**Insect Flight Behavior and Flight Mill Bibliography**

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Hardie, J. 1993. Flight behavior in migrating insects. J. Agric. Entomol. 10(4):239-245.

Hardie breaks down the advantages and cautions to using a flight mill to experiment insect flight behavior. His overall message is that flight mills are useful in that they can help distinguish migratory flight behavior from foraging flight behavior. However, in order to do so, researches should be aware that flight mills are often semi-sensory depleted because are used in the lab and not the field. In the field, it is possible that insects interrupt, change, and/or terminate their flight due to the introduction of a calling female, a food plant, or a suitable oviposition site (241). For that reason foraging flight behavior demonstrates targeted flight (when an insect changes flight in response to a stimuli) and periods of upwardly directed flight between approach-provoking target presentations. On the other hand, migratory behavior demonstrates insects ignoring any stimuli presented by the researcher. In turn, we might want to consider introducing stimuli to the soapberry bugs; this could include (as suggested by the review paper) using cards or screens that present or flash the host plant of the bug within their chamber, introducing juvenile hormones, or introducing a food odor like apple cider vinegar or host plant/nonhost plant volatiles.

Taylor, R. A. J., et al. 2010. Flight performance of *Agrilus planipennis* (Coleopetera: Buprestidae) on a flight mill and in free flight.J Insect Behav. 23: 128-148.

Taylor, et al. provided a very categorical approach to testing their bug of choice, the emerald ash borer (EAB), on a flight mill. They split their testing groups by 1.) sex, 2.) mated and unmated, 3.) light duration (24-hours or 16:8 Light:Dark cycle), and 4.) rest and food or no rest and food. No external motivation was given (like blowing on the bug)– it was a very sensory depleted environment except for light. Then after flight mill trials, free-flight experiments using a geometric setup with a wall, mirror, and camera was used (Fig 1.). We could replicate this experiment with lab raised soapberry bugs. What’s interesting to note is that mass was not a huge factor and “mated females flew almost 2.5 times as far as unmated females” (137). Also quoted from the discussion, “It has often been found that reproductive apparatus in migratory insects develop as flight muscles atrophy, the oogenesis-flight syndrome (Johnson 1969). Insects that exhibit the oogenesis-flight syndrome also exhibit a strong correlation between size and flight performance” (144). Finally, bout patterns were analyzed (which we didn’t because of the structure of our experiment) and they found that the pattern of flight by unmated females in constant light and in 16:8 (L:D is more like the male illustration than the female (Fig 3).

Minter, M., et al. 2018. The tethered flight technique as a tool for studying life-history strategies associated with migration in insects. Ecological Entomology. 43: 397-411.

This paper reviewed 85 studies that have directly incorporated at least some aspect of and the various avenues of tethered flight into experiments, including population genetics techniques, behavioral experiments, and flight mill design. Below I quoted important/relevant passages:

“Rotational flight mills have seldom been used to understand the impact of photoperiod…so far, the best examples of experimental shifts in photoperiod disrupting migratory behavior come from flight orientation studies…It would be interesting to extend this work to see whether similar day-flying migratory species, besides the Lepidoptera, use time compensation as a navigational tool and whether gradual changes in photoperiod, experienced during development, influence flight propensity on the flight mills.” (404) We could do photoperiod and temperature experiments to better understand the window in which soapberry bugs fly?

“…age is a significant factor when flying insects on flight mills and *a priori* knowledge of the species’ biology is a necessity if using these assays to study migration. Indeed, age is the most common factor investigated using flight mills to determine the timing of peak flight activity as a proxy for characterizing the migratory window in the field.” (405) We saw that flight performance in male soapberry bugs was impacted by days from start. We could do a more developmental/age approach to flight?

“Flight performance is generally positively correlated with the body mass of the insect, largely reflecting the proportional increase in fuel load (Roff, 1991).” (406) What is mass doing for soapberry bugs? We have found negative correlations.

“For each species under study, efforts should be made to understand how factors such as the absence of tarsal contact or lack of natural lift required to support body mass influence flight outputs and how this relates to energetic expenditure of the insect. The biggest challenge still to be addressed is how flight patterns observed on rotational flight mills can be used to define true migratory from non-migratory behavior without relying on assumptions related to the biology of the insect.” (407)

Jones, H., et al. 2016. Quantifying interspecific variation in dispersal ability of noctuid moths using an advanced tethered flight technique. Ecology and Evolution. 6(1): 181-190.

