

Assessing Insect Flight Behavior in the Laboratory: A Primer on Flight Mill Methodology and What Can Be Learned

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Abstract

Dispersal is a key component in the population ecology and dynamics of insects and remains one of the most difficult and intractable ecological processes to study in the field. As a consequence, many researchers have looked to laboratory methods for investigating the myriad factors that govern and impact an insect's ability to move within its environment. A key tool in this effort since at least the early 1950s has been the insect flight mill. Nearly 260 studies have been published using flight mills covering 214 species in 61 families and 9 orders. This review explores the methodology and technology of tethered flight in insects using flight mills. The goal is to provide the reader with a historical context of the approach, an understanding of the available tools and technology, background on how best to apply these tools through a comparative lens, and to summarize the wide breadth of factors that have been explored to further our knowledge of insect flight behavior. Overall, it is hoped that the interested reader will understand the limits and benefits of flight mills and will know where to find the resources, and perhaps collaborators, to pursue this line of study.

Key words: flight mill, tethered flight, comparative experiment, flight assay, automation

Dispersal is a key component in the population ecology and dynamics of insects, and understanding this process impacts our ability to progress in a wide array of areas such as population genetics, biogeography, biodiversity, invasion biology, and integrated pest management. Despite many advances in methodology and analyses (Hardie 1993, Reynolds et al. 1997, Turchin 1998, Hagler and Jackson 2001, Reynolds and Riley 2002, Carriere et al. 2012), insect dispersal by flight remains one of the most difficult and intractable ecological processes to study in the field. It is perhaps no surprise then that many researchers have moved to the laboratory in an attempt to investigate the myriad factors that govern and impact an insect's ability to move within its environment.

A search of the literature using the key words 'tethered flight' or 'flight mill*' and examination of several recent reviews (Hardie 1993, Reynolds et al. 1997, Reynolds and Riley 2002, Minter et al. 2018) identified over 400 scientific studies in the peer-reviewed literature that employed tethered flight systems for the study of insect flight behavior in the laboratory (Fig. 1). Since the earliest studies in the 1940s and 1950s, there has been an exponential increase

in activity, but prior to Minter et al. (2018), there has never been a general review of tethered flight. These authors briefly described methodology and discussed the general advantages and limitations of tethered flight for learning about, and predicting, migratory flight behavior, and physiology (Kennedy 1975, Dingle 2014) in the field. Minter et al. (2018) also summarized many of the key findings related to potential biotic and abiotic factors that can impact insect flight behavior generally and migration specifically. The goal of this Special Issue was to highlight a few of the many tools available to entomologists, biologists, and ecologists for the study of insect behavior and biological pest control. In that spirit, this review builds and extends upon that of Minter et al. (2018) by exploring in more detail the methodology and technology of tethered flight in insects, with specific emphasis on insect flight mills. The goal here is to provide the reader with a brief history of the approach, an understanding of the available tools and technology of the trade, and some background on how best to apply these tools to further knowledge of insect flight behavior more broadly. Hopefully, the reader will learn where to find the resources, and perhaps collaborators, to pursue this line of study.

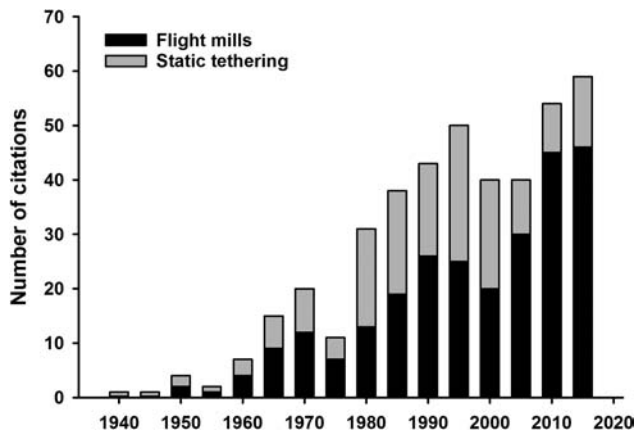


Fig. 1. Distribution of citations over time from the peer-reviewed literature on the use of active flight mills, and static tethered flight to study insect flight behavior, physiology, and mechanics.

Study of Insect Flight in the Laboratory

Scientists have been using laboratory-based systems to study and understand insect flight behavior, flight performance, and the physiology and mechanics of flight for many decades. Two fundamental approaches have been used. In one approach, the insect is allowed to fly freely within some sort of confined space where various observations and measurements can be made (Fig. 2). An example of such an approach is the vertical flight chamber (Kennedy and Booth 1963, David and Hardie 1988, Blackmer and Phelan 1991, Blackmer et al. 2004). Here, the flying insect circles upward toward a light cue (like a moth to a porch light) and an adjustable, downward laminar flow of air is used to counterbalance the lift generated by the insect. As a result of these competing downward and upward forces, the insect ends up flying in a horizontal circle in the center of the flight chamber. Over time, the downward airflow may need to be adjusted as the insect increases or decreases its lift to keep it flying in a consistent level plane. Parameters such as takeoff propensity, lift generated, flight duration, periodicity, and other variables can be measured. The system has typically been used for small insects, such as aphids, whiteflies, and small beetles. Gatehouse and Hackett (1980) suggest that it may not be generally amenable to stronger and faster flying insects due to issues with operator response time and airflow control. Automation is challenging, but some degree of mechanization is possible (David and Hardie 1988) and video can capture results that can be viewed and processed later (Byrne 1999). Recently, Stowers et al. (2017) describe a virtual reality free-flight arena where the insect can be presented with a realistic visual environment and movement is tracked with a series of motion-tracking video cameras. Wind tunnels are other common tools for the study of insect flight, although they are generally used to study the responses of insects to semiochemicals (Miller and Roelofs 1978) rather than flight behavior per se. The distinct advantage of free flight systems is that the insect is unencumbered by a tether or a restrictive flight path, allowing researchers to capture more realistic behavior. However, this approach also tends to be more labor intensive and generally fewer insects can be observed over shorter periods of time.

The second approach involves physically constraining the insect with a tether, a method that has been widely applied for many decades. Tethered flight can be categorized into two general approaches, static or active. In static tethered flight, the insect is typically attached to a rigid or flexible tether, the other end of which is fixed to some sort of stationary platform. When the insect flies, its

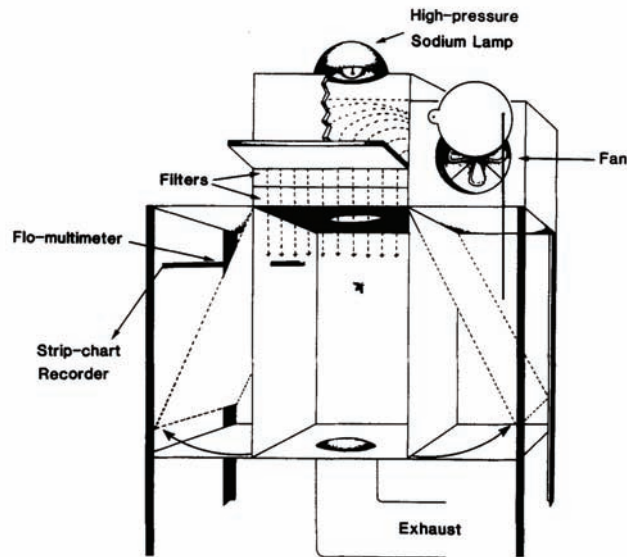
position remains fixed relative to the point of attachment—that is, it flies in place. The tethered insect might be placed in a wind tunnel or chamber, or simply on a laboratory bench where it can be observed and measured for various mechanical, physiological, and/or behavioral properties (Fig. 3). The insect is generally induced to fly once its tarsi are no longer in contact with the substrate; but often, an airstream is provided to the insect to further motivate flight (e.g., Dingle 1966, Brown 1972, Rankin and Rankin 1980). The static tethering approach has been, and continues to be, used widely. The main advantage is simplicity. Very little specialized equipment or construction is needed. It can be done virtually anywhere and is amenable to a variety of investigations such as the study of respiration, where the insect needs to be inside a sealed chamber so gas flow can be tightly regulated (e.g., Nachtigall et al. 1989, but see Lebeau et al. 2016). Static tethering also enables the measurement of wing beat frequency, which can be used to estimate velocity (Duistermars et al. 2007). A simple modification, in which the static tether arm rotates tightly around its attachment point, may allow the assessment of orientation relative to a sun compass (Mouritsen and Frost 2002). One of the earliest tethering systems described by Krogh and Weis-Fogh (1952) was somewhat of a hybrid between static and active. With this device, insects are statically tethered around the circumference of a large compressed air powered wheel that spins enough to overcome any drag and allows estimation of the speed the insect could fly untethered. The obvious disadvantage of static systems is that data collection is difficult to automate. Video can be taken; but generally, like free flight systems, an observer must be present to watch and record the insect's behavior. Other limitations are that metrics such as flight distance and velocity cannot be measured (but see Duistermars et al. 2007) and that study assays are generally shorter in duration.

Insect Flight Mills

The second type of tethered flight is the active approach or the flight mill, and this will be the focus of the remainder of this paper. I include flight balances here because the insect still moves a flight arm and such systems offer many of the same advantages as rotary mills in terms of automation. Flight mills have been used to study insect flight behavior and performance since the early 1950s and interest appears to be growing at an exponential rate with nearly 260 studies published (Fig. 1) since the pioneering work of Hocking (1953). In an active system, the tethered insect moves either in a circular horizontal plane (rotary system) or in a vertical plane about a central axis (flight balance). In a few systems, the insect moves both vertically and horizontally (Ribak et al. 2017, Barkan et al. 2018) requiring the insect to generate lift as well as forward momentum. On both rotary and flight balance systems, the lack of tarsal contact induces flight, and once flying the perceived flow of air over the body in rotary systems may reinforce continued flight activity.

The principles and the basic components of a flight mill are relatively straightforward (Hocking 1953, Chambers and O'Connell 1969, Cooter and Armes 1993). The insect is mounted to a tether that is simple to attach and adjustable. This tether, in turn, is attached to a lightweight and aerodynamic arm that rotates about an axle with minimal friction. In automated systems, a flag attached to the arm or a plate centered below the axle is used to trigger an electronic sensor that ultimately counts the rotation of the arm or logs the upward movement of the arm in a balance system. Hocking (1953) is generally credited with designing the first flight mill, but over a dozen papers have since been published with the sole purpose

(A)



(B)



Fig 2. (A) Free flight chamber in which the insect flies toward a skylight cue and with a controlled and measurable downward laminar draft resulting in the subject flying in a horizontal circle within the chamber (Kennedy and Booth 1963, Blackmer and Phelan 1991). (B) Free-flight arena that projects realistic landscapes and monitors insect flight with video-tracking software (Stowers et al. 2017). All images used with permission.

of describing the design of flight mill systems, and another three dozen papers or so have described ‘original’ designs as part of the scientific study undertaken (Table 1; Supp Table 1, Appendix A [online only]). The term original is used somewhat loosely because most of these are simply tweaks and modifications of earlier designs made to suit the need of the species under investigation and/or to introduce new technology, particularly that related to the automation of data collection. Note that in Supp Table 1 (online only), attempts have been made to credit the original designer when study authors have cited derivative work. Suffice to say, the diversity of systems used, and in use today, is nearly as great as the diversity of investigators using them. Necessity has been the mother invention (see Figs. 4–7 for a range of examples). Several flight mills have even been commercially produced and used over the years including one by W.R. Douglas (Riverside, CA; no longer in business) (Kishaba et al. 1967, Flint et al. 1975; Fig. 4) and another by Jiaduo Industry & Trade Co., LTD (Hebi, China, see Zhao et al. 2011, Chen et al. 2015, Yang et al. 2017). One other company (Crist Instrument Company,

Hagerstown, MD) manufactures a design apparently copied after Jones et al. (2010), but I am unaware of any study using these prohibitively expensive mills. In the following sections, more detailed information on flight mill construction, electronics, tethering, experimental design, and analysis will be discussed.

General Flight Dynamic Considerations

It goes without saying that flight mills do not mimic nature, and thus have limitations relative to the interpretation of results and their extrapolation to the field (Minter et al. 2018). First, in most rotary systems, the insect does not need to generate lift in order to fly because it is already suspended in a level plane. However, it does need to generate enough power to overcome the initial inertia of the flight arm, and the ensuing aerodynamic drag caused by bearing friction and resistance to the cross-sectional area of the arm (Hocking 1953, Rowley et al. 1968, Chambers and O’Connell 1969, Chance 1971, Taylor et al. 1992, Cooter and Armes 1993). Lift and drag are often thought to cancel one another energetically.

