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BEHAVIORAL MANIPULATION OF MOSQUITO (DIPTERA: CULICIDAE) HOST SEEKING USING ARTIFICIALLY APPLIED WIND AND VAPOR-PHASE REPELLENT: POTENTIAL FOR PERSONAL PROTECTION

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ABSTRACT

BEHAVIORAL MANIPULATION OF MOSQUITO (DIPTERA : CULICIDAE) HOST SEEKING USING ARTIFICIALLY APPLIED WIND AND VAPOR-PHASE REPELLENT: POTENTIAL FOR PERSONAL PROTECTION

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Flight-limit and dilution-mediated models for wind effects on mosquito host-seeking behavior are explored in this thesis. Field experiments on human subjects and CDC light traps tested these models and also assessed the efficacy of DEET vapor for increasing the protective effects of wind velocity. Fan-driven application of wind and DEET vapor at 2 m/s significantly reduced mosquito orientation to, landing on, and probing of a human subject. CDC light-trap catches of mosquitoes were also reduced by addition of wind. This reduction fit a negative logarithmic function over the applied velocity range (0 to 3.7 m/s) and closely matched the theoretical dilution function as wind increases over a constant-release source. Increasing the CO_2 release rate from 650 to 1,950 ml/min increased mosquito response for all wind velocities, but had no effect on the slope of the logarithmic response. There was no correlation between applied wind velocity and mosquito mass. Collectively, these data strongly support a dilution and not flight limit as the mode of action by which wind reduces mosquito host seeking. Outdoor fans as well as vertical or horizontal wind curtains are proposed as local protection tactics against nuisance mosquitoes in a backyard setting.

I would like to dedicate this thesis to my wife, Mandy, whose support and love are always reminders of how wonderful life is.

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KEY TO ABBREVIATIONS

Abbreviations for common mosquito genera

Ae. ....*Aedes*
An. ....*Anopheles*
Cq. ....*Coquillettidia*
Cs. .... *Culiseta*
Cx. ....*Culex*

CHAPTER I:

Mosquito biology, host seeking behavior, chemical cues and repellent strategies: Literature review

MOSQUITO BIOLOGY

Mosquitoes are hematophagous insects in the order Diptera. There are approximately 3,300 species of mosquitoes worldwide. Their habitats range from arctic biomes to the tropical regions near the equator (Gillett 1972, Service 1980).

Mosquitoes are notorious for the females' blood-feeding habits, both on humans and animals. Preferred hosts range from cattle to lizards and even frogs. Female mosquitoes bloodfeed in order to supply their eggs with the protein and other nutrients necessary for larval development. Some species accumulate sufficient reserves through the larval stage to allow females to mate and lay their first clutch of eggs without a blood meal. Such autogenous egg production is useful in areas where hosts are difficult to find or bloodfeeding itself is dangerous (O'Meara 1985). Subsequent egg production in these species must be fueled by blood meals. Anautogenous species lack the nutrients to provision their eggs immediately after eclosion and mating, and must take a blood meal before all egg-laying bouts (Clements 1992). Without the need to provision eggs, male mosquitoes do not bloodfeed. They are nectar feeders whose primary role is finding and fertilizing the female.

Eggs are typically laid in water or in an area that will eventually be flooded by water. Mosquito larvae (wrigglers) are completely aquatic and feed primarily on detritus, although a few are predaceous. They obtain oxygen from the air at the surface of the water. After four larval instars, they pupate. Unlike most insect pupae, those of mosquitoes, also known as tumblers, are still active despite not being able to feed. They are sensitive to shadows and movement and respond to apparent threats by swimming down in the water column. After completing metamorphosis, the adult mosquito ecloses from its pupal case at the surface of the water, and the cycle begins again (Borror et al. 1989).

Many mosquitoes overwinter as eggs, either in floodplains that will fill the following spring, or in existing ponds. In some species, inseminated adult females overwinter in leaf litter or other protective detritus, ready to emerge and oviposit when the spring rains and warmth return (Gillett 1972).

HISTORICAL PERSPECTIVE

The impact of mosquitoes on humans is far from trivial. Mosquitoes are arguably one of the most dangerous insects to human health. In addition to nuisance biting, mosquitoes vector diseases such as malaria, yellow fever, dengue hemorrhagic fever, and several strains of encephalitis (Fradin 1998). The debilitating effects of yellow fever and malaria hindered colonization and development of sub-Saharan Africa for centuries (Diamond 1998). As human population densities grew, so did the incidence of mosquito-borne illnesses, whose influence continues to the present.

Development in the Americas has also been hindered by mosquito-borne disease. One of the most famous cases was the Panama Canal Project of the 1900s. Thousands of workers died of mosquito-borne yellow fever while digging this Pacific-Caribbean waterway. Intensive larval and adult mosquito control measures were used throughout the project area, without which it is unlikely the canal ever would have been finished (Garrett 1994).

These historical anecdotes are instructive, especially since humans are still not free of mosquito-vectored diseases. Every year, malaria alone kills an estimated three million people worldwide (Fradin 1998). While the mortality aspect of disease vectoring is staggering by itself, morbidity has an even farther reach. With perhaps 700 million people being infected by a mosquito-borne disease every year (Fradin 1998), the lost productivity and drains on the global health care system due to sublethal cases are enormous and difficult to calculate. Malaria is again an informative case study; its estimated social (medical and productivity) cost is over $1.8 billion per year in Africa (Takken and Knols 1999).