Jones and team built a flight mill similar to ours, but it instead had 16 possible slots for bugs to hop on and bugs were attached via a small handle that was glued to their thorax, which hooks onto the flight mill arm. Their flight data also was formatted slightly different. Also, there was no flag on the flight mill arm; instead, there was a stripped disk with a banded pattern and attached to the axis so that it turns with the arm and crosses a light detector which detects the movement of the bands which records speed and distance. Their system recorded flight distance to the nearest 10 cm every 5 seconds. So one data file has the following columns: No., Date, Time, Channel 1, Channel 2, Channel 3, Channel 4, Channel 5, Channel 6, Channel 7, Channel 8, where the channel columns record the distances in cm travelled in those five second intervals. The circular trajectory of the arm had a circumference of 50 cm. look at Fig 1 (A) for more details. Furthermore, these were the limitations of the flight mill: harder to simulate sensory cues, lack of tarsal contact, effort of pushing the flight mill, and tether restricts natural flight and obviates the need for insects to produce sufficient lift to overcome their body weight.

Main interesting finding of this paper was that, after calculating 16 tethered flight performance variables across 24 different species of British noctuid moths and then asking 5 ‘experts’ to rank on a scale of 0-2 the flight capability of these species (0=low, 1=medium, 2=high), “total distance flown over night” and “maximum speed” were the most informative variables for distinguishing among the 24 study species. They also found that for the moths ranked high by experts, they also flew farther and had faster speeds. For species where there was confusion about whether they had a low or medium flying capabilities, there also uncertainty in how well they flew in the flight trials. Finally, here was an interesting quote: “We are currently developing calibration methods that will enable the comparison of distances flown on different arm types, thereby opening up new possibilities to compare a much wider range of taxa.” (188) This made me wonder about what “calibration” methods they’re concerned about (e.g. having arms that are different weights and so produce different drag?) It would definitely be interesting to learn more about this because every machine attempts to calibrate for their materials (e.g. the laser cutter I used to build the acyclic frame of the mill).

Kennedy, J.S. and Ludlow, A.R. 1974. Co-ordination of two kinds of flight activity in an aphid. J. Exp. Biol. 61: 173-196.

First, the authors define their investigative terms:

*migratory flight* – flight oriented vertically and horizontally towards a large overhead light source | it has the same meaning as ‘flight’ in previous experiments, namely, climbing flight directed phototactically toward a large overhead source of light in a laboratory flight chamber.

*targeted flight* – flight oriented towards a yellow leaf-like object seen to one side against a dark background | horizontally directed approach-response of the flying aphid to a visually-contrasting object at its own level, in this case a leaf-shaped yellow card seen against the black walls of the flight chamber.

*antagonistic induction* – when the after-effect of the temporary inhibition was a net strengthening

*antagonistic depression* – when the after-effect of the temporary inhibition was a net weakening

This research was also interesting because the flight mill of 50 years ago seemed very theatrical. There was a lot of manual work and a series of devices used in order to test the flight dispersal of insects. Here are some examples that illustrate what I mean by ‘theatrical’:

The flight chamber itself was a vertical wind tunnel with a closed-circuit airflow system.

Chambers, D. L., Sharp, J. L., and Ashley, T. R. 1976. Tethered insect flight: A system for automated data processing of behavioral events. Behavior Research Methods & Instrumentation. 8: 352-356.

Main takeaways from this paper is that their flight mill system was a lot larger and more expensive than modern day systems. It took a lot of electrical and data encoding infrastructure to make an automatic event recording system (something we take for granted now). But thanks to the progress of data acquisition and data recording devices, we are able to cheaply and efficiently record a lot of data. Here are then just a few notes and quotes that were interesting about the paper:

They mounted insects on the rotor arm by using rubber sleeves ( Chambers & O’Connell, 1969 used that too).

Head-down posture = “Earlier empirical data indicated that optimal performance was obtained by positioning the insect with the body tipped, head down, at an angle of 12-14 deg from horizontal.” (We did not do this also they sited no one.)

One thing could do is weigh everything on the flight mill like they did.

One interesting thing about this paper is that its written structure could be used as inspiration for formatting my own paper.

After running experiments on *Anastrepha suspense* with different hub geometrics, they note “As Hocking (1953) points out, most energy loss on a mill system is metabolic rather than aerodynamic. Thus, decisions on hub and rotor size may be governed more by the logistics of mounting, inertial weight, and flight velocities amendable to system reaction time than by considerations of aerodynamics and angular velocities.” (355)

Benefit of their system (during their times) (355) “Also, the components of the system are separable for adaption to types of tests other than those described.” And on (356) “The most salient features are low friction, flexibility, and high data output, with low cost in terms of man hours in data transduction.”

Blackmer, J. L., Naranjo, S. E., and Williams III L. H. 2004. Tethered and untethered flight by Lygus Hesperus and Lygus lineolaris (Heteroptera: Miridae).