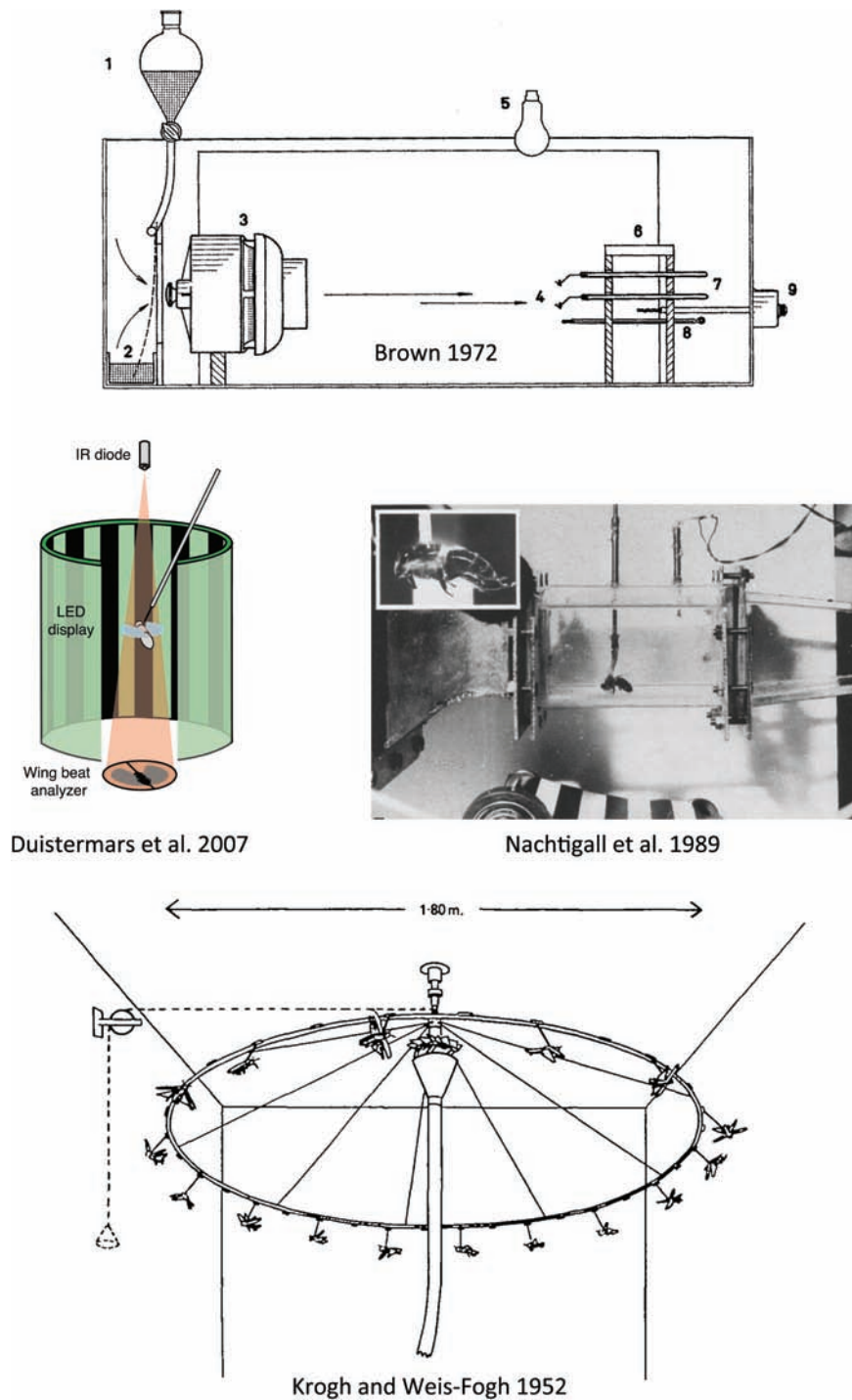


Fig. 3. Examples of static tethered flight systems for measuring such aspects as respiration, wing beat frequency and general flight activity. The Krogh and Weis-Fogh device is somewhat of a hybrid between static and rotary systems. With this device insects are statically tethered around the circumference of a compressed air powered wheel that spins enough to overcome any drag and allows estimation of the speed the insect could fly untethered. All images used with permission.

However, the circular motion of the flight path potentially generates additional energetic demands as the insect attempts to fly in a linear path and is, thus, constantly fighting against being forced to fly in a circular path (Ribak et al. 2017). In the roughly dozen studies that have compared flight speed from flight mills to free flight (see Supp Table 1 [online only]), most have shown lower velocities on flight mills (e.g., Atkins 1961, Niehaus 1981, Tsunoda and Moriya 2008, Maharjan and Jung 2009, Taylor et al. 2010,

Ribak et al. 2017), suggesting these additional energetic costs are real. The reader is referred to Ribak et al. (2017) for an excellent discussion of the aerodynamic issues and recommendations for minimizing some of them, by for instance, banking the orientation of the insect relative to the radial plane of the flight arm. In vertical balance systems, the insect must generate lift to remain aloft, but issues associated with drag, aerodynamics, and turning angles are less important or absent.

Table 1. Selected summary of the evolution of flight mill design and technology

Citation	Flight mill type	Flight path (m)	Arm	Bearings	Sensor	Software/hardware	Notes
Hocking (1953)	Rotary w/ landing platform	0.5–2	Feeler gauge steel	Glass	Photo cell	Chart recorder	Original design (Fig. 4)
Smith and Furniss (1966)	Rotary	1	Glass rod	Glass	Photo cell	Event recorder	Original design
Kishaba et al. (1967)	Rotary	Unspecified	Feeler gauge steel	Teflon	Photo cell	Event and chart recorders	W.R. Douglas, Riverside, CA (Fig. 4)
Rowley et al. (1968)	Rotary	1	Stainless spring steel	Jewel	Photo cell	Event and chart recorders	Original design (Fig. 4)
Chambers and O'Connell (1969)	Rotary	0.88–1.44	Feeler gauge steel, flat brass	Magnetic	n/a	Visual observation	Original design
Dybovskiy (1970)	Rotary	Unspecified	Plant straw	Magnetic	n/a	Visual observation	Original design
Schoenleber et al. (1970)	Rotary	2	Balsa wood	Jewel/magnetic or magnetic	Photo cell	Chart recorder	Original design
Chambers et al. (1976)	Rotary	0.63–1	Stainless steel hypodermic tubing	Teflon bearings w/ magnetic levitation	Infrared	Chart recorder	Original design (Fig. 4)
Gatehouse and Hackett (1980)	Vertical balance w/ landing platform	n/a	Stainless steel hypodermic tubing	Steel	Infrared	Event recorder; electronic interface added later by Barfield et al. (1988)	Original design (Fig. 7)
Ruzicka (1984)	Rotary, rotary w/ landing platform	1	Stainless steel wire	Steel	Photo cell	Chart recorder	Original design
McKibben (1985)	Rotary	0.96	Glass rod	Glass	Photo cell	Custom BASIC program, custom I/O interface	Original design, Original design (Fig. 7)
Wales et al. (1985)	Vertical balance w/ landing platform	n/a	Stainless steel hypodermic tubing	Steel	Photo cell	Chart recorder	Original design (Fig. 7)
Resurreccion et al. (1988)	Rotary	1	Aluminum	Jewel	Infrared	Custom program and I/O interface	Original design
Taylor et al. (1992)	Rotary	0.57	Glass rod	Magnetic	Infrared	Custom Fortran program, custom I/O interface	Original design (Fig. 5)
Cooter and Armes (1993)	Rotary w/ landing platform	1.49	Alloy	Glass	Photo cell	Chart recorder for remote work, Clarke et al. (1984) electronics	Original design (Fig. 5)
Weber et al. (1993)	Rotary	1	Stainless steel hypodermic tubing	Teflon bearings w/ magnetic levitation	Infrared	Custom BASIC program, custom I/O interface	Original design (Fig. 5)
Beerwinkle et al. (1995)	Rotary	1.92	Stainless steel hypodermic tubing	Teflon bearings w/ magnetic levitation	Infrared	Custom C program, custom I/O interface	Original design (Fig. 6)
Cheng et al. (1997) (Chinese, English abstract)	Rotary	Variable	Copper wire	Magnetic	Infrared	Custom software	Original design (Fig. 5)
Schumacher et al. (1997)	Rotary	0.7	Plastic drinking straw	Magnetic	Infrared	Custom software, I/O interface	Original design (Fig. 5)
Dubois et al. (2009)	Rotary	1.5	Aluminum	Steel	Unspecified	Tachometer and magnets	Original design
Jones et al. (2010)	Rotary	0.95	Stainless steel hypodermic tubing	Teflon bearings w/ magnetic levitation	Infrared	Custom LabView software, commercial I/O interface	Original design (Fig. 6)

Table 1. Continued

Citation	Flight mill type	Flight path (m)	Arm	Bearings	Sensor	Software/hardware	Notes
Zhao et al. (2011)	Rotary	0.58	Unspecified	Steel	Infrared	Unspecified	Jiadoo Industry & Trade Co., LTD, Hebi, China, Original design
Lopez et al. (2014)	Rotary	0.95	Steel	Steel	Infrared, slotted disk for velocity	Custom Labview program	Original design
Attisano et al. (2015)	Rotary	0.75	Stainless steel hypodermic tubing	Magnetic	Infrared	WinDAQ software (free w/ purchase of interface board), Python program for analysis	Original design
Jones et al. (2015)	Rotary	0.5	Twisted wire	Magnetic	Infrared, striped disk for velocity	Unspecified	Original design
Marti-Campoy et al. (2016)	Rotary	1–1.5	Carbon rod	Steel	Infrared	Custom C++ program, commercial I/O interface	Original design
Hahn et al. (2017)	Rotary	n/a	n/a	n/a	Infrared	DASylab Basic program, Arduino board interface, High-speed video	Rowley et al. (1968)
Ribak et al. (2017)	Rotary w/ vertical lift	1.25	Carbon rod	Steel	n/a		Original design (Fig. 7)

Magnetic bearings: axle of flight arm supported between two magnets, resting on one end, in general, not touching the second (see Figs. 4 and 5); teflon bearings with magnetic levitation = axle of flight arm spins within a teflon rod levitated by two opposing magnets (see Fig. 6)

Flight Mill Arms

A wide range of materials have been used to construct the main rotating arm of flight mills (Table 1; Supp Table 1 [online only]), including plastic soda straws (Schumacher et al. 1997), glass capillary tubes (Atkins 1961, Smith and Furniss 1966), balsa wood (Schoenleuter et al. 1970, Nilssen and Anderson 1995, Tsunoda and Moriya 2008), spring or feeler gauge steel (Hocking 1953, Koerwitz and Pruess 1964, Rowley et al. 1968), flat brass or aluminum (Chambers and O'Connell 1969, Resurreccion et al. 1988, Dubois et al. 2009), copper wires (Cheng et al. 1997, Wong et al. 2018), carbon rods (Bradley and Altizer 2005, Lopez et al. 2014, Barkan et al. 2018), and stainless steel hypodermic tubing (Chambers et al. 1976, Wales et al. 1985, Beerwinkle et al. 1995, Jones et al. 2010). Unusual materials like cereal stems, bamboo, and guitar wire have even been used (Dybovskiy 1970, Stewart and Gaylor 1994, Moriya 1995). The most commonly used material for arm construction has been stainless steel hypodermic tubing followed distantly by glass, copper wire, and balsa wood. The advantages of hypodermic tubing are low mass, high strength, uniformity, and availability in a variety of sizes. Carbon rods have been used since around 2005 and offer some of the same advantages. Regardless of material, the main consideration is mass and striking a balance between strength and the size and power of the test insect. Keeping the mass of the entire rotating assembly, which includes the arm and the axle, to a minimum is of paramount importance for aerodynamic reasons noted above. Thus, low arm mass, along with near frictionless bearings (see below), is critical. Arm length can vary considerably, and this impacts the mass as well as the total circumference of the flight path. The interaction between insect size and flight path length also influences the turning angle, and thus, the additional drag imposed by these turning angles (Ribak et al. 2017). Flight paths of about 1 m are most typical, but studies have varied from as small as 0.2 m (Henson 1962) to as large as 4.27 m (Bradley and Altizer 2005). Often, the choice of size appears to be dictated more by the available space than careful consideration of the specific needs of the insect. This further emphasizes the need to focus on the comparative approach in experimental design (see below). There has been very little research to investigate the importance of arm size. Chambers and O'Connell (1969) and Chambers et al. (1976) compared various arm sizes providing flight paths ranging from 0.63 to 1.44 m and found essentially no difference in multiple metrics of flight performance of tephritid fruit flies. They suggested that this demonstrated friction had been properly minimized in the axle bearing, but they did not examine any aerodynamic properties or insect energetics. Identifying the proper balance between insect and flight arm size is perhaps worthy of additional study.

