small insects." His horseshoe magnet, augmented by powerful alloy magnets at each pole, supported the point of an entomological pin as the bearing. This arrangement greatly reduces both the weight and friction which must be overcome by the tethered insect to fly.

The use of flight mills was criticized by Kennedy & Booth (1963a) as interfering with behavior and therefore unlikely to provide reliable estimates of insect flight. They invented the vertical flight chamber to study aphid flight behavior. This choice of insect was fortunate because, once flight is initiated, aphids usually fly continuously for long periods, provided that the flight thresholds are exceeded. For species that take off, fly for only a short period, alight, take off again, and repeat this pattern of short flights several times, the flight chamber is less satisfactory. Flight mills may offer a better alternative for insects when flight bout length and interval are critical parameters.

Determinations of behavioral variables such as threshold responses are best obtained under conditions of free flight in flight chambers, but quantitative estimates (of flight speed, for example) frequently require more intrusive methods such as flight mills. Because it is impossible to assess the influence of tethering on behavior, flight mills probably are used best in comparative studies, in which one assumes that the manipulation associated with tethering affects behavior of all experimental units equally; thus, only relative estimates are obtainable. Although this assumption may not be absolutely valid, it is probably a reasonably good first approximation. This was a key assumption made in the development of the apparatus we describe here.

We are interested in the dispersal and migration of members of the leafhopper genus *Dalbulus*. The corn leafhopper, *D. maidis* (DeLong & Wolcott), a serious pest and vector of several stunting pathogens of maize, is widespread in the American tropics and subtropics (Nault 1985, 1990). Some evidence suggests that the corn leafhopper is migratory (Bradfute et al. 1981), whereas other evidence suggests adults may disperse locally from maize fields to nearby refugia at the end of the growing season and return in the spring to invade newly planted fields (Larsen et al. 1992). *Dalbulus elimatus* (Ball) abandons harvested maize in fall to infest nearby irrigated winter wheat and winter annuals, where it develops breeding populations (Barnes 1954). In the spring, the leafhopper returns to maize. Other *Dalbulus* species that specialize on the gamagrasses, *Tripsacum*, reside in the host habitat year round (Larsen et al. 1991, Larsen et al. 1992). To answer questions regarding the potential of the corn leafhopper and perhaps other *Dalbulus* species to migrate, we flew leafhoppers in a vertical flight chamber, but few leafhoppers flew >1 min. Most deflected horizontally from the central beam of light and landed on the walls of the chamber. We decided then to turn to flight mills to study *Dalbulus*. In addition, we had questions concerning possible differences in flight behavior and performance between the sexes and between molicute-infected and noninfected individuals, both within and between species and sex.

Previous attempts to use flight mills to study insect flight were mostly with large, powerful insects: muscid flies, moths, locusts, and beetles. However, some quite small insects have been used (mosquitoes, black flies, drosophilids), but all of these are relatively powerful fliers which fly continuously for long periods. The problem of mill design is critical for leafhoppers because they are small and are generally weak fliers with erratic flight behavior. Our problem was to obtain flight performance data sufficient for analysis from these small, weakly flying insects. To do this requires a small lightweight apparatus and multiple (inexpensive) mills running simultaneously. We report here a computer-monitored, light-weight flight mill capable of operating on 16 channels simultaneously. To illustrate the type of data the monitor returns, we report also some preliminary results of flight tests with *D. maidis*.