The flight mill in this experience was very similar to the one we constructed except for any 3D printing. There were 24 individual units make of Plexiglass and wood, IR sensors, a flag to break the beam, and a mill arm where insects were tethered to. They split their trials between a tethered flight mill and an untethered flight chamber. They focused on determining how two species with different geographic ranges affected their flight periodicity, flight duration, and number of flights. They also assessed how sex, age-range, and reproductive/mating status (egg load and sperm sacs) within each species affected those flight parameters as well. (They also focused a lot on collecting data for and analyzing “trivial flights” or what we called bursting flight). Finally, they used a vertical flight chamber to study responses to varying light intensities as a way to compare rates of climb between the species. Overall, they found that the females of each species, were “well-adapted colonizers that are capable of lying with a full complement of eggs, allowing them to readily exploit new habitats.” There were also strong effects of age and sex throughout the paper, but species was often a weak effect except when analyzing flight periodicity. For *L. hesperus* females, sustained flights followed a diurnal to crepuscular periodicity, whereas sustained flights by *L. lineolaris* females were nocturnal. Finally, I thought flight periodicity was an interesting parameter to analyze and something we could analyze for the night flyers.

Notes:

They tethered with dental wax.

Gives a very accessible vertical flight chamber protocol. Flight mill vs. flight chamber = “ Rates of climate toward the skylight cue were about 0.5 m/s, indicating a capacity for strong, self-directed flight by both species.” (1389)

Similar to what we saw: “ Number of flights and flight duration were influenced by age and sex for tethered individuals and by sex for untethered individuals. Species differences were less apparent, but tethered *L. lineolaris* had more sustained flights (individual flights > 5 min) that were of longer cumulative duration compared with *L. hesperus*.” (1389)

Naranjo, S. E. 2019. Assessing insect flight behavior in the laboratory: A primer on flight mill methodology and what can be learned. Annals of the Entomological Society of America 112: 18-199.

As Naranjo iterates throughout the paper, there have been “nearly 260 studies that have been published using flight mills covering 214 species in 61 families and 9 orders.” Naranjo also clearly shows the evolution of the flight mill since the 1950’s (Hocking 1953 was the pioneering researcher behind the creation of the flight mill). He does so through a series of figures and graphs showing the growth in papers and the change in materials going from glass, photo cells, and chart recorders to automation, stainless steel hypodermic tubing, and IR (all the features we’re using). What makes our flight mill so unique is not only the use of magnetic paint but also our utilization maker space (aka the MADD center on campus). There has been no paper that has utilized 3-D printers nor any paper that attempted to make sure their structures were leveled using leveling screws. Our mill may not be unique in concept but it is unique is this aspect and it might incentivize other researchers to make use of the “future technical advances driven by the maker space revolution” (196).

Other things to note: Naranjo points to the lack of research investigating the importance of arm size (e.g. shorter vs. longer arm length effect on drag), the effect of the presence or absence of a landing platform, the effect of sensory cues (visual, airflow, or chemical) on flight behavior, and also the need to determine if smaller intervals of longer assays may be sufficient to address study needs (since these trials can be time consuming).

Finally, check out Fig. 10 to see where the soapberry bug falls in relation to other species that have been tested. Lepidoptera and Coleoptera have been the most studied groups with a large emphasis on migration.

Martini, X., et al. 2014. Abdominal color of the Asian citrus psyllid (Hempitera: Liviidae) is associated with flight capabilities. Annals of the Entomological Society of America, 107(4): 842-847.

Between soapberry bugs there wasn’t color variation and since this paper was short I read it out of curiosity. Not only what they found is this significant color factor but they also found no significant difference in flight performance between the two sexes and not much differences in age either. These results are almost the inverse of our results, which makes our question as to why polymorphism persists in field populations an overall intriguing question. This can also further make us ask more in an evolutionary framework than a modeling framework, what makes a trait important to flight?

Ribak, G., et al. 2017. The aerodynamics of flight in an insect flight-mill. PLoS ONE 12(11): e0186441.

This paper goes into a lot of detail (20 pages) on what is essentially really nicely summarized in the abstract. “Flight mill type did not affect flight speed or wing-beat frequency, but did affect flapping kinematics. The wingtip internal to the circular trajectory was always moved faster relative to air, suggesting that the beetles were attempting to steer in the opposite direction to the curved trajectory forced by the flight mill. However, banked beetles had lower flapping asymmetry, generated higher lift forces and lost more of their body mass per time and distance flown during prolonged flight compared to beetles flying level. The results indicate, that flapping asymmetry and low lift can be rectified by tethering the beetle in a banked orientation, but the flight still does not correspond directly to free-flight. This should be recognized and taken into account when designing flight-mills and interoperating their data.”