It is important to counterbalance the arm for the mass of the insect in order to ensure smooth operation and prevent the arm from tipping and creating additional drag, friction, and lift that the insect must overcome. This is typically accomplished by attaching mass to the opposite end of the arm equal to the mass of the insect and the tethering apparatus (see below).

Axle Bearings

Equally important to minimizing the mass of the arm assembly is minimizing the friction generated by the axle on which the arm is mounted. Fewer options have been applied to this aspect of design (Table 1; Supp Table 1 [online only]). Magnetic bearings, first introduced by Chambers and O'Connell (1969), have been the most widely used both in the past and presently. In this approach, a ferrous axle (typically a double-pointed pin) rests on a horizontally

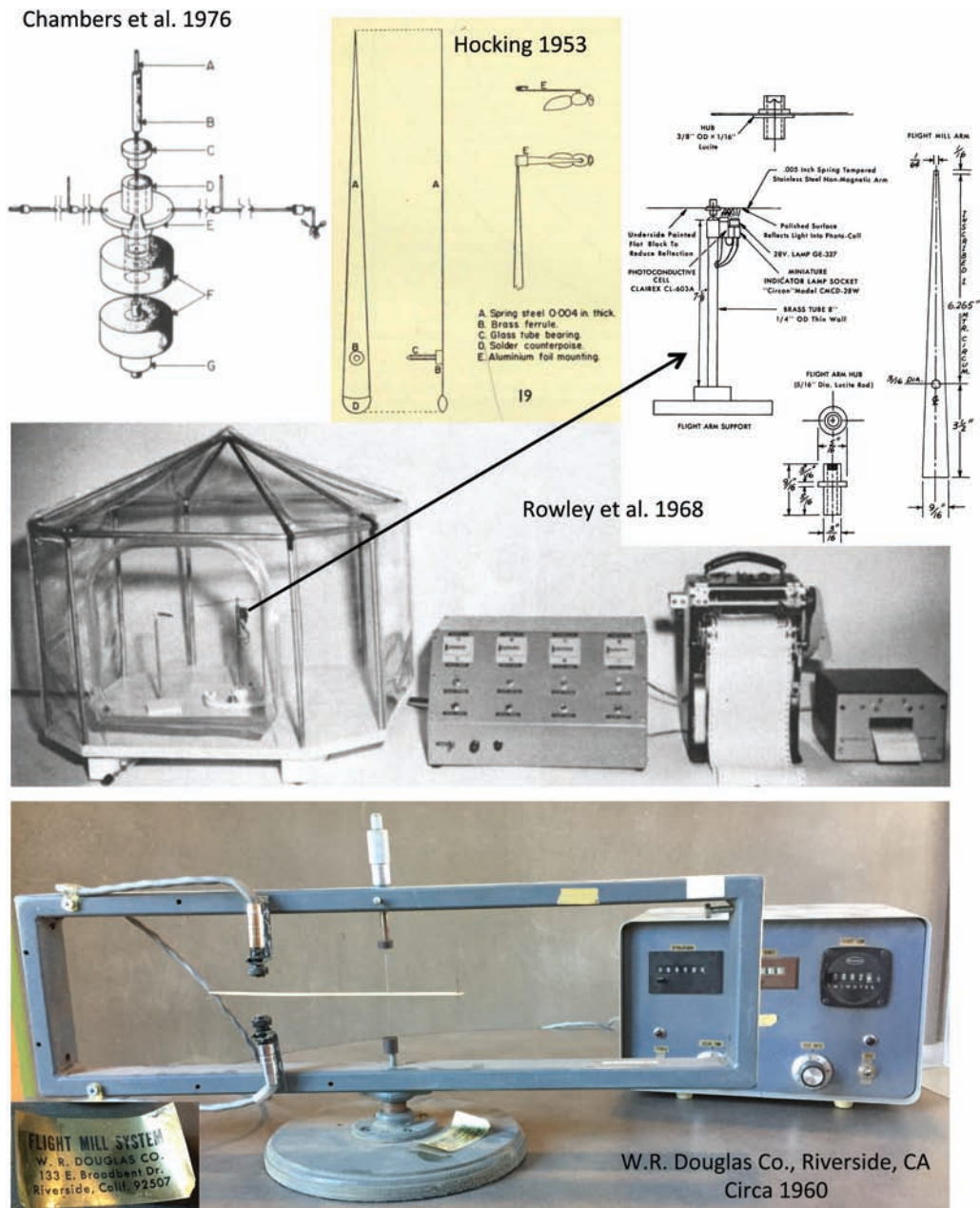


Fig. 4. Some early rotary flight mills that many scientists used or modified for flight behavior studies. These were developed at a time when computer technology was limited and analog recording of data was generally required. Hocking (1953) was one of the first to introduce the concept of a rotary flight mill and many subsequent designs cited and used his general ideas. All images by author or used with permission.

oriented magnet and a second magnet at the other end of the axle generates enough force to keep the axle vertical without actually touching the axle. As only one tiny contact surface (point of the pin) is touching one of the magnets, friction is greatly minimized (see Figs. 4 and 5). As the mass of the arm assembly and the insect mass and strength increases, so does the size and strength of the magnets needed in order to keep the arm parallel to the substrate once the insect is mounted and flying (Taylor et al. 2010). Jewel or glass bearings, introduced by Hocking (1953), have been the next most popular option, especially in early flight mill designs. Here, the axle pin rotates on the slick surface of glass or a jewel such as sapphire. Highly machined steel or ball bearing designs also have been popular over time. A few designs have employed commercially

available anemometers (ball-bearing based) as the rotation platform (Brown et al. 2017, Ribak et al. 2017, Barkan et al. 2018). A final common design, introduced by Chambers et al. (1976), employs an axle spinning inside a Teflon rod coupled with opposing magnets that levitate the arm assembly (see Figs. 4 and 6). Steel, ball, and levitated Teflon bearings work well for larger and more powerful flyers, because the axle is physically connected to the base. All of these approaches presented achieve the goal of stabilizing the arm assembly while also reducing friction. However, as the size (and power) of the insect declines, low arm mass and friction increasingly become more critical and magnetic bearings appear to be the more optimal choice in these situations. With larger and more powerful flyers, the options are broader.

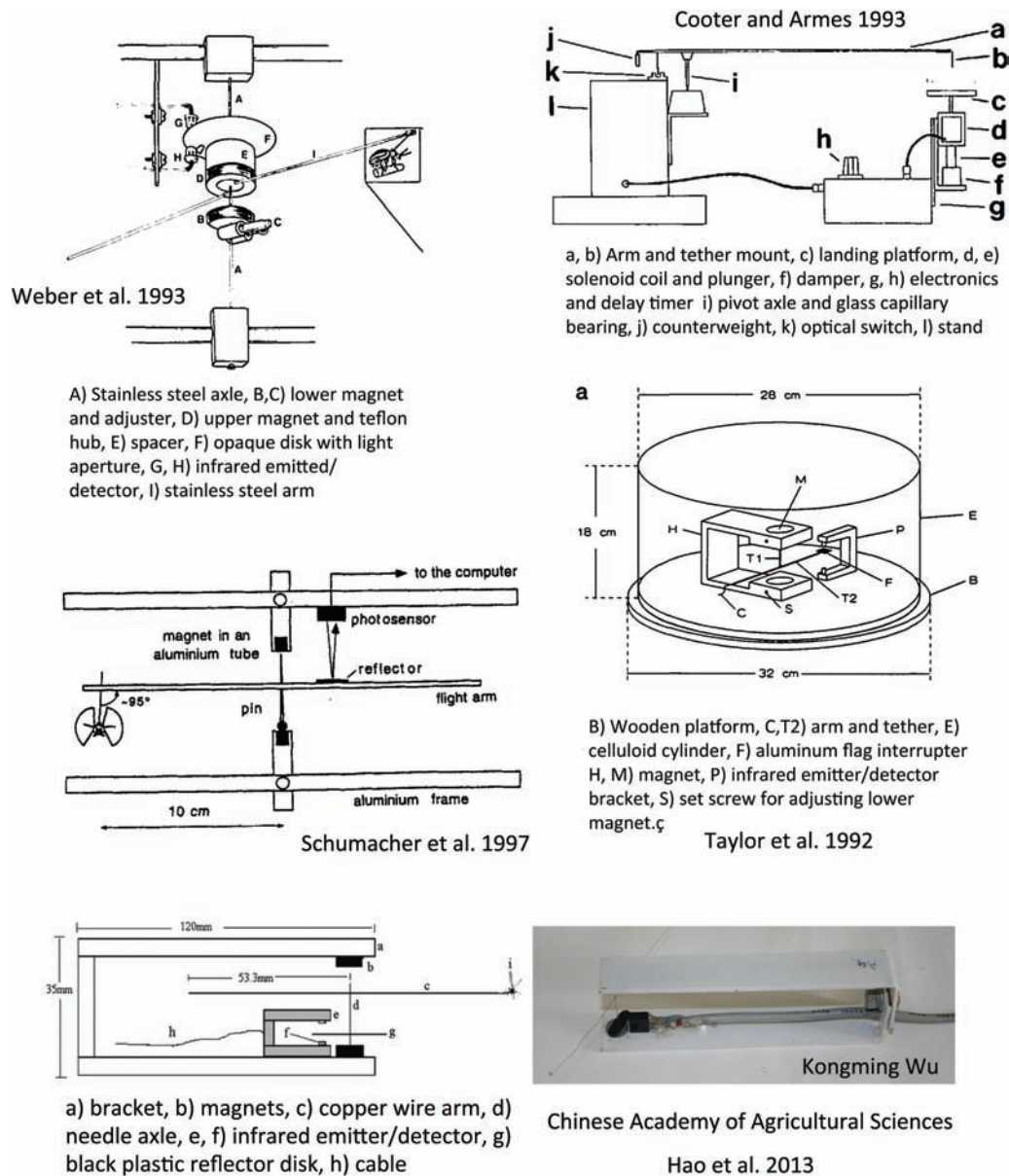


Fig. 5. More advanced rotary flight mill designs that include computer interfaces to collect, store and sometimes process the flight data. Represented are designs that many scientists have cited, used and modified for their own needs. The [Cooter and Armes \(1993\)](#) design is somewhat unique in that it provides a moveable landing platform for the insect when it ceases flying. The CAAS design has been used in a number of studies by Chinese scientists and others, and is designed for flying very small insects like aphids and minute parasitoids. All images used with permission.

Landing Platforms and Visual Cues

In the vast majority of flight mill designs, the insect remains suspended above the substrate during the flight assay. While this is clearly an unnatural situation, the lack of tarsal contact with a substrate assists in initiating flight. Likewise, the lack of tarsal contact also might inhibit cessation of flight and lead to biased estimates of flight performance. Sometimes the insect is manually provided a resting platform at the initiation of or during the assay (e.g., [Hocking 1953](#), [Green 1962](#), [Gmeinbauer and Crailsheim 1993](#)). Vertical balances allow landing platforms to be more easily integrated into the design; nonetheless, some intriguing approaches have been used. [Gatehouse and Hackett \(1980\)](#) devised an elaborate design wherein the platform drops away as the insect takes flight and returns when the insect ceases flying (see [Fig. 7](#)). The platform mechanism employed oil dampers for smooth operation and this mechanism was later simplified by [Parker and](#)

[Gatehouse \(1985\)](#). [Wales et al. \(1985\)](#) modified the Gatehouse design by providing a rotating landing platform that contained an oviposition substrate to allow the simultaneous measurement of flight and oviposition of a noctuid moth. The original rotary design of [Hocking \(1953\)](#) deployed a manually engaged landing platform where the insect was allowed to rest and feed periodically during an assay. This concept was automated in a complex design by [Cooter and Armes \(1993\)](#), where an electronic solenoid was triggered to raise or lower a platform via the decreased or increased speed of the insect's flight, respectively (see [Fig. 5](#)). To my knowledge, the effect of the presence or absence of a landing platform on insect flight behavior has not been examined experimentally.