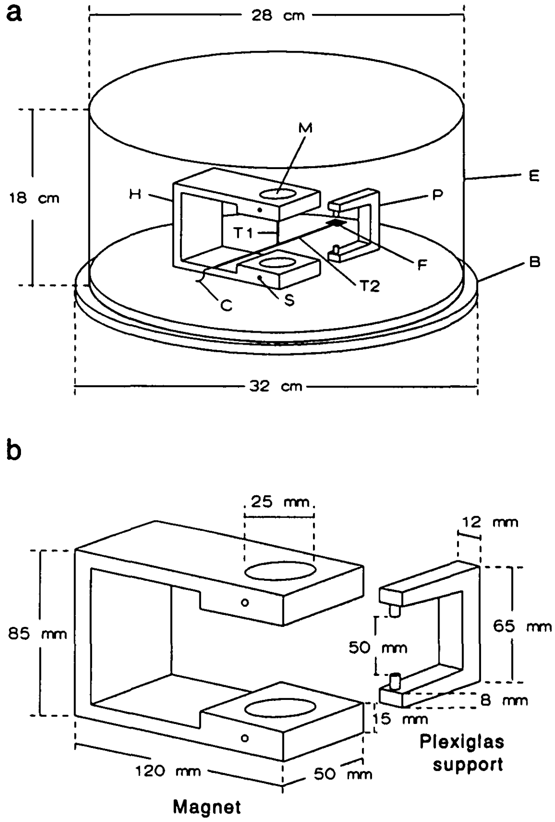

Fig. 1. Flight mill. (a) Each mill sits on a wooden base supporting a celluloid cylinder to limit drafts (see text for description and letters). (b) Dimensions of the magnet and Plexiglas support for the light-emitting diode and phototransistor.

Materials and Methods

Mill. A magnetic mount (Dybovskiy 1970) was used to minimize friction; to minimize weight, the arm was made from silica capillaries (McKibben 1985). In the description of the construction of the mill given below, the letters refer to those in the diagram of the mill (Fig. 1).

Horseshoe magnets of sufficient size and power are no longer commercially available, so the magnets were made by hand. The magnet consists of a strip of soft iron bent twice in the form of a horseshoe (H) (bending courtesy of S. Holmes of *The Speckled Band* public house). An additional piece of soft iron was welded at the end of each arm. A 25-mm hole was drilled through the arms near the end to accommodate a cylindrical magnet in a brass insulating sleeve (M). Care was taken to align the two holes exactly so that the magnets housed there formed a strong, straight, and vertical magnetic field. The magnets used are Magnetron, AlNiCo #5 magnets (Magni Power, Wooster, OH), rated at 3,000 oersted peak magnetizing force. To retain the magnets in place, a hole (2 mm diameter) was drilled and tapped in the end of each arm to take a setscrew (S). A steel no. 2 entomological pin suspended from the upper magnet forms the bearing.

A 2.5-cm piece of no. 1 GLC borosilicate micropipette (T1) was bonded to a piece of no. 2 micropipette (16 cm long) 7 cm from one end (T2) to form an extended "T." A decapitated no. 2 entomological pin was glued inside the stem (T1) so that the point extended 1 cm beyond the stem. A piece of aluminum foil (2 cm square) (F) was glued to the short end of T2 to interrupt the light beam. The leafhopper to be flown was glued at the pronotum to a coupling made of no. 2 gauge wire (C). The technique for attaching the wire was the same as used by Wayadande (1991) to attach gold wire tethers to leafhoppers for monitoring feeding. Once firmly attached to the insect's pronotum, the other end of the coupling was inserted into the free end of T2. The circle inscribed by a tethered insect is 57 cm.

Hocking (1953) and others have suggested that insects on a flight mill support their own weight when in flight, but some balancing may be necessary. This was accomplished by sliding the coupling in or out of the "T." The 5-gm mill assembly was suspended from the upper magnet (Fig. 1a).

The aluminum foil square interrupted an infrared light beam once each revolution. The principle employed is similar to that used in many security alarms. An infrared beam generated by a light-emitting diode was detected by an infrared phototransistor 5 cm below it. The diode and transistor were supported by the arms of a C-shaped Plexiglas support (P).

Each flight mill was attached to a circular base (B) supporting a clear plastic cylinder (E) to eliminate drafts (that might set the mill moving) or affect the behavior of the insect. The 16 mills were arranged in two rows of eight, in direct correspondence to 16 light-emitting diodes on the front of the monitor.

Electronics. The heart of the monitor was a 556 dual-timer integrated circuit (IC); this IC operates in one of two modes: monostable (suitable for timers, pulse detectors, and bounce-free switches) and astable (for pulse generation). We used it in both modes: one timer to generate the signal driving an infrared light-emitting diode, the other to detect the output from an infrared phototransistor.