Global travel elevates mosquitoes to a new and potent position intercontinental vectors of diseases. In 1999, a strain of encephalitis known as West Nile Virus, an arbovirus in the family Flaviviridae, was first documented in Long Island, NY and surrounding areas. It is vectored primarily by *Culex* mosquitoes, and is suspected to have been brought to the USA by ship traffic (Centers for Disease Control 2001). Over 4,323 birds were characterized as infected in the year 2000, and 74 people have been infected since the beginning of the outbreak in the year 1999. Human cases can be lethal; already nine deaths have been attributed to the virus. The resurgence of the disease in the year 2000 suggests that it will be a major public health concern in the United States, and aggressive monitoring and mosquito abatement campaigns have been implemented in eight eastern US states (Novello et al. 2000)

Despite worldwide participation in mosquito-reduction programs, it is unlikely that we will ever achieve high levels of regional mosquito control. Mosquito habitats are common and range widely in size. Breeding areas may be on the scale of several squarekilometer lakes down to cattle footprints with less than 100 ml of water. Given the difficulty in targeted chemical control and the expense of area-wide chemical application, we must continue to enhance existing mosquito-control programs. Areas needing further research are the behavioral processes by which mosquitoes locate their hosts and determining how these critical behaviors might be disrupted.

MOSQUITO ATTRACTION VIA HOST ODORS

Mosquitoes are sophisticated creatures when it comes to locating appropriate hosts for feeding. They are thought to rely mainly on the array of odors that emanate from their preferred host organisms rather than visual cues. Host odors form an odor plume, which is distributed downwind of the odor source in a meandering fashion, rather than a straight line (Murlis et al. 1992). Entry into a stimulatory odor plume elicits a series of orientational behaviors allowing assessment of the direction from which the odors are being released and begin upwind flight (Murlis et al. 1992). Upwind flight is assessed primarily through optomotor response (Kennedy 1939) and will be discussed in more detail later in this chapter. Once upwind flight is achieved, the mosquito maintains the proper heading toward the odor source via its chemosensory and visual systems. Upwind flight is maintained until the insect exits the plume. Once the odor plume is lost, a mosquito begins a series of ever-broadening upwind zigzags until it reenters the plume and can again begin directed upwind flight. Straight-line flight will often exit a meandering plume; this "casting" behavior enables an insect to reconnect with the odor plume with minimal lost time and energy (Murlis et al. 1992).

The major compounds attracting mosquitoes to humans are carbon dioxide (CO_2), lactic acid, acetone, and fatty acids (Takken 1991, Takken and Knols 1999), although some other chemicals are also reported to attract host-seeking females. Such stimuli are reported to be most attractive when presented as pulsed releases (breathing) rather than constant rate releases (Gillies 1980, Geier et al. 1999).

Carbon Dioxide (CO_2)

All living organisms emit CO_2 as a byproduct of respiration. It makes sense, then, that mosquitoes would be sensitive to increases in CO_2 concentration and use them as a means to locate their preferred hosts. Release rates of CO_2 depend on the organism; some benchmark rates are 25, 250, and 2500 ml CO_2/min for a chicken, human, and cow, respectively (Reeves 1953). Rudolfs (1922) first demonstrated that CO_2 was a stimulatory odor for mosquitoes, and in 1951, Reeves performed a series of experiments that showed CO_2 was attractive to mosquitoes in a field-trapping study. While these two research efforts are cited as the "classic" studies, many researchers have contributed to the qualitative and quantitative research involving carbon dioxide's attractant role in mosquito host seeking. No credible refutations have endured, and much data have been gathered to quantify species' reactions to various levels of CO_2.

Increased release rates of CO_2 have varied effects depending on the mosquito species present. Laboratory-reared *Aedes aegypti* females are significantly more attracted to dry air plus CO_2 in comparison to their response to dry air alone (Brown et al. 1951). Similarly, *Culex tarsalis, Ae. nigromaculis, Ae. vexans, Anopheles franciscanus,* and *An. walkeri* are more effectively caught in field traps as CO_2 release rates increase (Reeves 1953, McIver and McElligott 1989). *Culex* and *Culiseta* species may be less responsive to CO_2; *Cs. inornata, Cs. morsitans, Cx. restuans,* and *Cs. pipiens* showed no increase in response as CO_2 release rates increased from 200 to 4000 ml/min (McIver and McElligott 1989). Reeves' 1953 study showed that the attractive response of two species, *Cx. thriambus* and *Cx. quinquefasciatus* actually decreased at the highest (2500 ml/min) CO_2 release rates. Kline and Mann (1998) caught fewer mosquitoes at their highest rate (2000 ml/min CO_2) for *Ae. dupreei, An. perplexens, Cs melanura, Cx. erraticus* and *Mansonia titillans*. These data suggest that individual species have a preferred stimulus concentration and upper threshold for CO_2 rather than a uniform continuous positive response for all Culicidae (Reeves 1953, Constantini et al. 1996, Dekker and Takken 1998).

While these data are generally compelling in their support of CO_2's role, removal of CO_2 from exhaled breath is one of the strongest tests of this compound's effect on mosquito host seeking. Snow (1970) filtered out the CO_2 from the exhaled breath of humans, and found an 81% reduction in the number of *Cx. thalassius* mosquitoes attracted relative to breath with CO_2. A lesser reduction occurred in *An. gambiae* s.s. and *An. melas*, which adds further support to the idea that different species have different CO_2 concentration requirements.

The trend toward increased numbers of mosquitoes attracted to traps at higher CO_2 release rates may be related to increased effective distance of CO_2 attractant plumes. McIver and McElligott (1989) found that the range of attractiveness for *Ae. vexans* and *An. walkeri* increased from 3-7m to 7-11m with an increase of CO_2 from 1000 to 4000 ml/min. Gillies (1980) proposed a linear increase for the range of attractiveness for CO_2 release rates up to 1000 ml/min. This model was based on four different trapping studies using only CO_2 as bait.