Guo, J., et al. 2020. Flight performance of mamestra brassicae (lepidoptera: noctuidae) under different biotic and abdiotic conditions. Journal of Insect Science, 20(1): 1-9.

Guo et al. found the age, temperature, and relative humidity to significantly impact flight performances and wing beat frequency (sex didn’t). They used a flight mill and a stroboscope in order to test this pest insect for 12 hours form 8 pm to 8 am, using multiple trials per moth. There are multiple figures that show the nonlinear regressions fit for all the flight performance parameters in order to find the optimal temperature, age and RH in which the M. brassicae performed. They found that the optimal age was around 3 days, temperature around 24 C and RH from 64-75%. Also, across all biotic parameters, as flight distance, duration, and speed increased, the number of flights decreased. This could be due to the fact that when the moth had more energy, it’s able to sustain longer distances on a single bout and not have to stop and start flying multiple times. In addition, the paper addressed muscle histolysis as the reason behind flight performance degradation after the age of 3 days (page 7).

Irvin, N.A. and Hoddle, M. S. 2020. Assessing the flight capabilities of fed and starved Allograpata obliqua (Diptera: Syrphidae), a natural enemy of Asian citrus psyllid, with computerized flight mills. Florida Entomologist, 103 (1): 139-140.

This study tries to determine how sex and feeding treatment (fed or starved) effect flight parameters (distance, time flying, time resting, number of bouts, valid bouts, mean bout times(s), time to first bout in (min), and % loss weight) during 24 hour flight trials for *A. obliqua*. The motivation of studying this fruit fly species is that they are a natural enemy of *D. citri*, which have been a pest in cropping systems, especially citrus trees. In turn, the researchers took 3-9 potted citrus trees with *D. citir* nymphs in a grapefruit block as a way to collect *A. obliqua* from the field. Then, they transferred the trees to the lab in a plastic cage untilf fly pupae were observed. Those fly pupae that were newly emerged were sexed and randomly allocated to either fed or starved treatments (although there were not many – only 17 and 13 died within the 24 hour flight time). Then they were weighed, flight tested, and reweight after. They found that ther were no significant effect of feeding or sex except for the flight parameter time to first bout (min). In addition, the fruit flies did not travel far. The farest flight burst was 82 m. Ultimately, they determined that A. obliqua would not be a long dispersal fly and instead their flight activity may resemble “hovering” more.

Reynolds, D.R., and Riley, J.R. 2002. Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques.

This is an extensive review paper. I went ahead and copy and pasted the abstract because it summarizes it best and then listed important quotes and ideas extracted from the paper.

Abstract: This paper provides an overview of the recent literature on electronic, remote-sensing and computer-based techniques for observing and monitoring insect movement in the field and in the laboratory. Topics (such as entomological radar) which are covered in detail elsewhere in this Special Issue are deliberately omitted. Techniques which have been used, or which have potential for use, in monitoring insects in the field, include optical and opto-electronic devices, videography, thermal imaging, radio frequency identification (RFID), radio-teleme- try, X-ray radiography and computed tomography, sodar and sonar. The discussion includes optical sensors and insect trapping, instrumented beehives, acoustic detection of insects in grain, fruit and soil, and various laboratory methods for studying insect movement, such as actographs, treadmills, automatic flight mills, and the video recording and analysis of movement in wind tunnels and in indoor arenas. Airborne and satellite imaging of insect habitats is mentioned, but only in the context of the use of these techniques to deduce changes in population distribution in some migratory species. Finally, some of the main constraints to progress in the sensing of insect movement, and areas where rapid advances seem possible, are discussed.

Quotes and Ideas:

Cameras have been used not only to get wing beat frequency but also body temperature (thermal imaging cameras). Some also promising papers to read include Reynolds et al. (1997) and Room et al. (1998), which made a survey of techniques for quantifying a particular class of insect movement. We already talked about MIR and CT scans but they’re expensive. Cameras are versatile – can be used to sensory cue experiments like that ant trail experiment paper we read. Also, some questions I had in response: what is the vision of the soapberry bug – good/bad? And how good I their flight control?

“recently some simple radio transmitters have been made light enough to be carried by large insects (e.g. Kutsch, 2002 and Hedin and Ranius, 2002).”

biochemical and molecular biology techniques to investigate insect movement and gene flow (e.g. Loxdale and Lushai, 1998, 2001)

“Scientists at the Centre for Plant Architecture Informatics in Brisbane, are currently using a GTCO Freepoint 3D sonic digitizer (GTCO CalComp Inc., Columbia, Mary- land) to measure plant co-ordinates for virtual plant/insect models (http:// www.cpai.uq.edu.au/), and various other new measurement technologies may be applicable in the future (Room et al., 1998).”