The timer used as the signal generator (astable mode) has pins 2 and 6 (Fig. 2a; parts list in Table 1) connected so that the circuit triggers itself each timing cycle, thereby functioning as an oscillator. Capacitor C2 charges through resistors R1, R2, and R7 and discharges through R7. The resistance across R1 and R2 is 8.2 KX, sufficient to generate a 30-Hz square wave to the LED with a pulse length of 0.03 s, and off-cycle of 0.003 s. When a break in the infrared beam

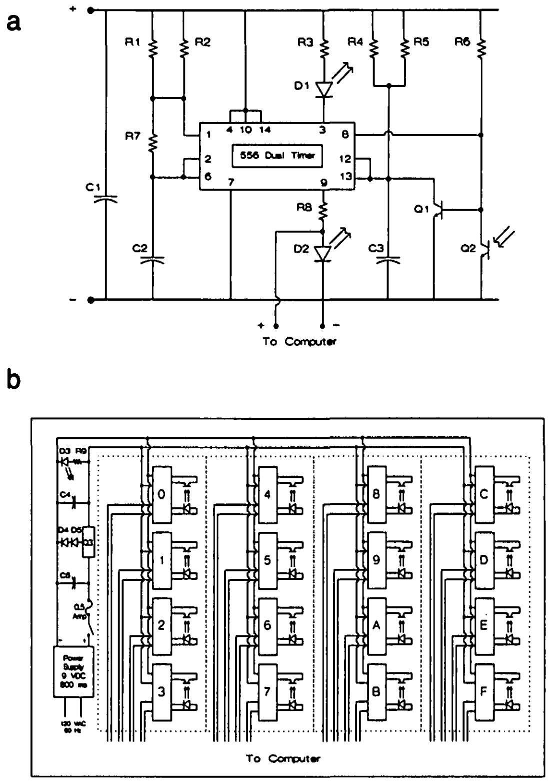

Fig. 2. Monitor circuit diagram. (a) A single channel uses a 556 dual-timer IC to generate a signal via D1 which is detected at Q2. While Q2 is receiving infrared light from D1, the voltage across the tell-tale light-emitting diode D2 is 0.67 volts DC; when the infrared beam is interrupted, the voltage across D2 goes to zero, which is detected by the computer, (b) Block diagram of the 16-channel monitor. The monitor has a 9-V DC power supply (to the left) and four printed circuit boards, each containing four channels (numbered from 0 to F) (see Table 1 for the parts list).

opens the circuit at the phototransistor (Q1), the detector circuit (monostable mode) detects the negative trigger pulse at pin 8. This turns off a transistor in the timer that otherwise shorts C3 to ground. Capacitor C3 charges through R4 and R5, and when the charge reaches 0.67 V, it discharges to ground and the output goes low for C3·R4·R5/(R4 + R5) s. The 46-KX resistance across R4 and R5 drops the output to the computer from 0.67 V to zero for 0.046 s. This period is long enough to ensure that the computer will "read" the signal but short enough to ensure that the voltage goes high again before a leafhopper can complete a rotation of the mill. For the computer to miss a break in the beam, the insect must fly at >20 revolutions per second. The trim resistors R2 and R5 were chosen to make this the limiting condition.

The electronics to generate the signal to the infrared emitter and process the return single were constructed in modular form: Fig. 2a shows

Table 1. Parts list, computer-monitored 16-channel flight mill

Symbol[a]	Quantity	Description
R1, R5	32	47 KΩ 0.25 watt resistor
R2	16	10 KΩ 0.25 watt resistor
R3, R8, R9	48	220 Ω 0.25 watt resistor
R4	16	2.2 MΩ 0.25 watt resistor
R6	16	220 KΩ 0.25 watt resistor
R7	16	1 KΩ 0.25 watt resistor
C1	16	0.1 μF 25-V ceramic capacitor
C2	16	4.7 μF 35-V capacitor
C3	16	1.0 μF 50-V capacitor
C4	1	4700 μF 35-V capacitor
C5	1	250 μF 25-V capacitor
D1	16	Infrared light-emitting diode
D2	17	Red light-emitting diode
Q1	16	2N3906 transistor
Q2	16	Infrared phototransistor
Q3	1	7805 voltage regulator
	16	556 dual timer IC
	16	IC sockets
	17	LED snap holders
	1	SPST switch
	1	0.5-amp fuse
	1	Fuse holder
	1	BUD cabinet TV 2155
	1	9-V 800-milliamp adapter

[a] See Fig. 2 for location.

the circuit diagram for one channel, and Fig. 2b is a block diagram of the monitor with its power supply. The monitor consists of four printed circuit boards, each with four infrared transmitting and receiving channels. Each channel is independent and operates in parallel. The telltale light on the front of the monitor is on when the infrared beam is unbroken and blinks off each time the infrared beam is interrupted by the passage of the aluminum foil.