L-Lactic acid and other mosquito-attractant metabolites

While it is effective as a stimulatory odor, CO_2 is not the only chemical mosquitoes use in orienting to their hosts. CO_2 combined with human odor was more effective than CO_2 alone (Constantini et al. 1996, Gibson et al. 1997, Dekker and Takken 1998). Price et al. (1979) concur that emanations *in addition to* CO_2 are likely to be responsible for human attractiveness to mosquitoes. One of the isolated components of human odor attractive to mosquitoes is L-lactic acid. Lactic acid is excreted through the skin as a waste product of anaerobic muscle metabolism, and volatilizes into the air. Lactic acid may act alone as an attractant (Smith et al. 1970, Geier and Boeckh 1999), or it is possibly just a synergist with CO_2 (Acree et al. 1968). Mosquitoes possess grooved-peg (A3) receptors on their antennae for this chemical, but these receptors are not also sensitive to CO_2 (Davis and Sokolove 1976). Therefore, the central nervous system must mediate any synergism between lactic acid and CO_2 rather than the sensory apparatus itself. Lactic acid alone does not elicit takeoff responses in *Ae. aegypti*, but it does enhance the landing and probing responses in combination with CO_2 (Eiras and Jepson 1991). These results suggest that lactic acid may be a required short-range cue (perhaps an arrestant) at the very end of the host-seeking behavioral chain.

Some other chemical metabolites have been shown to either increase the number of mosquitoes caught in traps or effect a behavioral change in the laboratory. 1-Octen-3-ol has species-dependent attraction effects. For some mosquitoes, it is both an attractant on its own and a synergist with CO_2, while for other species, notably *Cx.* spp., 1-octen-3-ol seems to have only a minor attractive effect (Takken and Kline 1989, Takken et al. 1997*a*, Kline and Mann 1998). *Cs. melanura* does not fit this trend, though, since it is the only species that responds less to 1-octen-3-ol when the chemical is deployed at a release rate of 2-5 mg/h (Kline and Mann 1998). Two other components of human/animal breath have also been found to be important for host-seeking mosquitoes; acetone has an attractive affect (Takken 1991, Takken et al. 1997*a*) as does butanone (Kline and Mann 1998).

There is evidence that some non-host metabolites also enhance the attractiveness of humans to mosquitoes. One group of chemicals that has been researched is fatty acid metabolites of bacteria. This research was spawned from observations of mosquito preference for human feet during bite location assays (Knols et al. 1997*a*). Foot odor resembles that of Limburger cheese, and the chemistry of these odors is very similar in composition (Knols et al. 1997*b*). Wind-tunnel studies by Knols et al. (1997*b*) showed that mosquitoes responded positively to the odor of Limburger cheese. This was the documentation of mosquito attraction in the absence of CO_2 or a human subject. In later field studies, worn socks proved attractive to mosquitoes with and without CO_2 (Kline 1998). EAG responses were significant to C_5 to C_{14} aliphatic fatty acids (Knols et al. 1997*b*).

Many of the above studies examined mosquito responses to specific release rates of particular compounds in the field or wind tunnel. One might be tempted to project these results to human release rates and generate an all-in-one human odor for field trapping experiments. Unfortunately, using human equivalents for compounds such as

CO_2 and lactic acid has not resulted in equivalent numbers of mosquitoes caught relative to actual human subjects. There appear to be minor components in breath and skin secretions that together create the full stimulatory odor profile of a human. This poses a challenge in field studies when researchers wish to attract high numbers of mosquitoes using biologically relevant chemical release rates. The apparently simple solution of just using human subjects does not solve this problem. While people are more stimulatory than their known synthetic chemical equivalents, they are also highly variable in attractiveness to mosquitoes (Schreck et al. 1990). This person-to-person variation is unwelcome in field experiments, which are already heavily affected by uncontrollable environmental factors and uneven mosquito density. The need for consistent replication of chemical treatments (e.g. release rates) often outweighs the ideal of using humans in mosquito choice assays.

PERSONAL PROTECTION

It is apparent that mosquitoes are sensitive to many chemicals humans release into the environment. This ability, combined with *in*ability to reduce our attractive odor output, encourages humans to devise methods for interfering with the female mosquito's ability to effectively find hosts.

There are two primary categories for personal mosquito control. The first category is physical control, and includes such measures as protective clothing and netting that provide a barrier to mosquito biting. While largely effective, these measures are often cumbersome and can be unreasonable in hot and humid conditions. Physical control is not limited to barriers, however. Any non-chemical environmental manipulation can be considered physical control, and mosquitoes are acutely sensitive to abiotic factors like humidity, temperature, and wind velocity.

The second major class of personal mosquito protection is chemical control. In areas of high mosquito pressure or when barrier methods are undesirable, chemical methods are the method of choice. There are several mosquito repellents currently available, and all strive to create an active space around a human or area that is avoided by host-seeking mosquitoes.

Physical Control and Environmental Parameters

Humidity

Mosquitoes show preferences for certain ranges of relative humidities. While different species have their own optima, it is apparent that survivorship and activity patterns are influenced by moisture. It is unclear whether biting likelihood is affected by humidity.

Mosquito activity patterns seem to be heavily influenced by humidity regardless of species. Grimstad and DeFoliart (1975) observed and collected 23 species of mosquito in Wisconsin under various wind, temperature, and humidity conditions. Mosquitoes were collected at relative humidities lower than 48% only once over a three-year study period.

Platt et al. (1957) performed a detailed series of experiments on *An. quadrimaculatus* (Say) to clarify humidity effects on this species. An experimental chamber with relative humidity gradients ranging from 40 to 90% was used to assess preferences, and this group found that *An. quadrimaculatus* preferred relative humidities between 70 and 80 % at 15°C. One of the critical questions regarding atmospheric moisture was whether mosquito response was driven by relative humidity, absolute humidity, vapor pressure (of water), or vapor pressure deficit. The preference experiments were performed at 5°C increments from 15 to 30°C to separate these possible models. Platt's group found that the preferred relative humidity was 70% at both the highest and lowest temperatures; there was no correlation between preference and the other measures. It is not possible for mosquito preference to be simultaneously correlated with absolute moisture measures (humidity, vapor pressure or vapor pressure deficit) and relative humidity. Therefore, all the absolute models are falsified. It would seem more parsimonious for mosquitoes to assess absolute humidity in the same concentration-dependent manner as other chemicals, but these organisms apparently possess a mechanism to integrate the moisture content and temperature into a single measure.