The ‘shadow’ method is when you are “able to reconstruct the three-dimensional position of cecidomyid midges swarming over a marker, by using the relation of the midges to their shadows.”

“A possible avenue for tracking many big insects within very large cages may be through systems like the 3D-iD indoor location and tracking system devel- oped by PinPoint Corporation of Billerica, MA…”

High spatial resolution images from earth observation satellites have, however, been useful for detailed mapping of potential locust habitats (Bryc … The coarse spatial (1–5 km), but high temporal, resolution images from meteorological satellites are routinely used for migratory pest forecasting…

Other methods/devices used: servosphere to analyze walking, acoustic sounder detection, virtual reality simulators, high-speed video recording of insect wing motions; and supercomputer simulation of the two-dimensional fluid motion around wings (Wang, 2000). Then the paper ends with flight mill/tethering techniques

“Among the tethering techniques we have static tethering, flight balances, roundabouts and mills (see reviews by Hardie, 1993; Cooter, 1993; Dingle, 1996; Reynolds et al., 1997). Recent descriptions of computer-monitored flight mills are given in Taylor et al. (1992), Weber et al. (1993), Beerwinkle et al. (1995), and Schumacher et al. (1997)….Preiss and Kramer (1984) developed a sophisticated flight mill which presented the test insects with a moving visual environment of the type which they might experience in free flight, and thus allowed them to adjust their flight speeds…In the ‘barber’s pole’ tunnel, the optomotor effect is produced by the movement of an outer tube marked with a helical stripe pattern which rotates around a transparent inner tube containing the insects (David, 1982). In each of the above cases, detailed flight manoeuvres in the wind tunnel can then be recorded by video camera and the flight path reconstructed….The resultant flight paths are usually two-dimensional (in the horizontal (yawing) plane), but a smaller number of wind tunnel/flight chamber studies have employed two video cameras with an overlapping field of view, thus enabling a three-dimensional analysis of flight tracks (Young et al., 1993; Mankin and Hagstrum, 1995; Hardie and Young, 1997; El-Sayed et al., 2000; Fry et al., 2000; Hardie and Powell, 2002).

And finally: “The spatial resolution and frame rate of video systems were, at the time, considered inadequate to measure the body orientation during flight of the blowfly *Calliphora* T*icina* in a 40×40×40 cm cage, due to the insect’s relatively fast movement and very sharp turns (several thousand degrees per s) (Schilstra and van Hateren, 1998). These authors, therefore, recorded the position and thorax orienta- tion of the flies using a variation of the induction coil technique often used for measuring head and eye movements in humans and other animals (cf. magnetic motion capture systems like the Polhemus FASTRAK mentioned above). A time-varying magnetic field was produced around the experimental cage with three orthogonal pairs of field coils, carrying sinusoidal currents at frequencies of 50, 68, and 86 kHz, respectively. Each pair of field coils induced a voltage at the corresponding frequency in each of three tiny orthogonal sensor coils mounted on the insect. The sensor coils were connected via very fine (12 mm) wires to a set of nine lock-in amplifiers, each locking to one of the three field frequencies (Schilstra and van Hateren, 1998). It was possible to measure orientation with a typical accuracy of 􏰀0.5 degrees, and position accuracy to 1 mm. The maximum weight of the sensor coils and leads was approximately 5.7 mg, and this apparently did not hinder normal flight in an insect weighing about 80 mg.”

Davis, M.A. 1986. Geographic patterns in the flight ability of a monophagous beetle. Oecologeia. 1-6.

Davis studied the flight performance of a monophagous insect, *Tetraopes tetraophthaimus* (Cleopetra: Cerambycidae), the red milkweed beetle, using a still air tethering technique. He collected 9 males and 9 females from each of the 10 populations across two distinct regions (river valleys and mountainous areas across the US Northeast) and tested only one flight parameter - the time a bug flew within a 30 minute trial. He also measured the body size of the insects by measuring the body width at the anterior edge of the elytra using a hand held micrometer. From a previous study he did, he stated that body width is strongly correlated with body mass (r=0.93; Davis 1984). He analyzed the data using a multiple analysis of variance to compare flight capacities and body sizes between regions and between population within regions (worked with means not individuals). He found that his study supported what is known as the FLY hypothesis (long distance migraters = Females, Large, and Young). He also found that region differences did impact flight performance where mountainous populations were more migratory (flew more) and were more heterogenous (had more flight variety). Mostly the females were driving these relationships among regions because when only the males were compared, the comparisons were not significant. In addition, there were no significant differences in body sizes of the beetles between the two regions but mountainous populations were more heterogenous when only females were considered. Finally, the magnitude of the size differences between males and females correlated with female size, “meaning that the magnitude of the sexual dimorphism varied considerably between populations” (4)

Dingle, H. et al. 1980. Variation in body size and flight performance in milkweed bugs (oncopeltus). Evolution. 371-385.