Output from the monitor is fed by 16 grounded leads to a 16-channel multiplexor board (Qua-Tech, PXB-721 expansion board with ADM8-10 analog-digital converter [ADC]; Qua-Tech, Akron, OH) which can sample at up to 30 KHz. A much lower sampling rate is sufficient because the monitor's 0.046-s negative pulse output ensures that all 16 channels can be examined during one channel's negative pulse (equivalent to 350 Hz). This leaves ample computer cycles to record the revolution on the hard disk.

A less-expensive alternative to the ADC multiplexor is to attach the output directly to one of the computer's serial ports via a RS-232C connector. The disadvantage of this approach is that a maximum of 12 channels may be monitored, and the programming required is increased, because the multiplexing must be done in software. The parameters controlling the dual-timer IC were chosen to ensure that the system would work using a serial port. However, the low cost (<$300) of 16-channel analog-digital multiplexors for PCs normally will not be a limiting factor.

Software. The value of the input voltages are "read" by the multiplexor and placed in buffers accessible by a FORTRAN program. The program checks each buffer for a zero value (the light beam is broken), and if it finds a zero, the computer time and channel number are written to a file on the hard disk. At the end of each cycle, the program pauses before returning to the beginning to read the first buffer again. Each buffer is read at exactly 0.046-s intervals (21.7 Hz) to ensure that no channel is still zero from the previous read. On a 4.77-MHz PC, this is ample time to read and write all 16 channels before it is time to examine the ADC buffers again.

This program cycles continuously until it is interrupted by pressing the ESC key on the keyboard. At this point, the log file is reread and each channel analyzed. The time of every occultation on each channel constitutes the basic data. From these data, intermediate statistics are calculated: total number of revolutions and the duration of each revolution. From the intermediate data, the frequency distributions of flight speed, distance flown, and flight bout length are computed for each channel. All three levels of data are then stored as files and printed out, and the program terminates. A copy of the program is available from R.A.J.T. upon request.

Results

The reason for building this apparatus was to compare flight performance among several species of the genus *Dalbulus*. Some are thought to be migrants, whereas the majority are comparatively autochthonous. Our main interest was to ascertain whether their supposed migratory status could be supported by relative flight performance.

A previous study had indicated that flight is strongly crepuscular, probably the result of a balance in temperature and light thresholds for flight. As a preliminary exercise to test the equipment and to develop methods for later experiments, 96 *D. maidis* females were tethered to flight mills and recorded for 3 h. One group of 32 was left to fly in bright light (150 lux) to simulate daylight; another group was left in total darkness; and the third was given low light (30 lux) equivalent to crepuscular conditions. Fig. 3 shows the frequency distribution of distance flown in 3 h for the three groups.

Discussion

Tethering insects for flight mill studies entails both practical and theoretical problems. Kennedy & Booth (1963a, b) found that preflight handling of aphids increased their inclination to settle. Thus, any interference by the experimenter is likely to influence the results of flight mill studies by reducing the time spent flying. The