Several other laboratory experiments have solidified the link between relative humidity and general mosquito activity. In a dual-port olfactometer, *An. gambiae* s.s. did not enter traps when the humidity was less than 40%. However, significantly more mosquitoes entered ports with rising humidities than those where the humidities were constant or falling. This result was independent of whether host odors or clean air was used in the olfactometer (Takken et al. 1997*b*).

Field experiments have also borne out the humidity effect on mosquitoes. *Ae. vexans* (Meigen) shows a near perfect positive correlation with humidity and activity in the field (Platt et al. 1958). The humidity range activating this species as assessed by light trap catches was 60-90%. Wright and Knight found a comparable range of 40-90% for *Ae. vexans* in their field experiments, and observed an activity range of 32-98% relative humidity for *Ae. trivittatus* (1966).

Flight performance may not be affected by relative humidity in the same way that general activity is. Female *Ae. aegypti* was not affected by relative humidity in sustained flight-mill experiments (Rowley and Graham 1968). Similarly, biting activity may not be correlated with relative humidity, as Lumsden (1947) found no significant effect of humidity on biting likelihood in wind tunnel experiments.

In the field, humidity influences mosquito biting and flight indirectly by modifying the behaviors that precede active host seeking. With high surface area: volume ratios, mosquitoes are ill-equipped to manage rapid moisture loss in dry conditions.

With respect to personal probability of receiving mosquito bites, humidity is an informative factor. But humidity alone is not an appropriate candidate for active manipulation of these pests. The extremely dry or humid conditions required to achieve repellency are impractical with respect to both cost and human comfort.

Temperature

Temperature dramatically affects mosquitoes, as it does with most flying insects. Low temperatures reduce flight muscle efficiency and inhibit proper function of many physiological systems (Chapman 1998). Insects that fly at low temperatures are likely to exhibit warm-up behavior, such as shivering of thoracic flight muscles to endothermically increase flight muscle temperatures above low ambient levels.

Lower temperatures are required to reduce activity of thermoregulating mosquitoes. Grimstad and DeFoliart found that it took temperatures below 10°C to reduce mosquito nectar-feeding activity for 23 Wisconsin species of mosquito (1975).

California *Cx. tarsalis* (Coquillett) initiated flight at 13°C and began biting at 15°C (Bailey et al. 1965).

While mosquitoes may be physiologically able to host seek at low temperatures, individual species have preferred temperature ranges for activity, probably driven by the mosquito's physiology and also host availability. Upon examining two North American temperate species Wright and Knight (1966) found *Ae. vexans* has a preferred range of 16-27°C and *Ae. trivittatus* had a preferred range of 18-29°C. Lumsden (1947) found that *Ae. aegypti* was most likely to feed at 35°C.

When preferred temperature ranges are exceeded, mosquitoes lack any mechanism for cooling beyond that of shade seeking. Hyperthermia in insects can be remediated with evaporative cooling, but few insects possess the excess water to allow for this behavior (Chapman 1998). Thomson's 1938 work with *Cx. fatigans* illustrates how physiological status shapes mosquito responses to high temperature. Females at various stages of reproduction were presented with temperature gradients of 1 – 5°C increments over a range of 10–30°C. Blood-fed, hungry, and newly-emerged mosquitoes responded slightly differently to these temperature gradients, but there was a general avoidance of temperatures that approached 30°C. These results were corroborated by later work on *An. quadrimaculatus*. Behavioral alterations were observed at 36-38 °C and there was only 57% survivorship after 20 minutes at 40°C for this species (Platt et al. 1957).

Temperature manipulation is a potential candidate for personal protection. Mosquitoes cannot escape the lower and upper temperature thresholds for biological processes, and heat is easier to manipulate than humidity. The major fault, however, is that temperatures necessary to deter mosquitoes are quite high (or low) for human comfort. Another obstacle is the technical challenge of generating heat. Increasing temperatures from 15 to 21°C at a distance of 1.5 m required a 220-volt heater/fan combination in a laboratory test (Hoffmann and Miller, unpublished). Such equipment represents a significant capital investment to which must be added the cost of power consumption.

While current technology makes temperature modification expensive, the possibility of behavioral control via temperature modification should not be dismissed. Eventually, more efficient and targeted heaters could become available for behavioral manipulation of insects. Since changing the ambient temperatures also changes the relative humidity, this strategy has the potential of affecting two parameters that influence host seeking with one manipulation.

Wind Velocity

While temperature and humidity may individually have limited use in personal mosquito control, wind velocity has potent behavioral effects on mosquitoes at levels quite acceptable for human comfort. Wind is one of the most spatially and temporally variable of the abiotic factors mosquitoes encounter. Ambient breezes are transitory by nature, and intermittency is increased by the influence of terrain elevation and obstructions. Such features generate wind shadows and wind breaks where wind speeds are much lower than for open areas (Bidlingmayer 1985, Bidlingmayer et al. 1985).

A discussion of wind velocity would be incomplete without addressing contemporary theory on mosquito flight and the physics of wind. Mosquito (and other insects') navigation in wind is thought to be driven largely by optomotor responses

(Kennedy 1939, Klassen and Hocking 1964, Snow 1980, Gillies and Wilkes 1981). Optomotor theory argues that there is a preferred rate at which the ground images move across the insect's eyes (perceived ground speed) and that air speed, altitude, and/or direction will be adjusted to maintain this preferred rate. While ground speed is a fixed measure, perceived ground speed shifts relative to altitude. At higher altitudes, ground features appear to pass beneath the insect more slowly, even though actual ground speed may be unchanged. Airspeed is yet another complicating issue, as that measure is based on how fast the insect is moving relative to the wind being encountered. If a mosquito is flying at 0.5 m/s with no wind, its air- and ground speeds are both 0.5 m/s. With a 0.5 m/s headwind, however, the mosquito's ground speed drops to 0 m/s (hovering) despite the energy output for 0.5 m/s of forward motion. In such cases, a mosquito must reduce altitude, fly faster, or fly at an angle to the wind to maintain the ideal perceived ground speed.