They do not talk much about the ‘tethered flight’ that they used to test their mix of lab-raised and field-collected milkweed bugs. They only mentioned that they were tethered, and that each individual bug was tested 5 times in 30 minute intervals. Only their flight duration was recorded (in minutes). On the other hand, they went into extensive detail about body size variation between milkweed bugs collected across 10 different populations. That detail is worth it though because they did a comprehensive analysis across a huge geographical gradient from the continental mainland (Florida, Mexico) to the large island of Puerto Rico and finally to the smaller more isolated island of Guadeloupe. They also collected milkweed bugs in Michigan, Iowa, Maryland, Georgia, Jamaica, Trinidad, and St. Vincent. A lot of their data isn’t entirely comparable, like their museum data, but their wing length measurements, which they used as a proxy of body size, showed that females were generally larger than males and the tropical species were generally smaller than the continental species. Moreover, they found that the proportion of bugs that flew over 30 minutes was generally higher for populations collected on the continental mainland than those collected from the oceanic islands (goes from .24 to .045). However, across all populations, it seems like most bugs were what we call bursters or as what they define as bugs who flew for < 5 minutes.

Pollack, G. S. and Martins, R. 2007. Flight and hearing: ultrasound sensitivity differs between flight-capable and flight-incapable morphs of a wing-dimorphic cricket species. The Journal of Experimental Biology, 210: 3160 – 3164.

No flight trials were conducted in this piece, but there were a lot of useful neurobiological observations and explanations for the link between flight and hearing in insects. This study focused on testing a lab-reared cricket species, *Cryllus texensis,* because of its flight dimorphism (there is a long-wing and short-wing morph). They compared auditory sensitivity between flight-capable, or long-wing and not-histolized crickets, and flight-incapable, or short-wing and long-wing, histolized crickets during the crickets’ scotophase. To test this, they produced sound stimuli via digital-to-analog boards with sampling rate of 100 kHz. They then monitored the abdominal position of an individual cricket by using photocells masked by a V-shaped covering, so that the shadow of the abdomen varied as the abdomen was flexed to the left or right. Then, they stuck the cricket’s pronotum to a stick and issued a wind stream to induce flight. They also dissected the cricket to expose its prothoracic ganglion and cervical connectives in order to record ON1 and AN2 (AN2 is sensitive to high frequencies) concentrations in real-time. The photocell output and stimulus marker was recorded to computer files with a sampling rate of 1 kHz. Each individual had 5 trials, 1 minute apart, with increasing or decreasing stimulation in order to determine thresholds, or the lowest sound level that evoked a response on at least three of five stimulus presentations. They found that ultrasound hearing is poorer when flight is no longer possible, meaning that they observed a significant loss of high-frequency hearing (thresholds were higher) in individuals that cannot fly (short-wing and long-wing with histolyzed muscles). They theorized that this sensitivity to ultrasound is closely linked to flight ability and to the risk of predation from bats (need to hear high-frequency call of bats if you’re a flyer flying with bats at night).

Chen, M., et al. 2015. Flight capacity of Bactrocera dorsalis (Diptera: Tephritidae) adult females based on flight mill studies and flight muscle ultrastructure. Journal of Insect Science, 15(1): 1-7.

This study compared flight data with the flight muscle ultrastructure of *B. dorsali* adult females of similar age, including myofibril diameter, sarcomere length, and volume content of mitochrondria which are regarded as the main indicators of flight capacity of insects. “In general, flight capacity in insects has been shown to increase with longer myofibril diameter, shorter sarcomere length, and higher volume amounts of mitochondria.” (1) However, they did not use the same bugs that were flew with the bugs that were dissected and they only flight tested each female bug once. All of their bugs were also collected from Yunnan Province, China and then lab raised for 10 generations. Female bugs of the same size and age range were tested together in order to determine the effect of age on flight duration, flight distance, flight speed, and flight max speed in a 13 hour flight trial window. They found that female bugs of age 15 days after adult emergence had the highest significant flight parameter values. Similarly but not truly comparably, female bugs at age 15 had the longest myofibrillar diameter, shortest sarcomere length, and highest volume percentage of mitochondria in the muscle fiber (see page 5 for descriptions on what is advantageous in flight muscle ultrastructure). All the flight muscle ultrastructure data was obtained via transmission electron microscopy.