The use of visual cues to provide the insect with a sense of motion on flight mills appears to be rare. In some studies, it was noted that visual cues were not provided, but in the vast majority of studies,

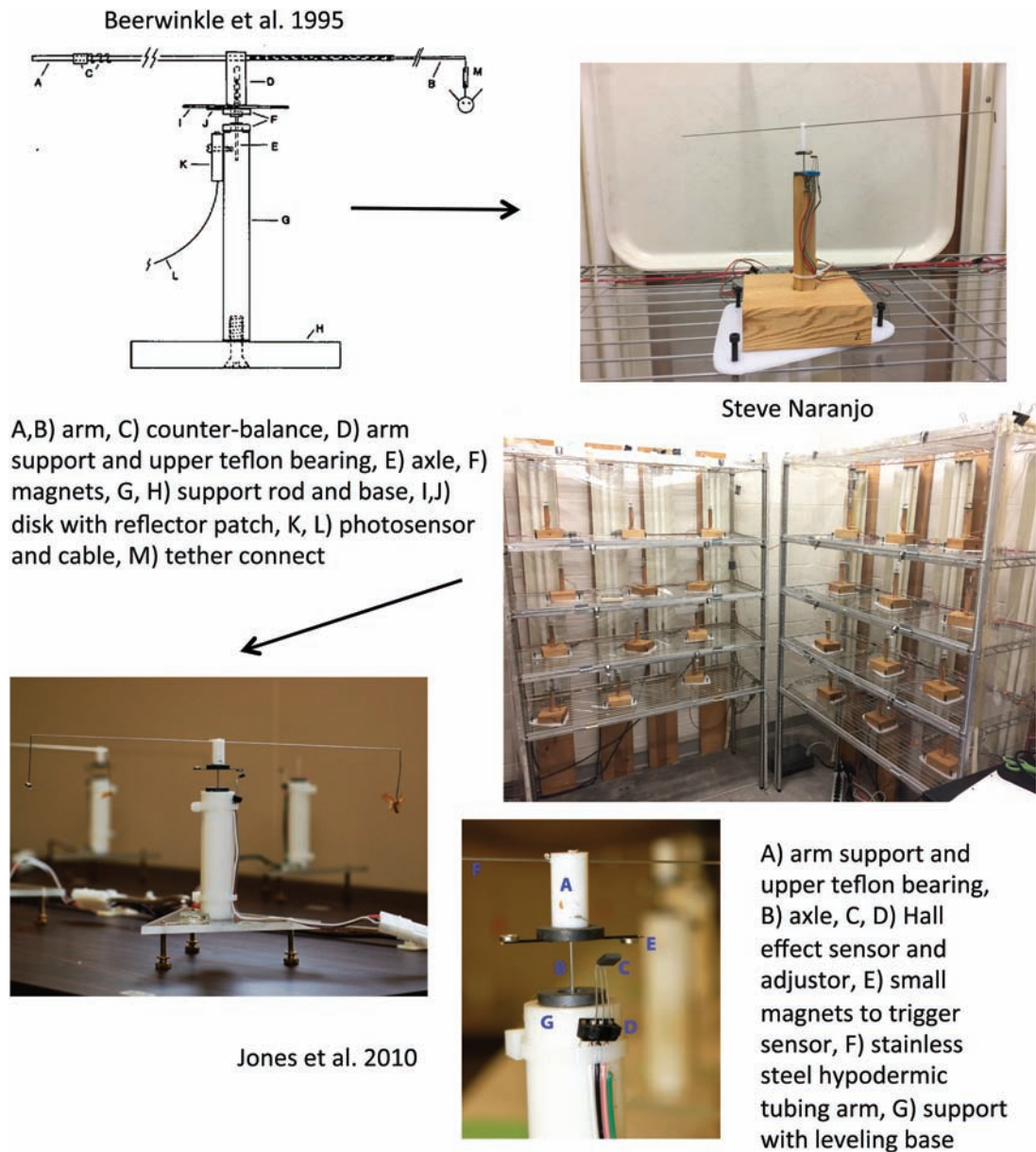


Fig. 6. The evolution of design in a flight mill system initially introduced by Beerwinkle et al. (1995). Material selection can be flexible, simple, and align with the users background skill set. For example, Naranjo is a woodworker and used 2 x 4s and wooden dowels. Complete plans for the more advanced version along with a parts list and downloadable software is provide by Jones et al. (2010). All images by author or used with permission.

such cues were never mentioned, so it is difficult to know if they were deployed or not. When provided, they are typically composed of either contrasting patterns (e.g., pinwheel) on the floor of the mill (Stanfield and Hunter 2010, Evenden et al. 2014), on walls surrounding the mill (Gmeinbauer and Crailsheim 1993, Villacide and Corley 2008, Wells et al. 2016), or both the floor and walls (Barkan et al. 2018). Visual cues would be unimportant for assays performed on nocturnal insects that are generally only flown in the dark (e.g., Sappington and Showers 1991). If visual cues are important, this could influence, in part, the decision on whether to employ a rotary or flight balance system for diurnal assays as it would be difficult to provide such cues for the latter. Some study authors noted that they provided some sort of air stream stimulus (Jutsum and Goldsworthy 1974, Shelton et al. 2006, Wong et al. 2018). Other studies have examined the role of chemical cues, but more with the

goal of understanding the effects of semiochemicals on flight behavior (Borden and Bennett 1969, Roitberg et al. 1984, Stelinski et al. 2014), and not whether they are important to flight initiation or maintenance. Overall, there has been no study of whether or not visual, airflow, or chemical cues are an important element of the flight assay. It is likely that in most cases, the background of the room or chamber in which the flight mill is placed automatically provides some visual motion cues, and often, mills are placed within enclosures to minimize distracting airflow or chemical cues.

Sensors, Electronics, and Software

Some early studies (Atkins 1961, Dybovskiy 1970, Heinrich 1971, Baker et al. 1980) and even some more recent ones (Vogt et al. 2000, Shelton et al. 2006, Stelinski et al. 2014) have used simple visual observations to measure flight metrics. However, most flight

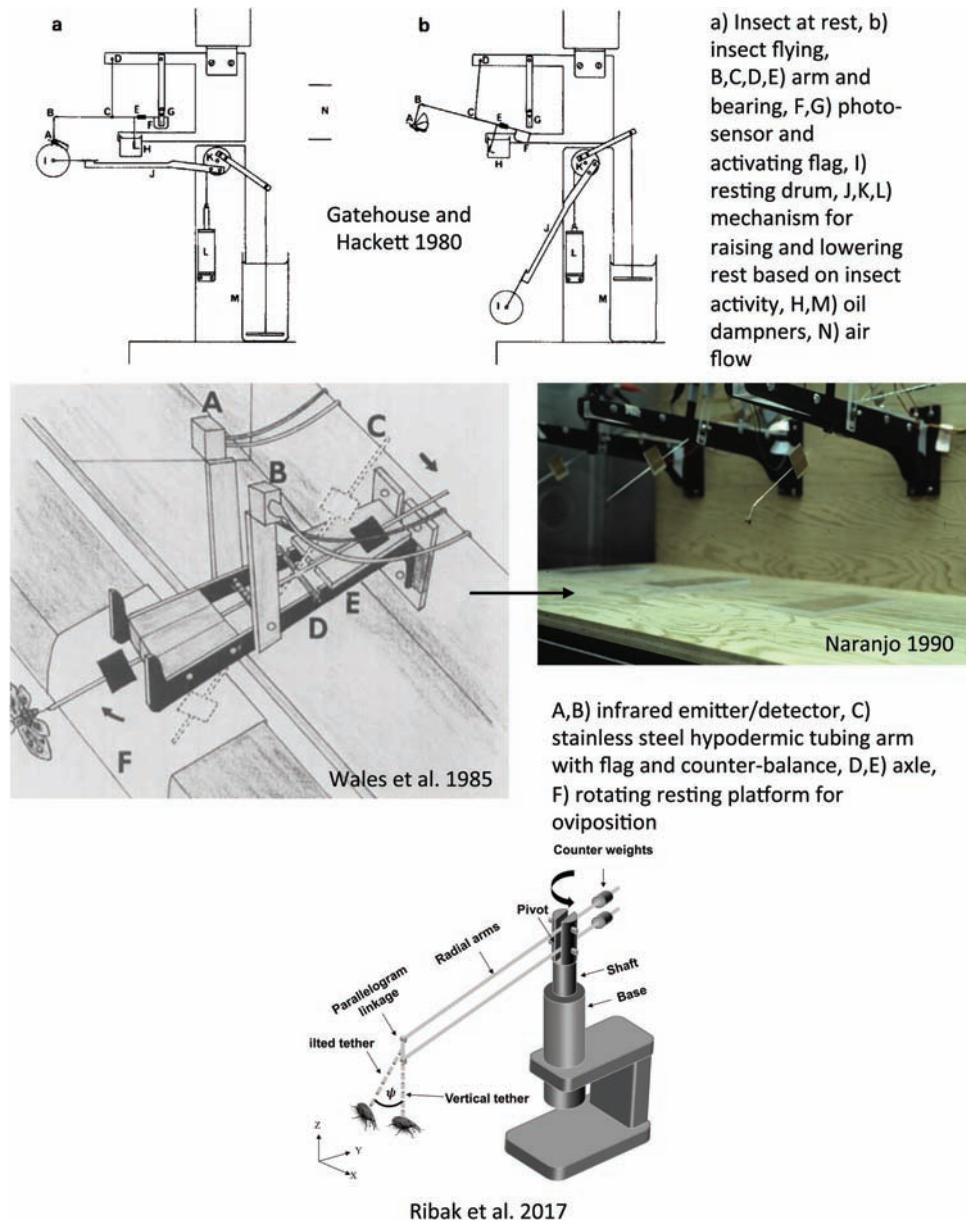


Fig. 7. Flight mills based on vertical rather than rotary motion. Here, the insect moves up and down in a vertical plane and can rest on a platform when not flying. The original design of Gatehouse and Hackett (1980) was modified by Wales et al. (1985) with a rotating landing drum to measure the periodicity of oviposition. This design was modified again by Naranjo (1990) to fly smaller insects without a landing platform. The Ribak et al. (2017) system is a hybrid in which the insect must generate lift to raise the arm but then flies in a rotary fashion. The tether mechanism also allows the insect to be banked during flight and can be used to study flight mechanic variables. All images by author or used with permission.

mill systems have been automated to some degree going back as far as Hocking (1953). A flag on the arm, the arm itself or a disk mounted below the arm axle are typically used to trip a sensor that then counts the rotations of the flight arm. In the early days, visible light electronic photocells were used as the sensors. These were replaced by infrared sensors beginning in the mid-1970s Chambers et al. (1976) and Hall effect (magnetic) sensors started to be used around 2010 (Dubois et al. 2010, Jones et al. 2010). All flight mills with automation now use one of these two latter technologies for detecting arm rotations.

In early automated systems, sensor signals were logged onto simple analog event recorders and sometimes strip-chart recorders, which then had to be deciphered by hand to extract useful data (see Fig. 4). The advent of personal digital computers in the early 1980s

changed all of that, finally allowing true automation in which the disruptor signal could be logged and processed by a computer. The earliest references to personal computer-based systems were Clarke et al. (1984), who modified the Rowley et al. (1968) flight mill for fully automated data collection, and McKibben (1985). By the end of the 1980s, such systems were the norm rather than the exception. Digital input/output (I/O) interfaces between the flight mill and the computer were primarily custom fabricated by the users, and there are numerous examples in the literature of custom schematics that would allow others to reproduce them (e.g., Barfield et al. 1988, Resurreccion et al. 1988, Taylor et al. 1992, Weber et al. 1993, Beerwinkle et al. 1995). By the late 1990s, off-the-shelf digital I/O interfaces were commonly available (Schumacher et al. 1997, Alyokhin et al. 1999, Blackmer et al. 2004), and these, along with

commercially available, open-source software and microcontroller kits such as Arduino (Hahn et al. 2017, Wong et al. 2018) represent the state of the art today. These modern digital I/O boards coupled with modern PCs are capable of extremely high sampling rates and a single interface can accommodate multiple (e.g., 24–40) flight mills.

Unlike commercially available digital interface boards, there are not commercially available software programs to log and process the data collected (but see the WinDaq system used by Attisano et al. 2015). Instead, each researcher has either had to develop their own programs or use those produced by others. A wide variety of programs have been developing using languages, such as BASIC, Fortran, Pascal, C++, Python, and MATLAB, and programs, such as LabView (Fig. 8) and DASyLab, that are graphical-based languages designed specifically to program the operation of electronic equipment (see Table 1; Supp Table 1 [online only]). Some of these programs have user settings that help eliminate false rotation counts after the insect has ceased flying (Jones et al. 2010, Marti-Campoy et al. 2016).

Tethering

An integral component of the overall flight mill assay is tethering of the subject insect. Researchers have used a wide variety of techniques and materials (Fig. 9; Supp Table 1 [online only]). The key qualities of a tethering system are straightforward; the tether should be relatively simple to attach and should not unduly affect the subsequent behavior of the insect (beyond the fact that a tether is already unnatural). Typically, the insect needs to be sedated and/or constrained in some fashion to allow mounting of the tether. Chilling the insect for

a few minutes or asphyxiating the subject with a small dose of CO₂ is a common approach. Asphyxiation with ether has also been used, but less commonly. Sometimes the insect can be sufficiently subdued with vacuum suction, typically moths with larger wing areas. At other times, the insect is sufficiently large and durable enough (e.g., larger beetles) that sedation is not required. Gatehouse and Hackett (1980) developed an interesting system for tethering noctuid moths in which the tether was attached to the unsedated pharate adult in the pupal case. When the moth emerged its tether was already attached and it did not need to be subject to any additional stress or manipulation.