At some point, a mosquito will be unable to satisfy both upwind flight and apparent ground speed criteria. Given the goal-oriented nature of host seeking, mosquitoes faced with excessive wind will likely desist or take refuge from the wind. Such a wind refuge is the boundary layer, a cushion of air, typically up to 2 m altitude, in which wind speeds are significantly less than those at higher altitudes (Taylor 1974). In fact, one sees 50% reductions in effective wind speeds at a height of 0.25 m above the ground (Snow 1982). The reduction is due to fluid viscosity and friction over the surface (Vogel 1981), and, the thickness of the boundary layer is inversely related to wind speed (Snow 1977, Vogel 1981).

Boundary layer use by mosquitoes has been demonstrated through simultaneous suction trapping at various heights. Snow (1977) found that flight heights and wind speed are inversely related. Significantly more *An. melus* (Theobald) were caught lower to the ground at winds in excess of 0.8 m/s (Snow 1977). This does not imply that mosquitoes do not fly above the boundary layer in high wind conditions. It merely demonstrates a population-level probability for how individuals are distributed under various wind conditions (Bidlingmayer 1974). There are situations, such as dispersal, where flight above the boundary layer is preferred. The higher winds make host orienting nearly impossible but allow for rapid and efficient downwind flight to new feeding, mating, or oviposition areas (Snow 1977, Snow 1979, Service 1980, Snow 1982). Upon reaching an appropriate location, a mosquito could drop back into the boundary layer and resume orientation behaviors in more favorable wind conditions.

In addition to providing a refuge from wind's disrupting effects, the boundary layer may also play a role in upwind navigation in the absence of visual cues. Despite strong evidence against flight in the absence of visual cues (Daykin et al. 1965), Gillett (1979) suggests that a mosquito at level, sustained flight within the boundary layer can assess the direction from which the wind is originating by merely changing altitude. If the mosquito is flying downwind, and lowers its flight elevation, it will encounter an apparent deceleration, as the boundary layer reduces the tailwind. An assumption is made that the change in velocity would be sensed by inertial receptors. Similarly, if the mosquito is flying upwind and lowers its altitude, its impeding headwind is reduced and it senses positive acceleration. This model assumes relatively constant winds at a given altitude; but even with this limitation, it is a feasible mechanism for upwind flight in the absence of visual cues and optomotor input. It also relaxes some of the light intensity requirements intrinsic to optomotor-based host-seeking models. It should be noted that upwind orientation does not equal upwind flight. A mosquito in complete darkness may be oriented upwind, but it has no way of assessing whether it is making headway relative to the ground or is actually experiencing negative displacement as the headwind pushes it *backwards*. While this inertial model is theoretically possible, blinded insects are not successful in wind-tunnel flight (J.Miller, personal communication).

While the boundary layer may be a partial refuge from wind, its protection is not complete. Mosquitoes almost always engage headwinds while host seeking, and they are not equipped to counter high wind velocities. Lewis and Taylor (1967) developed a relationship between mean wind speed and insect size based on their experimental as well as literature data for these parameters. Their theoretical values of about 1 m/s for mosquitoes are borne out well in field experiments. Wind velocity limits have been established for several American and African mosquitoes. Wind velocities of over 0.9 m/s reduce flying and biting of both *Ae. vexans* and *Ae. trivittatus* (Wright and Knight 1966). *Cx. neavei* and *Cx. poicilipes* are also affected by higher wind velocities; suction trap catches were consistently lower at wind speeds above 1 m/s compared to speeds below this level (Snow 1979). Bite-count experiments in Gambia, West Africa showed mean flight speed estimates for *Ae. melas* and *Cx. thalassius* to be 1.12 and 1.14 m/s, respectively (Snow 1980). These two species were caught at higher wind velocities, but numbers were greatly reduced at wind velocities above these calculated mean flight speeds. For North American species, Grimstad and DeFoliart (1975) found that winds over 2.8 m/s halted nectar feeding in 23 common Wisconsin mosquito species. The general range for successful orientations (as assessed by trap catches) seems to be around 0.8 m/s; trap catches are drastically reduced by winds exceeding this rate. An exception appears to be arctic mosquitoes, some of which have an incredibly high flight capacity -- up to 8 m/s (Neilson and Neilson 1953, Service 1980). However, this thesis focuses primarily on temperate North American species, thought to share more modest flight capacities.

Reductive effects of wind are not restricted to velocities approaching the absolute flight limit for mosquitoes. Bidlingmayer el al. (1985) found that while winds below 0.24 m/s had no discernable effect on the number of mosquitoes caught in suction traps, winds between 0.25 m/s and 0.49 m/s reduced catches by 75%. In their collections of 23 species of Wisconsin mosquitoes, Grimstad and DeFoliart found velocities of about 1.5 m/s reduced nectar feeding in some species (1975).

Bidlingmayer, Day and Evans published one of the most comprehensive studies of mosquito response to ambient wind in 1995. Suction trap collections and average wind velocities were automatically taken every 15 min rather than at typical nightly intervals. Trap catch for all species declined as wind velocity increased; winds of 0.5 m/s reduced overall numbers caught by 50% relative to numbers caught at 0.25 m/s (the lowest velocity class measured). 75% reductions were seen at velocities of 1.0 m/s. While this study did not encounter wind velocities below 0.25 m/s, the authors found no evidence of a velocity plateau below which there is no significant difference in catches. They posit that even winds of 0.1 m/s would reduce mosquito catches when compared to perfectly calm conditions. Another important finding of this work was that mosquito response to wind velocity appears to be logarithmic (Bidlingmayer et al. 1995) rather than linear or quadratic. Previous studies had simply lacked the rigorous sampling design required to adequately describe the relationship mathematically.