Taylor, R.A.J., et al. 1992. Computer-monitored, 16-channel flight mill for recording the flight of leafhoppers (homoptera: auchenorrhyncha). Entomological Society of America. 85(5): 627-632.

Since it’s more relevant to flight mill technology, I’ll start with the end of the paper. Taylor and collegues list the limitations of the flight mill, stating 1) preflight handling of aphids increased their inclination to settle (reduced time spent flying), 2) the flight mill eliminates lift force expended by insects when they takeoff for flight, and 3) parasite drag occurs where the flight mill parameters are underestimates of real flight activity because insects must overcome inertia when accelerating and overcome friction and air resistance. Despite these limitations, flight mills are great for comparison analyses. The mill they constructed was very similar to ours (not in look but in concept) except for the monitor circuit diagram, which recorded grounds not peaks in the voltage. The value of the input voltages are then read by a multiplexor and placed in buffers accessibly by a FORTRAN program. The program checks each buffer for a zero value, in order to identify when a bug completed a full revolution.

Nachtigall, W., et al. 1995. Flight of the honey bee VII: metabolic power versus flight speed relation. Journal of Comparative Physiology B. 165: 484-489.

Nachitgall et al conducted both a metanalysis and their own study in order to evaluate the power vs. flight speed relationship of honey bees. They conducted the metanalysis through critiques on methodology and a series of conversions. Moreover, they conducted their own study by taking long-term measurements of tethered flying bees between the speed range of 3.3 m/s and 5.1 m/s using a two component aerodynamical balance (they followed Hanauer-Thieser and Nachtigall, 1995) They already had an idea, from airplanes, that the relationship was going to be a 2nd order polynomial and they double checked that relationship with their data by comparing models by their residuals. The best fit model was the 2nd order polynomial. Thus, they presented a minimum metabolic power curve for honey bees where the minimum metabolic power was approximately 37.5 mW for medium-weight bees and the minimum power speed was about 3 m/s.

Cheng, X. and Sun, M. 2016. Wing-kinematics measurement and aerodynamics in a small insect in hovering flight. Scientific Reports: 1-11.

Chen and Sun used an enclosed flight chamber with three orthogonally aligned synchronized high-speed cameras to record wing and body kinematics of a vegetable leafminer. They also took detailed morphological measurements on the wing shape, wing length, the area of one wing, the radius of the second moment of wing area (radius of gyration), and the total mass of the insect. Finally, they computed the flows and aerodynamic forces experienced by their model specimen in hovering flight. They found that the small fly vegetable leafminer uses relatively high wingbeat frequency (~265 Hz) and large stroke amplitude (~182). Therefore, even if its wing-length is small (1.4 mm), the mean velocity at the radius of gyration of the wing reaches about 1.5 m/s, about the same as that a of a fruit fly (an average-size insect, R ~ 3 mm; but the Reynolds number, Re, of its wing is still low (~ 40) owing to the small wing size (Re for average- and large-size insects is above 100). Moreover, in order to have a large stroke amplitude, ϕ, the wings have a follow a ‘clap and fling’ motion. Finally, although the small fly can produce enough lift to support its weight, it needs to overcome a larger drag to do so.

Makumbe, L. M., et al. 2020. Effect of sex, age, and morphological traits on tethered flight of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) at different temperatures. Physiological Entomology. 45: 110-119.

Makumbe et al. tested the oriental fruit fly like we would test soapberry bugs except that the fruit fly was lab-raised 18+ generations and they tested the effect of sex and age on tethered flight. They had three flight mills set up in a 238-L cooling incubator (MIR-254 Panasonic Healthcare Company, Japan). Temperature was randomly set to one of these seven temperatures – 12, 16, 20, 24, 28, 32, 36 – for each set of flights attached to the three flight mills. This helped account for diurnal differences in flight activity, which we see in our data too. Soapberry bugs that fly in the morning will fly longer than those in the afternoon. 3 -, 10- and 21-day-old *B. dorsali*, adults were grouped and tested each day (total of 82 flies). They recorded date of the experiment, cohort number, weight prior to flight, age, and sex of the fly, fly label, flight mill number, and test temperature. They found that temperature had an effect on most recorded tethered flight parameters in *B. dorsalis* (such as total distance, number of bouts, and max speed). However, only variables such as mean total distance flown and number of bouts followed a typical thermal performance curve, with a thermal optimum within the range of 20-24 C. No variable effected average speed (null model was the best fit**). They also calculated wing loading which was equal to body mass (Newtons) / wing area (m^2), which is something we could calculate**. They found that wing loading increased with age, but we may not see that in soapberry bugs because of their muscle histolysis. They also found that body mass and wing loading were associated with flight distance. Congruently, **total flight duration across groups** can also be graphed/analyzed. Age also effected distance flown where 10- and 21-day-old flies covered a greater distance than 3-day-old flies. Sex was not always significant but it effected max speed when it varied with age. Max speeds were lowest at 10 days and the highest at 21 days of age in females.