Researchers also have used a variety of attachment substances and tethers (Fig. 9; Supp Table 1 [online only]). The most common adhesive is some sort of superglue (cyanoacrylate), sometimes with an accelerant (Beerwinkle et al. 1995, Wong et al. 2018) to further hasten drying. Other common materials include contact and rubber cement, dental and other waxes, low temperature hot melt glue, and wood glue. Relatively unique options include skin adhesives, nail polish, magnetic paint, and poster paint. Water-based Arabic glue offers the advantage of being dissolvable with water so that the tether can be easily removed. The live insect can then be subject to other experimental measurements after flight (Chen and Feng 2004). Often, the cuticle must be prepared by removing the scales, particularly for moths, or de-waxing before the adhesives will bond properly. There also are nonadhesive options employing wire or fishing line nooses (Heinrich 1971, Castro et al. 2014). Ideally, adhesives should be nontoxic, dry quickly so that sedation periods are brief, and provide strong adhesion on the smooth and waxy surface of insect cuticle. Rarely are adhesives tested and then only for lethal

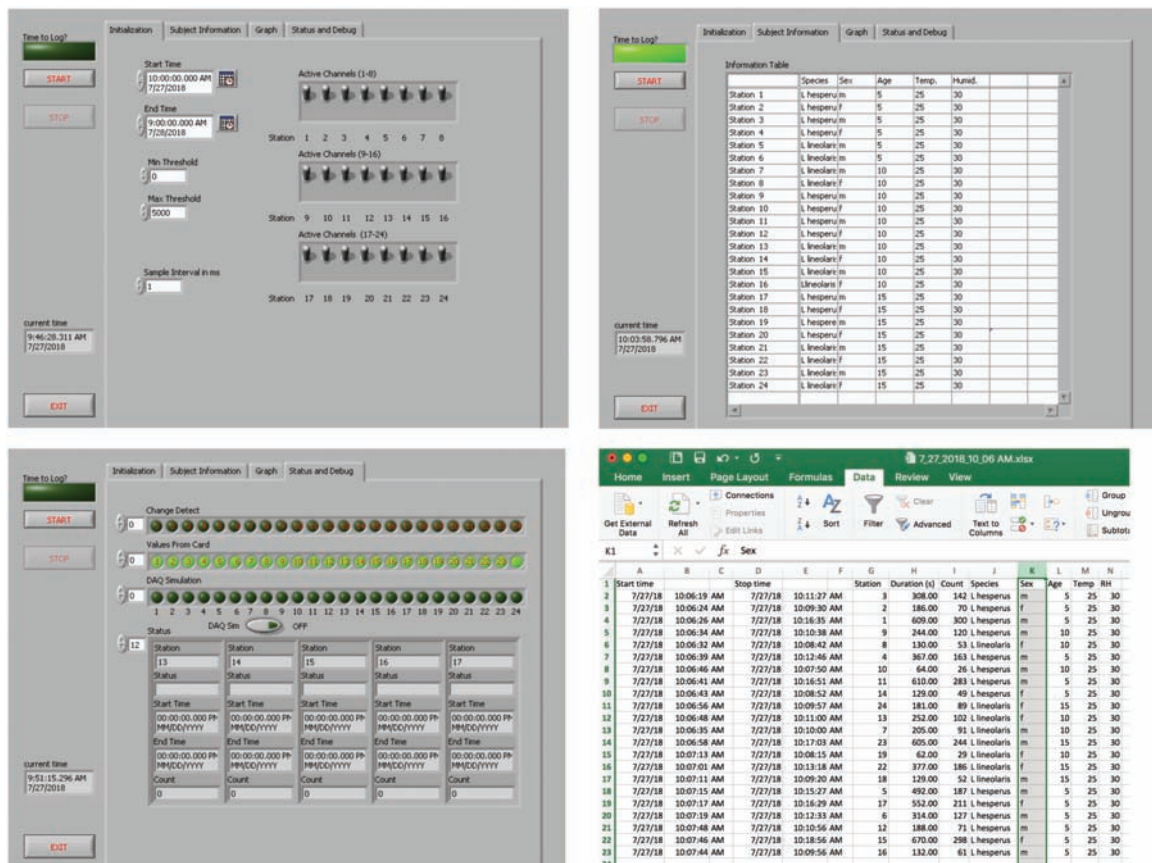


Fig. 8. An example of computer software for testing flight mill operation and logging flight behavior. This is a LabView program coupled with a 24-channel digital I/O board in a PC (National Instruments, Austin, TX). Data are output to an Excel spreadsheet for further processing (see Jones et al. 2010).



Fig. 9. Examples of insects tethered to the flight mill arm. All images by author or used with permission.

effects (Akbulut and Linit 1999). In reality, testing adhesives and the tethering process in general is difficult because a control treatment is problematic. This again emphasizes the important role of the comparative approach in experimental design (see below).

Tethers can be flexible or nonflexible and can be an extension of the arm or the end of the arm itself (Fig. 9). Gatehouse and

Hackett (1980) and Wales et al. (1985) suggests that a flexible tether allows the insect to adjust its own pitch and yaw somewhat, but Resurreccion et al. (1988) tested flexible and rigid tethers and found no advantage to flexibility. Chambers et al. (1976) suggest that the insect should be pitched forward (head down) about 12–14 degrees for maximum generation of thrust, but Hocking (1953) suggested

that the subject be mounted near its center of gravity (mesothorax) and parallel with the flight arm. In practice, the point of attachment (pronotum, mesonotum, or even abdomen) is dictated more by the morphology of the insect than anything else. For example, while it might be relatively easy to mount the tether on the mesonotum of a moth or fly, this would be difficult for a beetle or true bug because that would require the wings to be unnaturally pried open to do so. Another option is to mount the tether to the ventral side of the thorax (Zhang et al. 2008, Liu et al. 2011, see Fig. 9). Zhang et al. (2009) suggest that tethering to the ventral surface ensures horizontal orientation and eliminates issue of interference with wing motion or muscle flexion. However, none of the studies using this technique discuss how such tethering might interfere with tarsal contact and stimulation. As for the angle of mounting on the flight arm and tether flexibility, many authors fail to describe their methodology. Overall, tether orientation is something that will need to be adjusted for each species through trial and error (but see Ribak et al. 2017). I have had good success flying relatively small beetles and true bugs with flexible tethers attached to the pronotum with horizontal mounting orientation on both vertical balance and rotary systems (Naranjo 1990, 1991; Blackmer et al. 2004; Stebbing et al. 2005; Brent et al. 2013).

Other Considerations

One element that is not frequently addressed in rotary flight mill design is the ability to level the platform of the system (see Fig. 6). Along with balancing the flight arm, this ensures that the arm rotates in a level plane and once again does not introduce any additional drag or friction. Solutions can be as simple as providing a leveling platform on which the mill sits or integrating leveling screws into the base of the flight mill itself (Fig. 6). It is likely that most flight mills are automatically level based on their design, but the substrate upon which the mill is placed may not be. Overall, this is a factor that should be considered and addressed as needed.

Often, flight assays are conducted in environmentally controlled spaces in the laboratory. This is clearly critical if controlled factors such as temperature, humidity, and photoperiod are being investigated. Frequently, flight mills also are contained within enclosures to control for variable airflow that might unduly affect assay results (Rowley et al. 1968, Naranjo 1990, Taylor et al. 1992, Attisano et al. 2015). While directional airflow might be useful in inducing flight, turbulent or unpredictable airflow patterns from building air-handling systems could introduce unwanted variation into the flight assay.

What Can Be Learned from Flight Mills?

Metrics

The variety of information that can be collected from flight mills depends on the type of system and the degree of automation. Assuming a modern level of automation for vertical balance designs, the basic metrics include flight propensity (did the insect fly or not), the number of flights initiated, when they were initiated (periodicity), and the duration of each of these flights. Additional metrics can be estimated from this basic information including total flight time over the entire assay period. Because the insect only moves up and down, no information on distance traveled is available, and as a consequence, flight velocity cannot be estimated. Distance and velocity, along with all the other metric mentioned, can of course be measured in rotary systems. Velocity is a potentially useful metric in many studies and it is common for flight mill results to be corrected for drag and other aerodynamic forces (e.g., Hocking 1953,

Green 1962, Chance 1971, Taylor et al. 2010). With automation, data can be examined with the finest or coarsest grain desired from the length, timing, and speed of a single flight to the total distance flown in a day. The particular metrics captured and reported in any given study varies considerably based on the questions asked (Supp Table 1 [online only]). Sometimes the goal is to measure how far an insect can fly in a day to estimate invasion potential (e.g., Taylor et al. 2010, Hoddle et al. 2015), whereas at other times, researchers might want to know how flight potential changes with age and temperature (e.g., Naranjo 1991, Weber et al. 1993). Sometimes the flight mill might just represent a tool for getting an insect to fly so that other physiological parameters like body temperature and metabolics can be assessed (e.g., Heinrich 1971, Gmeinbauer and Crailsheim 1993). Overall, flight mills lend themselves to addressing a wide range of research problems.

Experimental Design

Despite the technological advances in flight mill systems over time (Table 1 and Supp Table 1 [online only]) and the careful attention to details in design and tethering, the fact remains that tethered flight—or any study of flight behavior in the laboratory—is only a facsimile of reality. There will always be limitations in what the data can reveal about behavior in the field even if the results are calibrated to some extent (Hocking 1953, Chance 1971, Gatehouse and Hackett 1980, Jactel and Gaillard 1991, Taylor et al. 2010, Jones et al. 2016). These limitations might be further exacerbated by the use of laboratory-reared insects, a concern common to all laboratory studies (Sorensen et al. 2012, Hoffmann and Ross 2018) and certainly not unique to the study of flight behavior. Several studies have indeed shown that the flight performance of laboratory-reared insects is different when compared with their wild counterparts (e.g., Baker et al. 1980, Nakamori and Simizu 1983, Wales et al. 1985, McKibben et al. 1988). For all these reasons, the optimal approach to the study of insect flight behavior in the laboratory, including flight mills, is through a comparative process. The long record of study in this field bears out a strong adherence to this general philosophy in a wide range of insect species.

Since the early 1950s, flight mills have been used to study various aspects of insect flight behavior, physiology, and mechanics in 214 species in 61 families and 9 orders (Fig. 10). Lepidoptera and Coleoptera have been the most studied groups with a large emphasis on migration, particularly in moths (Minter et al. 2018). Hemiptera, Hymenoptera, and Diptera also have been relatively well studied, with particular focus on insects affecting human health, such as mosquitoes (Hocking 1953, Clements 1955, Rowley and Graham 1968,

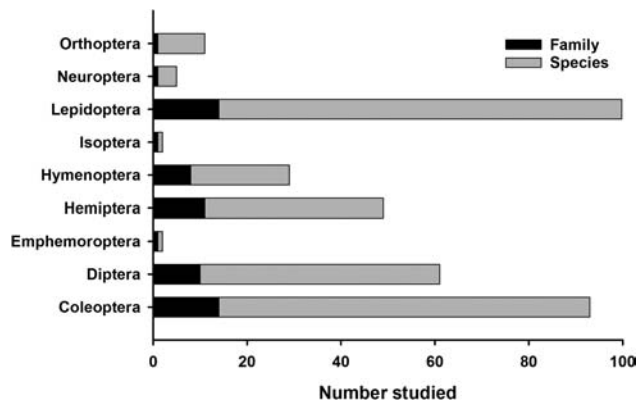


Fig. 10. Taxonomic distribution of flight mill studies in the literature.

Nayar and Sauerman 1971, Briegel et al. 2001). Species in other orders have been less studied or not at all, even though the range of insect sizes and diversity examined to date suggests no barriers from a technological standpoint.

A comparative approach obviates, to a large extent, many of the limitations already discussed, because all treatments in the study are subject to the same background issues. Unless there are interactions with these limitations and the experimental factor, which seems extremely unlikely, then they play no role in the final outcome. Through a comparative lens, the focus is on the relative changes in behavior (e.g., flight speed, duration, or frequency) due to a specific factor (e.g., gender, age, or temperature), rather than on the absolute value of that behavior per se. For example, even if flight velocity (and thus distance covered) can be corrected for aerodynamic and energetic influences (Hocking 1953), there is still no guarantee that an insect will fly in a straight line from one point to another. Thus, predictions of distance traveled in the field can hardly ever be accurate. However, we might learn that females fly further than males or that younger insects disperse further than older insects and this provides important insight even while not providing absolute metrics.