In addition to their robust conclusions on ambient wind effects, Bidlingmayer et al. (1995) also suggest three possible causal mechanisms of reduced mosquito catch at higher wind velocities. First is the impact on optomotor response resulting in either lower flight height or turning downwind in order to maintain a preferred apparent ground speed. This model predicts a higher density of mosquitoes in the boundary layer at higher wind speeds, and thus higher trap catches up to the velocity limit. This model was inconsistent with the Bidlingmayer et al. (1995) findings; traps within the boundary layer caught fewer mosquitoes as wind velocity increased.

A second model focuses on the direct effects of wind velocity on the mosquito. Higher winds reduce the maximum recruitment rate to the traps over time, although the traps may still be fully attractive if operating over long periods. A third model involves the impact of energy reserves of mosquitoes. The most efficient time to fly would be at low wind velocities, and the largest percentage of the population would be able to host seek. Host seeking would also occur at higher velocities, but only by mosquitoes having the energy reserves to overcome the physical flight barrier. The latter two models are not exclusive, and both may be involved in reducing trap catches of mosquitoes at higher wind velocities. (Bidlingmayer et al.1995).

While field studies of ambient wind are useful in addressing wind as a monitored factor, there are few studies where wind velocity is directly *manipulated* in field settings. Gillies and Wilkes (1981) tried this approach with a wind tunnel in Gambia, West Africa. They used a calf as odor bait and a grid to electrocute mosquitoes that successfully navigated the tunnel. Catches of *Mansonia* species fell sharply above 0.5 m/s and became negligible above 1.8 m/s. While the restricted entrance of this wind tunnel limited the host-seeking responses of the mosquitoes, it was a first step in showing that wind velocity could be manipulated in the field with meaningful results.

Wind has behavioral activity on mosquitoes at levels comfortable to humans, and is inexpensive to generate. Thus, it is an appropriate option to manipulate for mosquito deterrence. However, there is an appreciable gap in knowledge of mosquito response to manipulated wind in outdoor settings frequented by humans.

CHEMICAL CONTROL

DEET

Chemical repellents have become a mainstay for easy and inexpensive mosquito control. The most commonly used chemical mosquito repellent in the United States is DEET (*N,N*-diethyl-*m*-toluamide or, alternatively, diethyl methyl benzamide). DEET is a potent mosquito repellent first marketed in 1956. It is considered the most effective broad-spectrum repellent, working not only against mosquitoes, but also on ticks, deerflies, and sandflies. An estimated 200,000,000 people worldwide and one-third of the US population use DEET every year (US Environmental Protection Agency 1980), which amounts to billions of applications. DEET has an effective repellent duration in excess of 5 h, and various studies using highly concentrated (>90%) formulations see greater than 90% effectiveness 8 h after application (Schreck and McGovern 1989, Chou et al. 1997, Frances et al. 1999). DEET has an oral LD_{50} of ~ 2000 mg/kg and an inhalation LC_{50} of 5.95 mg/L.

DEET's mode of action upon mosquitoes is unclear. Davis and Sokolove (1976) found grooved-peg (A3) lactic acid sensilla on the antennae of *Ae. aegypti* and determined through neurophysiological recordings that DEET inhibited the firing of these neurons, or reduced impulses in an already active cell. DEET may also affect other sensilla on the mosquito antenna, as evidenced by DEET's interference with sensitivity to ethyl proprionate, an oviposition stimulant (Kuthiala et al. 1992). These lines of evidence have generated a series of pore-blocking models for DEET action (Wright 1975, McIver 1981), which unfortunately suffer from inconsistencies with experimental results. Insects encountering a repellent stream show increased turn angles and often exit the stream immediately (Daykin et al. 1965, Davis and Bowen 1994). From these data, the insect must perceive the repellent as a negative stimulus. If the pore-blocking model were correct, then the mosquito should have continued on through the plume - oblivious to the attractant (or repellent) odors (Davis 1985). McIver's 1981 analgesic model does have some subtleties that bear pointing out, even if the data are not corroborating. DEET may interact with membrane components, causing a general modification in the sensory pattern. Alterations in mosquito behavior may stem from this distorted pattern (McIver 1981)

DEET's action as an inhibitor does have some support, however. Recent olfactometer experiments failed to show repellency (displacement away from the source) under the experimental conditions (Dogan and Rossignol 1999, Dogan et al. 1999). Regardless of its mode of action, there is general agreement that DEET interacts in some manner with the mosquito nervous system, be it inhibition of sensors, stimulation of sensors, or some across-fiber behavioral modification.

Despite these recommending traits, there is a strong public misperception that DEET is a toxic chemical that should be used only if absolutely necessary. DEET has a remarkably strong safety record after almost 50 years of intensive worldwide use. Toxic reactions to DEET are extremely rare; since 1956, only 14 individuals have been diagnosed with encephalopathy (neurotoxicity) associated with DEET. Three of these individuals died; the others made a full recovery (Osimitz and Grothaus 1995). From 1986 to 1989, The American Association of Poison Control Centers reported taking calls for 9,086 cases of DEET exposures. Upon follow-up of these cases, 5 patients reported major effects and one patient had died (after intentionally ingesting 8oz of DEET) (Osimitz and Grothaus 1995). These toxicological data compare favorably with alternative "natural oil" repellents, which have themselves been implicated in at least 3 human deaths since 1990 (Goodyer and Behrens 1998).

Alternative Chemical Strategies

While the toxicity of DEET is very low, it does have some negative physical attributes. It is a plasticiser, and can cause damage to synthetic fabrics and finishes. This, coupled with a chemical smell and fears of toxicity, has contributed to mistrust of DEET. The desire for less harsh repellents has spurred governments and corporations to seek longer-lasting and more broadly-effective compounds that meet the requirements of the concerned end user. One of the new classes of chemical mosquito repellents is piperidine, which is being tested as a replacement for DEET in US military applications. Several piperidine compounds have been tested (Yap et al. 1998, Debboun et al. 1999, Frances et al. 1999); one of the most promising is KBR 3023 (Bayer AG, Germany). This compound is remarkably non-toxic; its oral and dermal LD_{50} toxicities are 4,743 and over 2,000 mg/kg body mass, respectively (Yap et al. 1998). KBR 3023 is under consideration for EPA registration for consumer use and is likely to be available in the United States in the next few years (Spagnoli, personal communication).