Liu, Y., et al. 2020. Strong impacts of smoke polluted air demonstrated on the flight behavior of the painted butterfly (*Vanessa cardui* L.). Ecological Entomology. 1-14.

Liu Y et al. asked whether smoke polluted air (from unscented incense sticks) impacted flight speed, duration, and distance of the painted butterfly, using OLS regressions and t-tests (n=20, age = all the same, lab-raised; 9 parts water and 1 part honey diet; mixed sexes). To find out, they first did preliminary trials to test how quickly the butterflies got fatigued by testing all butterflies under controlled conditions in a tethered flight mill. They found that after 30 minutes the speeds and distances significantly dropped (t-test, compared first 10 minutes to fourth 10 minute chunk). In turn, they set their main trials at 30 minutes to minimize the impacts of fatigue. They had a control condition with no pollution and a polluted condition that varied at three levels: low smoke (LS), medium smoke (MS), and high smoke (HS). In addition, during polluted conditions, butterflies first had a pre-treatment, nonpolluted 10 minute period before adding the treatment – the incense sticks. They tested each butterfly in each condition 3 times in order to increase the study’s statistical power. They found that between the pre-treatment and treatment conditions, flight distance was 25% less in the 10 min of the MS smoke treatment than in the subsequent treatment period, and average speed 26% lower. Also maximum speed in the MS condition was lower than that in the pre-treatment period by 43%. LS and HS treatments were not significantly different. Then, comparisons between the control and treatment conditions showed that average speed declined by 54% for LS, 58% for MS, and 56% for HS; maximum speed declined by 43%, 42%, and 40%; and flight duration declined by 32%, 15%, and 10%. Finally, figures 6 and 7 are particularly notable as they plotted the relationships between flight speed and PM2.5 concentration. They found as PM2.5 concentrations increased, the variation and extremes in speed decreased, suggesting that under polluted conditions the painted butterfly has a narrower speed window.

Barros, L. S., et al. 2020. Sublethal effects of diamide insecticides on development and flight performance of Chloridea virescens (Lepidoptera: Noctuidae): implications for bt soybean refuge area management. Insects. 11: 1-18.

Insecticides used to kill off tobacco budworms, a pest, from attacking cotton, vegetative, and reproductive structures of soybeans are frequently in use in Canada, the US, and throughout South America where the tobacco budworms inhabit. However, there is the concern of resistance evolution where survivors of lethal dosages of insecticides will go on to mate with each other and great an insecticide-resistant gene pool among budworm populations. There are multiple soybean management strategies to combat this, so this study focuses on one of its latest strategies which is a refuge area management meant to enclose the crops in a buffer zone. In turn, Barros et al. tests how two types of common insecticide – flubendiamide (Flu) and chlorantraniliprole (Chlo) – can impact the development and flight performance of tobacco budworms. To test this, they feed lab-raised larvae on treated and nontreated diets and then count larval, pre-pupal, and pupal mortality, deformity, and days of development. Once adults, survivors are placed into rearing cages where eggs and fertile eggs laid by pairs are counted daily until female death, and male and female adult longevity are recorded. The life-history parameters analyzed included intrinsic rate of increase, the finite rate of increase, net reproductive rate, and mean generation time. Then, to test flight performance, flight assays were conducted in a flight mill with adults. Male and females were flown on different days to avoid any disturbance form sex pheromones. The fill mill was set to 25 +/- C and 60 +/- 10% RH and assays were conducted at night from 7 PM to 7 AM. Two flight type categories were observed: sustained (>30 min) and unsustained (<=30 min). ANOVA was used to determine differences between flight parameters (speed, distance, duration, arrest, and number of bouts). They found that the insecticides increased development times for all growing stages and that weight and survival was lowest for Chlo. For flight trials, they did find that regardless of treatment, tobacco budworms would be able to disperse beyond the refuge area in a single or multiple flight bouts; both sexes flew > 6400 m in a single flight, which can lead to the mixing of resistant and susceptible populations from refuge pools. Sustained flights were generally unaffected by insecticide exposure. Only for unsustained flights were there differences where Chlo increased flight propensity relative to the control and Flo decreased flight propensity relative to the control.