Using a comparative approach, there has been a wide array of questions asked in flight mill studies (Fig. 11; Supp Table 1 [online only]). Not surprisingly, many studies have asked how flight behavior differs between males and females and as a function of age. A relatively large number of studies also have compared mated and virgin insects, compared effects of environmental variables like temperature and humidity, examined the role of differential nutrition and the types and quantities of flight fuels utilized, and contrasted related species and different populations of the same species. Flight mills have been used to ask how parasitism or microbial infections might alter flight performance, how insect movement relates to reproductive status, particularly with regards to the interaction of migration and oviposition, how body size or rearing density affects flight, and how flight behavior changes with season. Related to insect pest management, flight mills have been used to look at sublethal effects of insecticides and transgenic crops on flight potential as well as the comparative effects of pheromones and of irradiation used in sterile insect release technology. Practical questions like the effect of laboratory rearing and the potential effect of marking on insect dispersal have been addressed. More details on how some of these factors can potentially affect insect flight performance are discussed in Minter et al. (2018). There is almost no limit to the way in which

comparisons can be made to address important questions in insect flight behavior, physiology, and ecology to enhance our understanding of insect movement.

A Few Notes on Analyses

A common hallmark of flight mills is the high intersubject variability in performance that can lead to non-normal distributions and introduce challenges in data analyses (e.g., Cooter 1982, 1983; Naranjo 1990; Jactel 1993). Some of this variability is undoubtedly related to the length of the assay period, which can range from ≤ 30 min to 12 h during the scotophase, or more commonly, a full 22–24 h (Supp Table 1 [online only]). Insects may be flown variable amounts of time until exhaustion, or only for a fixed distance or number of rotations. Some of the inherent variability can be attenuated in several ways. While the particular questions being asked likely determine the assay interval, it also may be worthwhile to determine if smaller intervals of longer assays may be sufficient to address the study needs. Other facets of study design may help reduce variation as well. For example, researchers generally require that the insect survives the entire assay period to be included (e.g., Naranjo 1990, Blackmer et al. 2004, Brent et al. 2013). Researchers also commonly pretest insects for activity to eliminate nonflyers (Nakamori et al. 1983, Attisano et al. 2013, Ferrer et al. 2013, Fahrner et al. 2014, Blanken et al. 2015). Others have screened out subjects based on their performance. For example, insects that failed to demonstrate a single continuous flight of at least 1 h (Sappington and Showers 1991), a total flight duration of at least 30 min (Zhao et al. 2011), or failed to initially fly for at least 1 min (Briegel et al. 2001) or 10 s (Akbulut and Linit 1999) were not included in the analyses. Using a hybrid system, (Barkan et al. 2018) required the insect to lift the arm 30 degrees from the ground and move forward for the flight to count. These screening methods may reduce variation, but they also contribute to more sound data by eliminating insects that may have been injured during tethering or handling. However, eliminating insects that fail to fly or fly only briefly also may reduce a fuller understanding of real individual differences within a species. Thus, such screens should be used cautiously relative to the goals of the study.

It goes without saying that the statistical approach taken for analysis should be tailored to the data, and often this might require nonparametric analysis or use of more sophisticated generalized linear models to effectively deal with the distributional oddities of flight

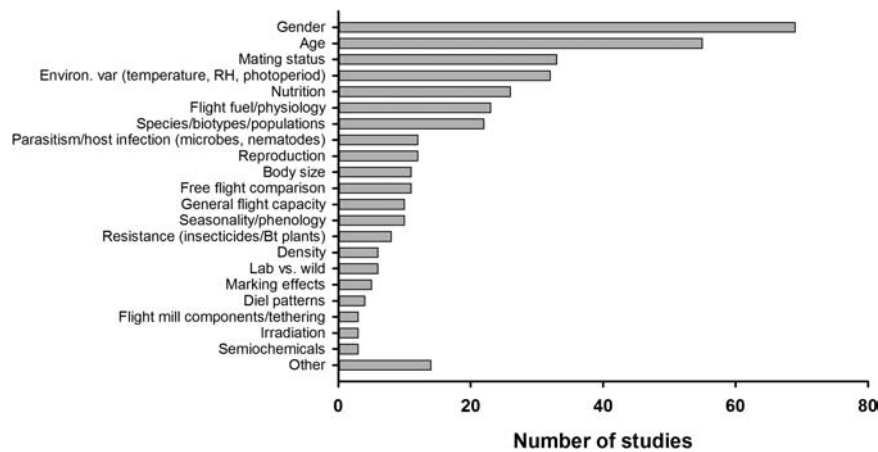


Fig. 11. Summary of the comparative factors examined in flight mill studies. The other category includes factors such as antennal length, cryo-preservation, diapause status, gallery construction, gene expression and genetics, parental care, phototaxis, and wing morphology. Note that these factors may have been examined on multiple species in a given study and that individual studies often examined multiple factors.

mill outputs. Also, given the high variability frequently encountered, it would also be useful to conduct a priori power analyses from a small initial trial so that appropriate sample sizes and experimental designs are employed to enable the study to discern real treatment effects (Steidl et al. 1997, DiStefano 2003).

A Simple Example

I provide a simple example from my own work that demonstrates the kinds of data that can be generated with an insect flight mill (Naranjo 1990; Fig. 12). This comes from a comparative study of the flight behavior of two species of *Diabrotica* (Coleoptera: Chrysomelidae) that are major pests of corn in many parts of the United States. In both western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and northern corn rootworm (*Diabrotica barberi* [Smith and Lawrence]), the distribution of total flight duration and of the single longest flight for each individual beetle were highly skewed toward short flights. This was true for both genders and regardless of age. Further, flights of western corn rootworm were distinctly bi-modal suggesting more sustained flights in a small proportion of the population, but it remains unclear if this represents migratory flight given that it occurs in both sexes and even in older gravid females. Coats et al. (1986) observed a similar bi-modal distribution using rotary flight mills, but Stebbing et al (2005), using the same vertical balances as Naranjo (1990), found less defined bi-modal patterns. This could represent differences in populations and/or rearing conditions and suggests that further research, including field-based observations, may be needed to elucidate this phenomenon. Sustained flights were never observed in northern corn rootworm beetles. Shorter so-called ‘trivial’ flights differed in frequency and duration between the species and between genders within each species, but not with age. Finally, periodicity of individual trivial flights was invariable between species and genders and suggested primarily diurnal activity with perhaps a slight peak of activity in the

early morning. Sample sizes were large, and nonparametric statistics were used for analyses. Overall, these findings provided insight into the observed dispersal behavior and distributional patterns known in the field at that time.

Resources

As noted, a dozen papers have been published in the last 50 years specifically focused on description of how to build insect flight mills (see Table 1), and many others have provided some details on construction (Supp Table 1 [online only]), so there is no shortage of ideas and aid. I highlight here three recent resources that are particularly useful to those interested in pursuing rotary flight mill-based studies (Jones et al. 2010, Attisano et al. 2015, Marti-Campoy et al. 2016). These all provide quality details on construction and how to source the materials. Two of them also provide videos that are helpful in understanding how to tether insects and operate the mills (Jones et al. 2010, Attisano et al. 2015). The most significant bottleneck for new users is probably the software. These two latter resources provide access to available computer software for logging flight mill data. Attisano et al. (2015) used WinDaq software that is free with the purchase of a USB DAQ interface device (<https://www.dataq.com/products/windaq/>) at a nominal price and can be used out of the box for some data collection tasks. Jones et al. (2010) offer free download of their custom LabView program along with instructions and a run-time Labview engine that allows execution of the program without the cost of a software license (see Fig. 8; http://entomology.tfrec.wsu.edu/VPJ_Lab/Flight-Mill#section2) and that will work for a system configured as detailed on the website. Also see Hahn et al. (2017) for details on an Arduino-based system. Future technical advances driven by the maker space revolution are likely to ameliorate this final barrier to flight mill technology and application.

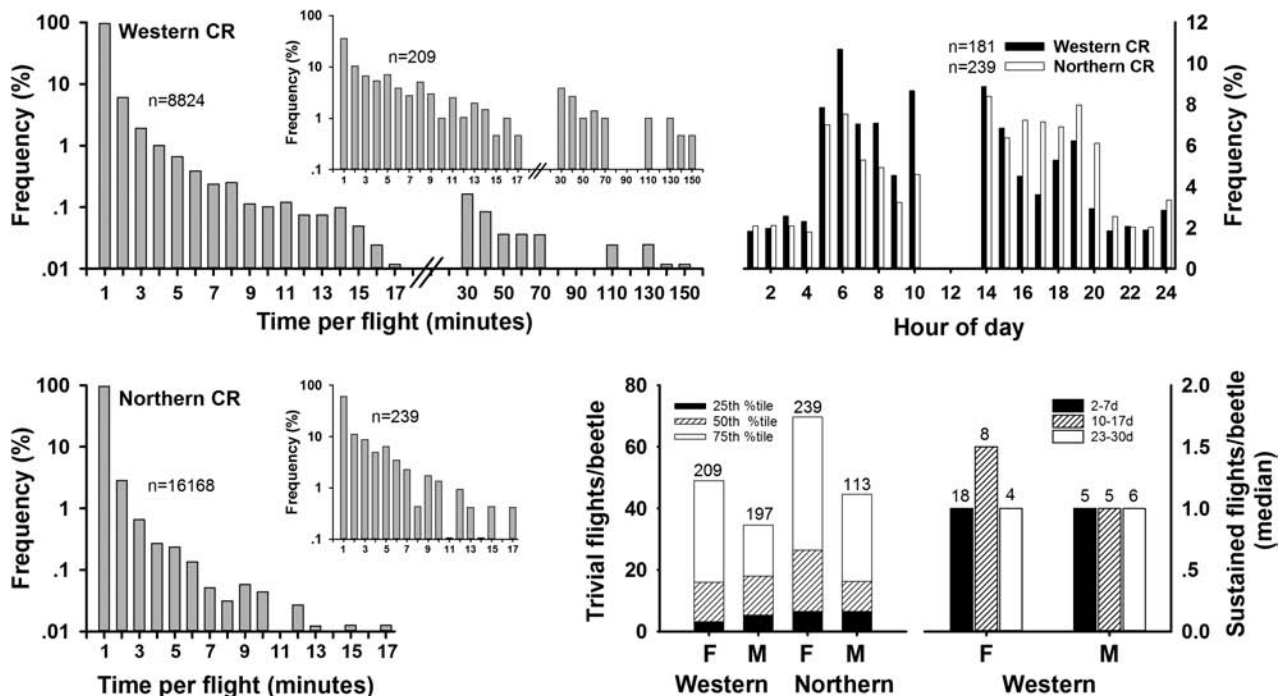


Fig. 12. Example results from a flight mill (vertical balance) study examining the comparative flight behavior of two *Diabrotica* spp., including distribution of total and individual (inset) flight durations by females, periodicity of individual trivial flights by females, and number of trivial and sustained flights of both genders. Assay period was 23 h at 25°C, 60% RH with a photoperiod of 14:10 (L:D) h (redrawn from Naranjo 1990, with permission).

Conclusions

Flight mills have played an important role in enabling a better understanding of flight behavior in more than 200 insect species over the past 70 years. The basic configuration of the insect flight mill has remained relatively unchanged over this period, but technological advances have improved designs and materials, and computers and modern electronics have made flight mills more accessible to more researchers while concurrently improving the automation of data collection. While flight mills may impose some limitations in measuring real insect behavior from the field, with calibration and proper attention to interpretation of results through a comparative lens, they can provide important insights into insect movement that remain difficult or even impossible to gather in any other way. Flight mills will continue to be an important tool of the behavioral trade with further growth of investigations into the effects of more biological and ecological factors on an ever-expanding range of insect species.

Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

Acknowledgments

I thank Jana Lee for putting together the symposium and Special Issue and for inviting me to speak and write about insect flight mills—one of the guilty pleasures I have dabbled with since my postdoctoral days. I thank Paul Wales for introducing me to flight mills, Carl Barfield for letting me cart the Wales mills to several new homes over the years, and John Westbrook for lending me a Beerwinkle mill so I could break into rotary mill technology. I also thank Jackie Blackmer for introducing me to the vertical flight chamber and leading a comparative study of *Lygus* spp. behavior with free flight and flight mill approaches. Finally, I thank Roberta Gibson for reviewing an early draft of this manuscript.