While chemical repellents are widely used with well-established safety profiles, it is apparent that there is a popular mistrust of dermal chemical applications for mosquito repellency. Oily feel, chemical smell, and uninformed fear reinforce public demand for "natural" alternatives to the various chemicals that are used for personal and area protection from mosquitoes. As a result, we see continued demand for bug-zappers, ultrasonic emitters, and home-remedies, despite limited evidence of their efficacy. The need remains for alternative mosquito management measures that are effective, inexpensive, and inoffensive to even the most sensitive individuals.

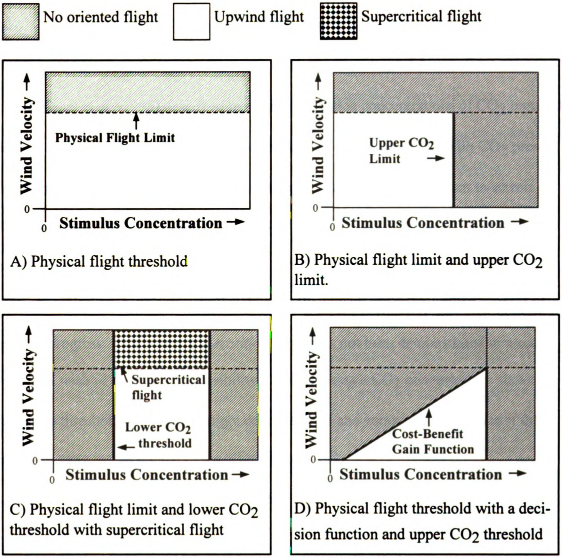
This thesis research was motivated by this need for such alternatives. Mosquito behavior is markedly affected by abiotic factors that can be manipulated to reduce mosquito presence or ability to host seek. There are significant gaps in research involving artificially-applied wind, and the field affects of this factor on mosquito behavior need to be quantified. The objectives of this research were to: 1) further quantify the relationship between mosquito host seeking and artificially-manipulated wind velocity, and 2) provide background data potentially supporting the development of wind application as a strategy for protecting humans from mosquitoes.

CHAPTER II

Proposed Conceptual Frameworks for Integrating Host Cues and Physical Factors Influencing Mosquito Host Seeking.

This chapter explores theoretical concepts on decision processes mosquitoes might use when finding hosts and proposes models of mosquito host seeking. In Figure 1, I present graphical representations of two factors that are reported to influence host seeking, wind velocity and stimulus concentration. Factors limiting mosquito host-seeking ability are proposed, and their interrelationships are expressed quantitatively.

Much mosquito behavioral data have been collected that suggests increasing wind velocity limits mosquito orientation to otherwise stimulatory host cues. In calm conditions, this flight limit would be equivalent to the maximum possible ground speed, but when ambient wind velocities exceed this velocity, reported to be between 1-2 m/s, host seeking is supposedly not possible. A refinement of this approach takes into account the idea of "preferred optomotor response" (Kennedy 1939, Snow 1980, Gillies and Wilkes 1981). If there were in fact a preferred rate of ground passage below the mosquito, the flight limit would be less than the theoretical maximum so the mosquito could maintain that preferred rate in the face of headwinds. A mosquito may "decide" whether or not to host seek in the presence of a stimulus like CO_2 based simply on whether or not ambient wind conditions are below this flight limit (Figure 1A). In acceptable wind conditions, the mosquito would fly until it finds the host, loses the plume, or succumbs to exhaustion.



A) Physical flight threshold

B) Physical flight limit and upper CO_2 limit.

C) Physical flight limit and lower CO_2 threshold with supercritical flight

D) Physical flight threshold with a decision function and upper CO_2 threshold

Figure 1(A-C): Four graphical illustrations of mosquito decision making when host-seeking decisions are based on instantaneous stimulus concentration and/or wind velocity.

CO_2 receptors on the maxillary palpi of *Aedes aegypti* exhibit a biphasic dosage-dependent response that initially increases then eventually decreases with increased CO_2 concentration (Davis and Bowen 1994). These observations lead to a refinement of the basic model (Figure 1B) such that a mosquito will host seek in winds below the physical flight limit but only at CO_2 concentrations under the neurological dosage limit.

Grant et al. (1995) described minimum activation concentrations of CO_2 required to elicit host-seeking behavior in mosquitoes at 150 – 300 ppm CO_2. With CO_2 present in the atmosphere at 300 – 400 ppm without host respiration, a mechanism to screen relevant concentrations (exhaled human breath contains 45,000 ppm CO_2 (Gillies 1980) from background "noise" is necessary. It is also possible that given a sufficiently high CO_2 concentration, mosquitoes will attempt to fly upwind regardless of the amount of forward progress. While such *supercritical flight* has not been demonstrated in insects thus far, I wish to point out its possibility. Upon sensing a CO_2 concentration above the minimum threshold, a mosquito might orient upwind and continue to fly – even if the mosquito were losing ground in the effort (Figure 1C).

Such absolute chemical maxima and minima may be too simplistic. A more sophisticated design would use a gain function that incorporates some measure of cost-benefit analysis for a given combination of wind velocity and stimulus concentration (Figure 1D). The cost is the amount of energy required to travel to the host, and the benefit is the complete blood meal. A mosquito would be more likely to orient to a host in high wind conditions under high CO_2 conditions compared to low CO_2. Given the diluting effect of wind, sensing high CO_2 would indicate very close proximity to the host and a higher chance of a positive investment return.