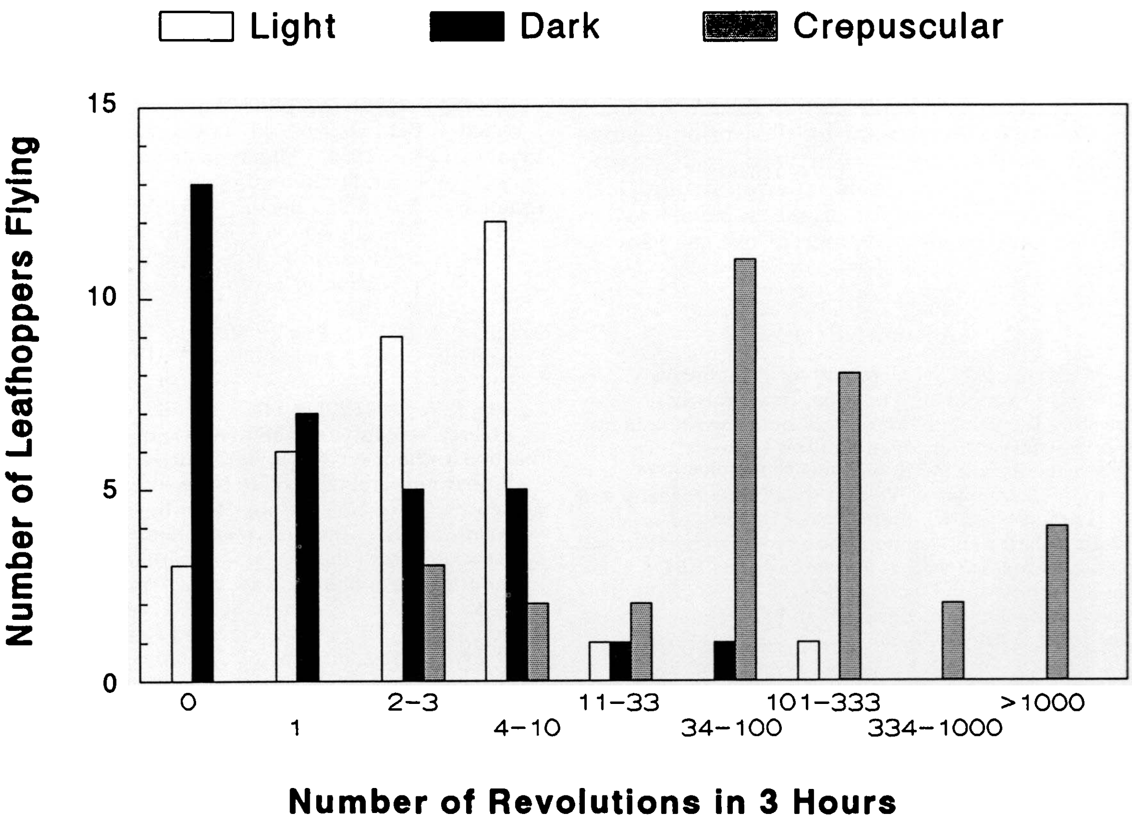

Fig. 3. Trial run of the flight mill. The frequency distributions of number of revolutions made by *D. maidis* females under different ambient light conditions confirm that this species is more likely to fly under crepuscular conditions.

smaller the insect, the more serious this is likely to be. Very small insects such as leafhoppers, may require more intrusive handling because of their size, whereas large, robust insects such as noctuid moths or scarab beetles are handled easily and offer a large attachment area. Another potential difficulty with flying leafhoppers on flight mills, which merits further work, is the actual process of takeoff. Leafhoppers take off by jumping, and a jump may or may not be converted to a flight. Thus, the wing opening response to loss of tarsal contact (Johnson 1969) may be an oversimplification for these insects.

One of the questions we hope to answer is whether *D. maidis* will fly for prolonged periods, sufficient to class it as a long distance migrant capable of crossing the Gulf of Mexico (Bradfute at al. 1981), or to fly from frost-free overwintering sites at low elevations in Mexico to high-elevation maize fields in spring (Nault 1990, Larsen 1991).

Once the practical difficulties of mill design and insect attachment have been overcome, the problem of interpreting flight mill results still exists. By comparing the performance of two or more experimental groups of comparable size, the problem of behavioral interference may be overcome partially (Rowley et al. 1968). As our preliminary results demonstrate, the distribution of flight tim of three groups subjected to different light conditions clearly are different. These preliminary results are consistent with our earlier observation that flight activity is crepuscular. The distances flown, as recorded by the computer, underestimate the true capabilities of all three groups. However, the shapes of the distributions are likely to be close to true. The relative differences between the distributions may be influenced by tethering only if there is an interaction between being tethered and the flight response to light, which seems improbable.

The recorded distances flown by insects flying on a flight mill are underestimates because the insects must first accelerate the arm up to speed (overcome the moment of inertia) when they initiate flight, and then overcome the friction and air resistance of the arm to which they are attached while in flight. The loss caused by these effects is called the parasitic drag. If the parasitic effects are too great, the insect may never succeed in initiating flight, or give up long before any normal threshold for quitting flight is exceeded. If they do fly, insects flying on a mill will consume fuel faster and fly slower than normally.

If necessary, corrections can be calculated to improve the estimate of flight speed and range (Hocking 1953, Chance 1971). Despite these limitations, however, replicated flight mills remain an important instrument for measuring relative flight performance of even very small insects. This is especially true for species such as leafhoppers, whose erratic flight behavior makes flight bout length and interval critical parameters.

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