References Cited

- Akbulut, S., and M. J. Linit. 1999. Flight performance of *Monochamus carolinensis* (Coleoptera: Cerambycidae) with respect to nematode phoresis and beetle characteristics. *Environ. Entomol.* 28: 1014–1020.
- Alyokhin, A., D. N. Ferro, C. W. Hoy, and G. Head. 1999. Laboratory assessment of flight activity displayed by Colorado potato beetles (Coleoptera: Chrysomelidae) fed on transgenic and Cry3a toxin-treated potato foliage. *J. Econ. Entomol.* 92: 115–120.
- Atkins, M. D. 1961. A study of the flight of the Douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae): III Flight capacity. *Can. Entomol.* 93: 467–474.
- Attisano, A., T. Tregenza, A. J. Moore, and P. J. Moore. 2013. Oosorption and migratory strategy of the milkweed bug, *Oncopeltus fasciatus*. *Anim. Behav.* 86: 651–657.
- Attisano, A., J. T. Murphy, A. Vickers, and P. J. Moore. 2015. A simple flight mill for the study of tethered flight in insects. *J. Vis. Exp.* 106: e53377.
- Baker, P. S., R. J. Cooter, P. M. Chang, and H. B. Hashim. 1980. The flight capabilities of laboratory and tropical field populations of the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae). *Bull. Entomol. Res.* 70: 589.
- Barfield, C. S., D. J. Waters, and H. W. Beck. 1988. Flight device and database management system for quantifying insect flight and oviposition. *J. Econ. Entomol.* 81: 1506–1509.
- Barkan, S., A. Hoffman, A. Hezroni, and V. Soroker. 2018. Flight performance and dispersal potential of red palm weevil estimated by repeated flights on flight mill. *J. Insect Behav.* 31: 66–82.
- Beerwinkle, K. R., J. D. Lopez, D. Cheng, P. D. Lingren, and R. W. Meola. 1995. Flight potential of feral *Helicoverpa zea* (Lepidoptera: Noctuidae) males measured with a 32-channel, computer-monitored, flight-mill system. *Environ. Entomol.* 24: 1122–1130.
- Blackmer, J. L., and P. L. Phelan. 1991. Behavior of *Carophilus hemipterus* in a vertical flight chamber: transition from phototactic to vegetative orientation. *Entomol. Exp. Appl.* 58: 137–148.
- Blackmer, J. L., S. E. Naranjo, and L. H. Williams. 2004. Tethered and untethered flight by *Lygus hesperus* and *Lygus lineolaris* (Hemiptera: Miridae). *Environ. Entomol.* 33: 1389–1400.
- Blanken, L. J., F. van Langevelde, and C. van Dooremalen. 2015. Interaction between *Varroa destructor* and imidacloprid reduces flight capacity of honeybees. *Proc. Royal Soc. B Biol. Sci.* 282: 20151738.
- Borden, J., and R. Bennett. 1969. A continuously recording flight mill for investigating the effects of volatile substance on the flight of tethered insects. *J. Econ. Entomol.* 62: 782–785.
- Bradley, C. A., and S. Altizer. 2005. Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. *Ecol. Lett.* 8: 290–300.
- Brent, C., C. Klok, and S. Naranjo. 2013. Effect of diapause status and gender on activity, metabolism, and starvation resistance in the plant bug *Lygus hesperus*. *Entomol. Exp. Appl.* 148: 152–160.
- Briegel, H., I. Knüsel, and S. E. Timmermann. 2001. *Aedes aegypti*: size, reserves, survival, and flight potential. *J. Vector Ecol.* 26: 21–31.
- Brown, H. D. 1972. The suitability of some crops to infestation by the wheat aphid, *Schizaphis graminum* (Homoptera: Aphididae). *Entomol. Exp. Appl.* 15: 128–138.
- Brown, S., V. Soroker, and G. Ribak. 2017. Effect of larval growth conditions on adult body mass and long-distance flight endurance in a wood-boring beetle: do smaller beetles fly better? *J. Insect Physiol.* 98: 327–335.
- Byrne, D. N. 1999. Migration and dispersal by the sweet potato whitefly, *Bemisia tabaci*. *Agric. Forest Meteorol.* 97: 309–316.
- Carrière, Y., P. B. Goodell, C. Ellers-Kirk, G. Larocque, P. Dutilleul, S. E. Naranjo, and P. C. Ellsworth. 2012. Effects of local and landscape factors on population dynamics of a cotton pest. *PLoS One.* 7: e39862.
- Castro, L. A., J. K. Peterson, A. Saldana, M. Y. Perea, J. E. Calzada, V. Pineda, A. P. Dobson, and N. L. Gottdenker. 2014. Flight behavior and performance of *Rhodnius pallescens* (Hemiptera: Reduviidae) on a tethered flight mill. *J. Med. Entomol.* 51: 1010–1018.
- Chambers, D., and T. O'Connell. 1969. A flight mill for studies with the Mexican fruit fly. *Ann. Entomol. Soc. Am.* 62: 917–920.
- Chambers, D., J. Sharp, and T. Ashley. 1976. Tethered insect flight: a system for automated data processing of behavioral events. *Behav. Res. Methods Instr.* 8: 352–356.
- Chance, M. 1971. Correction for drag of a flight mill with an example for *Agrotis orthogonia* Morr. (Lep. Noctuidae). *Quaest Entomol.* 7: 253–254.
- Chen, C., and M. G. Feng. 2004. *Sitobion avenae alatae* infected by *Pandora neoaphidis*: their flight ability, post-flight colonization, and mycosis transmission to progeny colonies. *J. Invertebr. Pathol.* 86: 117–123.
- Chen, M., P. Chen, H. Ye, R. Yuan, X. Wang, and J. Xu. 2015. Flight capacity of *Bactrocera dorsalis* (Diptera: Tephritidae) adult females based on flight mill studies and flight muscle ultrastructure. *J. Insect Sci.* 15: 141.
- Cheng, D., Z. Tain, J. Sun, H. Ni, and G. Li. 1997. A computer-monitored flight mill system for tiny insects such as aphids. *Acta Entomol. Sin.* 40: 172–179.
- Clarke, J. L. I., W. A. Rowley, S. Christiansen, and D. W. Jacobsen. 1984. Microcomputer-based monitoring and data acquisition system for a mosquito flight mill. *Ann. Entomol. Soc. Am.* 77: 119–122.
- Clements, A. N. 1955. The sources of energy for flight in mosquitoes. *J. Exp. Biol.* 32: 547–552.
- Coats, S., J. Tollefson, and J. Mutchmore. 1986. Study of migratory flight in the Western corn rootworm (Coleoptera: Chrysomelidae). *Environ. Entomol.* 15: 1–6.
- Cooter, R. J. 1982. Studies on the flight of black-flies (Diptera: Simuliidae). I. Flight performance of *Simulium ornatum* Meigen. *Bull. Entomol. Res.* 72: 303–317.
- Cooter, R. J. 1983. Studies on the flight of black-flies (Diptera: Simuliidae). II. Flight performance of three cytospecies in the complex of *Simulium damnosum* Theobald. *Bull. Entomol. Res.* 73: 275.

- Cooter, R. J., and N. J. Armes. 1993. Tethered flight technique for monitoring the flight performance of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Environ. Entomol.* 22: 339–345.
- David, C. T., and J. Hardie. 1988. The visual responses of free-flying summer and autumn forms of the black bean aphid, *Aphis fabae*, in an automated flight chamber. *Physiol. Entomol.* 13: 277–284.
- Dingle, H. 1966. Some factors affecting flight activity in individual milkweed bugs (*Oncopeltus*). *J. Exp. Biol.* 44: 335–343.
- Dingle, H. 2014. *Migration: the biology of life on the move*. Oxford University Press, Oxford, United Kingdom.
- DiStefano, J. 2003. How much power is enough? Against the development of an arbitrary convention for statistical power calculations. *Funct. Ecol.* 17: 707–709.
- Dubois, G. F., P. J. Vernon, and H. Brustel. 2009. A flight mill for large beetles as *Osmoderma eremita* (Coleoptera: Cetoniidae), pp. 219–224. In J. Buse, K. N. A. Alexander, T. Ranius and T. Assmann (eds.), *Saproxylic beetles—their role and diversity in European woodland and tree habitats*, Proceedings of the 5th Symposium and Workshop on the Conservation of Saproxylic Beetles. Pensoft Publishers, Sofia, Moscow.
- Dubois, G. F., P. J. Le Gouar, Y. R. Delettre, H. Brustel, and P. Vernon. 2010. Sex-biased and body condition dependent dispersal capacity in the endangered saproxylic beetle *Osmoderma eremita* (Coleoptera: Cetoniidae). *J. Insect Conservation.* 14: 679–687.
- Duistermars, B. J., D. M. Chow, M. Condro, and M. A. Frye. 2007. The spatial, temporal and contrast properties of expansion and rotation flight optomotor responses in *Drosophila*. *J. Exp. Biol.* 210: 3218–3227.
- Dybowski, V. V. 1970. A device for investigation of the flight of small insects. *Entomol. Rev.* 49: 435–436.
- Evenden, M. L., C. M. Whitehouse, and J. Sykes. 2014. Factors influencing flight capacity of the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae). *Environ. Entomol.* 43: 187–196.
- Fahrner, S. J., J. P. Lelito, K. Blaedow, G. E. Heimpel, and B. H. Aukema. 2014. Factors affecting the flight capacity of *Tetrastichus planipennis* (Hymenoptera: Eulophidae), a classical biological control agent of *Agrilus planipennis* (Coleoptera: Buprestidae). *Environ. Entomol.* 43: 1603–1612.
- Ferrer, A., S. Dorn, and D. Mazzi. 2013. Cross-generational effects of temperature on flight performance, and associated life-history traits in an insect. *J. Evol. Biol.* 26: 2321–2330.
- Flint, H. M., B. Wright, H. Sallam, and B. Horn. 1975. A comparison of irradiated or chemosterilized pink bollworm moths for suppressing native populations in field cages. *Can. Entomol.* 107:1069–1072.
- Gatehouse, A. G., and D. S. Hackett. 1980. A technique for studying flight behavior of tethered *Spodoptera exempta* moths. *Physiol. Entomol.* 5: 215–222.
- Gmeinbauer, R., and K. Crailsheim. 1993. Glucose utilization during flight of honeybee (*Apis mellifera*) workers, drones and queens. *J. Insect Physiol.* 39: 959–967.
- Green, G. W. 1962. Flight and dispersal of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.). I. Factors affecting flight, and the flight potential of females. *Can. Entomol.* 94: 282–299.
- Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: current techniques and future prospects. *Annu. Rev. Entomol.* 46: 511–543.
- Hahn, N. G., M. C. Hwang, and G. C. Hamilton. 2017. Circuitry and coding used in a flight mill system to study flight performance of *Halyomorpha halys* (Hemiptera: Pentatomidae). *Fl. Entomol.* 100: 195–198.
- Hardie, J. 1993. Flight behaviour in migrating insects. *J. Agric. Entomol.* 10: 239–245.
- Heinrich, B. 1971. Temperature regulation of the sphinx moth, *Manduca sexta*. I. Flight energetics and body temperature during free and tethered flight. *J. Exp. Biol.* 54: 141–152.
- Henson, W. R. 1962. Laboratory studies on the adult behaviour of *Conophthorus coniperda* (Coleoptera: Scolytidae). III. Flight. *Ann. Entomol. Soc. Am.* 55: 524–530.
- Hocking, B. 1953. The intrinsic range and speed of flight of insects. *Trans. Royal Entomol. Soc. Lond.* 104: 223–345.
- Hodde, M. S., C. D. Hodde, J. R. Faleiro, H. A. El-Shafie, D. R. Jeske, and A. A. Sallam. 2015. How far can the red palm weevil (Coleoptera: Curculionidae) fly?: computerized flight mill studies with field-captured weevils. *J. Econ. Entomol.* 108: 2599–2609.
- Hoffmann, A. A., and P. A. Ross. 2018. Rates and patterns of laboratory adaptation in (mostly) insects. *J. Econ. Entomol.* 111: 501–509.
- Jactel, H. 1993. Individual variability of the flight potential of *Ips sexdentatus* Boern. (Coleoptera: Scolytidae) in relation to day of emergence, sex, size, and lipid content. *Can. Entomol.* 125: 919–930.
- Jactel, H., and J. Gaillard. 1991. A preliminary study of the dispersal potential of *Ips sexdentatus* (Boern.) (Col., Scolytidae) with an automatically recording flight mill. *J. Appl. Entomol.* 112: 138–145.
- Jones, C. M., A. Papanicolaou, G. K. Mironidis, J. Vontas, Y. Yang, K. S. Lim, J. G. Oakeshott, C. Bass, and J. W. Chapman. 2015. Genomewide transcriptional signatures of migratory flight activity in a globally invasive insect pest. *Mol. Ecol.* 24: 4901–4911.
- Jones, H. B., K. S. Lim, J. R. Bell, J. K. Hill, and J. W. Chapman. 2016. Quantifying interspecific variation in dispersal ability of noctuid moths using an advanced tethered flight technique. *Ecol. Evol.* 6: 181–190.
- Jones, V. P., S. E. Naranjo, and T. J. Smith. 2010. Insect ecology and behavior: laboratory flight mill studies. Accessed 11 July 2015. http://entomology.tfrec.wsu.edu/VPJ_Lab/Flight-Mill
- Jutsum, A. R., and G. J. Goldsworthy. 1974. Some effects of mermithid infection on metabolic reserves and flight in *Locusta*. *Int. J. Parasitol.* 4: 625–630.
- Kennedy, J. S. 1975. *Insect dispersal*, vol. 103–119. Academic Press, New York.
- Kennedy, J. S., and C. O. Booth. 1963. Free flight of aphids in the laboratory. *J. Exp. Biol.* 40: 67–85.
- Kishaba, A. N., T. J. Henneberry, P. J. Hancock, and H. H. Toba. 1967. Laboratory technique for studying flight of cabbage looper moths and the effects of age, sex, food, and tepa on flight characteristics. *J. Econ. Entomol.* 60: 359–366.
- Koerwitz, E., and K. Pruess. 1964. Migratory potential of the army cutworm. *J. Kansas Entomol. Soc.* 37: 234–239.
- Krogh, A., and T. Weis-Fogh. 1952. A roundabout for studying sustained flight of locusts. *J. Exp. Biol.* 29: 211–219.
- Lebeau, J., R. A. Wesselingh, and H. van Dyck. 2016. Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes. *Proc. Royal Soc. B: Biol. Sci.* 283: 20160455.
- Liu, Z., K. A. G. Wyckhuys, and K. Wu. 2011. Migratory adaptations in *Chrysoperla sinica* (Neuroptera: Chrysopidae). *Environ. Entomol.* 40: 449–454.
- Lopez, V. M., M. N. McClanahan, L. Graham, and M. S. Hoddle. 2014. Assessing the flight capabilities of the goldspotted oak borer (Coleoptera: Buprestidae) with computerized flight mills. *J. Econ. Entomol.* 107: 1127–1135.
- Maharajan, R., and C. Jung. 2009. Flight behavior of the bean bug, *Riptortus clavatus* (Thunberg) (Hemiptera: Alydidae), relative to adult age, sex and season. *J. Asia Pacific Entomol.* 12: 145–150.
- Martí-Campoy, A., J. A. Ávalos, A. Soto, F. Rodríguez-Ballester, V. Martínez-Blay, and M. P. Malumbres. 2016. Design of a computerised flight mill device to measure the flight potential of different insects. *Sensors* 16. doi:10.3390/s16040485
- McKibben, G. H. 1985. Computer-monitored flight mill for the boll weevil. *J. Econ. Entomol.* 78:1519–1520.
- McKibben, G. H., M. J. Gradowitz, and E. J. Villavaso. 1988. Comparison of flight ability of native and two laboratory-reared strains of boll weevils on a flight mill. *Environ. Entomol.* 17:852–854.
- Miller, J. R., W. L. Roelofs. 1978. Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. *J. Chem. Ecol.* 4: 187–194.
- Minter, M., A. Pearson, K. S. Lim, K. Wilson, J. W. Chapman, and C. M. Jones. 2018. The tethered flight technique as a tool for studying life-history strategies associated with migration in insects. *Ecol. Entomol.* 43: 397–411.
- Moriya, S. 1995. A preliminary study on the flight ability of the sweetpotato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Apionidae) using a flight mill. *Appl. Entomol. Zool.* 30: 244–246.
- Mouritsen, H., and B. J. Frost. 2002. Virtual migration in tethered flying monarch butterflies reveals their orientation mechanisms. *Proc. Natl. Acad. Sci. USA* 99: 10162–10166.

- Nachtigall, W., U. Rothe, P. Feller, and R. Jungmann. 1989. Flight of the honey bee—III. Flight metabolic power calculated from gas analysis, thermoregulation and fuel consumption. *J. Comp. Physiol. B.* 158: 729–737.
- Nakamori, H., K. Simizu. 1983. Comparison of flight ability between wild and mass-reared melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae), using a flight mill. *Appl. Entomol. Zool.* 18: 371–381.
- Naranjo, S. E. 1990. Comparative flight behavior of *Diabrotica virgifera virgifera* and *Diabrotica barberi* in the laboratory. *Entomol. Exp. Appl.* 55: 79–90.
- Naranjo, S. E. 1991. Influence of temperature and larval density on flight performance of *Diabrotica virgifera virgifera* Leconte (Coleoptera: Chrysomelidae). *Can. Entomol.* 123: 187–196.
- Nayar, J. K., and D. M. Sauerman, Jr. 1971. Physiological effects of carbohydrates on survival, metabolism, and flight potential of female *Aedes taeniorhynchus*. *J. Insect Physiol.* 17: 2221–2233.
- Niehaus, M. 1981. Flight and flight control by the antennae in the Small Tortoiseshell (*Aglais urticae* L., Lepidoptera)—II. Flight mill and free flight experiments. *J. Comp. Physiol. A.* 145: 257–264.
- Nilssen, A. C., and J. R. Anderson. 1995. Flight capacity of the reindeer warble fly, *Hypoderma tarandi* (L.), and the reindeer nose bot fly, *Cephenemyia trompe* (Modeer) (Diptera: Oestridae). *Can. J. Zool.* 73: 1228–1238.
- Parker, W. E., and A. G. Gatehouse. 1985. The effect of larval rearing conditions on flight performance in females of the African armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae). *Bull. Entomol. Res.* 75: 35–48.
- Rankin, M. A., and S. Rankin. 1980. Some factors affecting presumed migratory flight activity of the convergent ladybeetle, *Hippodamia convergens* (Coccinellidae: Coleoptera). *Biol. Bull.* 158: 356–369.
- Resurreccion, A. N., W. B. Showers, and W. A. Rowley. 1988. Microcomputer-interfaced flight mill system for large moths such as black cutworm (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 81:286–291.
- Reynolds, D. R., and J. R. Riley. 2002. Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques. *Comp. Electron. Agric.* 35: 271–307.
- Reynolds, D. R., J. R. Riley, N. J. Armes, R. J. Cooter, M. R. Tucker, and J. Colvin. 1997. Techniques for quantifying insect migration, pp. 111–145. *In* D. R. Dent and M. P. Walton (eds.), *Methods in ecological and agricultural entomology*. CAB International, Wallingford, United Kingdom.
- Ribak, G., S. Barkan, and V. Soroker. 2017. The aerodynamics of flight in an insect flight-mill. *PLoS One.* 12: e0186441.
- Roitberg, B. D., R. S. Cairl, and R. J. Prokopy. 1984. Oviposition deterring pheromone influences dispersal distance in tephritid fruit flies. *Entomol. Exp. Appl.* 35: 217–220.
- Rowley, W. A., and C. L. Graham. 1968. The effect of age on the flight performance of female *Aedes aegypti* mosquitoes. *J. Insect Physiol.* 14: 719–728.
- Rowley, W. A., C. L. Graham, and R. E. Williams. 1968. A flight mill system for the laboratory study of mosquito flight. *Ann. Entomol. Soc. Am.* 61:1507–1514.
- Ruzicka, Z. 1984. Two simple recording flight mills for the behavioral study of insects. *Octa. Entomol. Bohemoslov.* 81:429–433.
- Sappington, T. W., and W. B. Showers. 1991. Implications for migration of age-related variation in flight behavior of *Agrotis ipsilon* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Am.* 84: 560–565.
- Schoenleuter, L., L. White, and B. Butt. 1970. Flight mill system for studying insect behavior. USDA, Agric. Res. Serv. Tech. Bull. 42–164, Washington, D.C.
- Schumacher, P., A. Weyeneth, D. C. Weber, and S. Dorn. 1997. Long flights in *Cydia pomonella* L. (Lepidoptera: Tortricidae) measured by a flight mill: influence of sex, mated status and age. *Physiol. Entomol.* 22: 149–160.
- Shelton, T. G., X. P. Hu, A. G. Appel, and T. L. Wagner. 2006. Flight speed of tethered *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) alates. *J. Insect Behav.* 19: 115–128.
- Smith, H. W., and M. M. Furniss. 1966. An automatically recording insect flight mill. *Can. Entomol.* 98: 249–252.
- Sørensen, J. G., M. F. Addison, and J. S. Terblanche. 2012. Mass-rearing of insects for pest management: challenges, synergies and advances from evolutionary physiology. *Crop Prot.* 38: 87–94.
- Stanfield, T. K., and F. F. Hunter. 2010. Honeydew and nectar sugars differentially affect flight performance in female black flies. *Can. J. Zool.* 88: 69–72.
- Stebbing, J. A., L. J. Meinke, S. E. Naranjo, B. D. Siegfried, R. J. Wright, and L. D. Chandler. 2005. Flight behavior of methyl-parathion-resistant and -susceptible western corn rootworm (Coleoptera: Chrysomelidae) populations from Nebraska. *J. Econ. Entomol.* 98: 1294–1304.
- Steidl, R., J. Hayes, and E. Schaub. 1997. Statistical power analysis in wildlife research. *J. Wildl. Manag.* 61: 270–279.
- Stelinski, L., R. Holdcraft, and C. Rodriguez-Saona. 2014. Female moth calling and flight behavior are altered hours following pheromone autodetection: possible implications for practical management with mating disruption. *Insects.* 5: 459–473.
- Stewart, S. D., and M. J. Gaylor. 1994. Effects of age, sex, and reproductive status on flight by the tarnished plant bug. *Environ. Entomol.* 23: 80–84.
- Stowers, J. R., M. Hofbauer, R. Bastien, J. Griessner, P. Higgins, S. Farooqui, R. M. Fischer, K. Nowikovsky, W. Haubensak, I. D. Couzin, et al. 2017. Virtual reality for freely moving animals. *Nat. Methods.* 14: 995–1002.
- Taylor, R. A. J., L. R. Nault, W. E. Styer, and Z. B. Cheng. 1992. Computer-monitored, 16-channel flight mill for recording the flight of leafhoppers (Homoptera, Auchenorrhyncha). *Ann. Entomol. Soc. Am.* 85: 627–632.
- Taylor, R. A. J., L. S. Bauer, T. M. Poland, and K. N. Windell. 2010. Flight performance of *Agrilus planipennis* (Coleoptera: Buprestidae) on a flight mill and in free flight. *J. Insect Behav.* 23: 128–148.
- Tsunoda, T., and S. Moriya. 2008. Measurement of flight speed and estimation of flight distance of the bean bug, *Riptortus pedestris* (Fabricius) (Heteroptera: Alydidae) and the rice bug, *Leptocoris chinensis* Dallas (Heteroptera: Alydidae) with a speed sensor and flight mills. *Appl. Entomol. Zool.* 43: 451–456.
- Turchin, P. 1998. Sinauer associates. Sunderland, MA.
- Villacide, J. M., and J. C. Corley. 2008. Parasitism and dispersal potential of *Sirex noctilio*: implications for biological control. *Agric. Forest Entomol.* 10: 341–345.
- Vogt, J. T., A. G. Appel, and S. West M. 2000. Flight energetics and dispersal capability of the fire ant, *Solenopsis invicta* Buren. *J. Insect Physiol.* 46: 697–707.
- Wales, P. J., C. S. Barfield, and N. C. Leppla. 1985. Simultaneous monitoring of flight and oviposition of individual velvetbean caterpillar moths. *Physiol. Entomol.* 10:467–472.
- Weber, D. C., D. N. Ferro, and J. G. Stoffolano. 1993. Quantifying flight of Colorado Potato Beetles (Coleoptera, Chrysomelidae) with a microcomputer-based flight mill system. *Ann. Entomol. Soc. Am.* 86: 366–371.
- Wells, T., S. Wolf, E. Nicholls, H. Groll, K. S. Lim, S. J. Clark, J. Swain, J. L. Osborne, and A. J. Haughton. 2016. Flight performance of actively foraging honey bees is reduced by a common pathogen. *Environ. Microbiol. Rep.* 8: 728–737.
- Wong, J. S., A. C. Cave, D. M. Lightle, W. F. Mahaffee, S. E. Naranjo, N. G. Wiman, J. M. Woltz, and J. C. Lee. 2018. *Drosophila suzukii* flight performance reduced by starvation but not affected by humidity. *J. Pest Sci.* 91: 1269–1278.
- Yang, F., Y. Luo, and J. Shi. 2017. The influence of geographic population, age, and mating status on the flight activity of the Asian gypsy moth *Lymantria dispar* (Lepidoptera: Erebididae) in China. *Appl. Entomol. Zool.* 52: 265–270.
- Zhang, Y., L. Wang, K. Wu, K. A. Wyckhuys, and G. E. Heimpel. 2008. Flight performance of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) under different temperature and humidity regimens. *Environ. Entomol.* 37: 301–306.
- Zhang, Y., K. Wu, K. A. Wyckhuys, and G. E. Heimpel. 2009. Trade-offs between flight and fecundity in the soybean aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 102: 133–138.
- Zhao, K. F., Z. P. Shi, and J. C. Wu. 2011. Insecticide-induced enhancement of flight capacity of the brown planthopper *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Crop Prot.* 30: 476–482.