Explanations for Wind and Odor-Mediated Host Seeking

While conceptual illustrations of possible behavioral outcomes are interesting, they lack the potency to explain "how" or "why" the variables involved would exert their effect. I explore three possible explanations for mosquito observed host-orienting behavior relative to wind velocity and stimulatory host odors and describe tests that could be conducted to determine their validity. The first two of these involve absolute measures of environmental conditions, while the third is an energetics-based model that focuses on available resources and perceived distance to target (host). An additional evolutionary model is also proposed to address mosquito host odor thresholds.

Wind Velocity and Flight Limitation

The first model, favored by current mosquito literature, focuses on the mosquito's flight limit as the dominant factor influencing mosquito host seeking. This model corresponds to Figure 1C and predicts that for any appropriate release rate of CO_2, a mosquito will fly toward the host. If mosquito host seeking is driven exclusively by wind velocity, then mosquito responses in a wind tunnel or trapping study should exhibit little response change up to the flight limit, after which a dramatic decline would be evident (Figure 2). Both "maximum flight velocity" and "optomotor response" considerations would yield this rapid decline. If mosquito host seeking were shaped entirely by flight limits, then mosquito response would be unaffected by increased in-air concentrations of CO_2 at a given wind velocity.

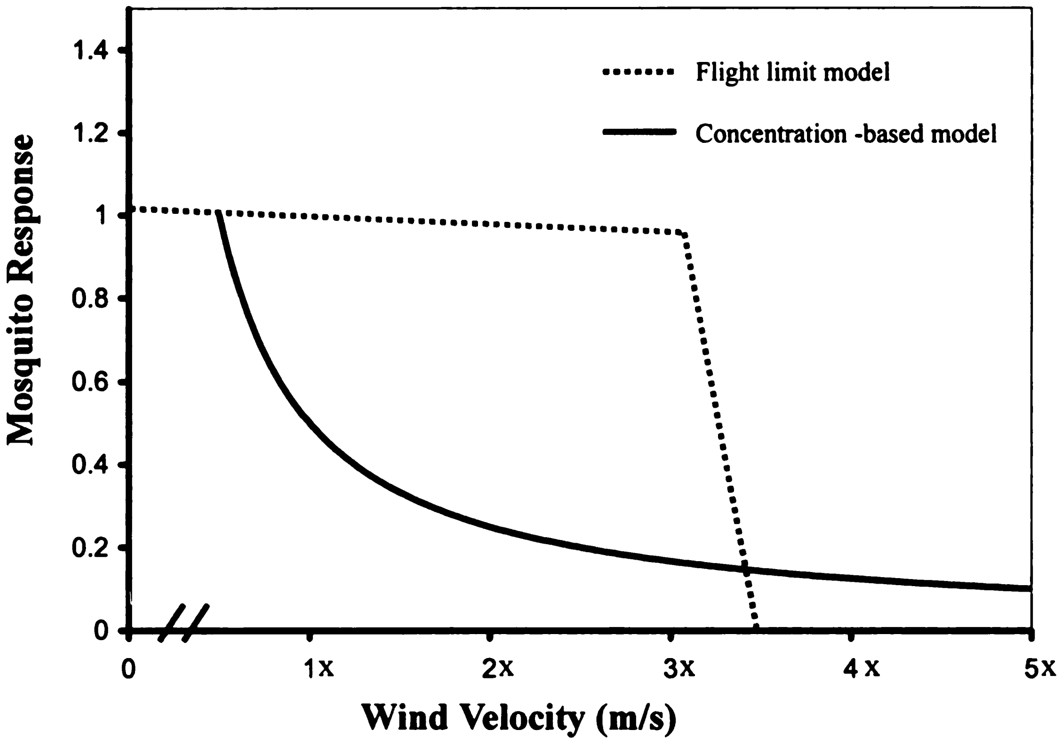


Figure 2: General forms of the flight-limit and concentration-based models of wind-mediated mosquito host seeking.

If the flight-limit model were correct, a positive relationship between mosquito mass and wind velocity would be expected; larger mosquitoes would be capable of negotiating higher velocities (Lewis and Taylor 1967). In addition to the ability to generate greater locomotory force, larger mosquitoes are likely to have higher energy reserves and greater muscle mass, supportive attributes when undertaking upwind flight in demanding winds. In addition to weaker flight, smaller mosquitoes may be more unstable than larger ones in turbulence because of decreased inertia.

Stimulus Concentration

Rather than forcefully influencing mosquito host-seeking behavior, it is possible that wind simply dilutes stimulus concentration. If stimulus concentration governs mosquito host seeking, then host seeking will take place whenever appropriate concentrations of CO_2 are present, other conditions permitting. Outcomes of decision-making so influenced could be illustrated by Figure 1C.

This model's uniqueness lies in the predicted response-curve shape for mosquito trap catch vs. wind velocity. It is well known and readily calculable that concentration of a chemical emitted into air from a point source and at a constant rate over time diminishes geometrically with wind velocity; each doubling of wind velocity halves concentration of the chemical. Moreover, this dilution is steepened by turbulence, which increases more than linearly with wind velocity. With this in mind, the idealized shape of a mosquito response curve in a trapping study would be a rapid drop in trap catch at low wind velocities and an asymptotic approach to zero as wind velocities further increased (Figure 2). In reality, the response level with no wind is finite and depends on the release rate from the source. Furthermore, turbulence and diffusion would modify the shape of this curve, resulting in a steeper initial drop at low velocities, and a more rapid approach to background levels of CO_2 at higher wind velocities. With all other aspects being equal, increasing initial stimulus release rates from the source would increase the number of mosquitoes at a given wind velocity relative to a lower release rate. In contrast to the flight-limit model, a positive relationship between body mass and wind velocity is judged unlikely in a host-seeking system relying only on the concentration of attractants.

Integrated wind and odor concentration model

Upon encountering a host-odor plume, a mosquito may choose whether or not to orient to that plume and fly towards the source host. This choice could be based on the determination of distance to a host and whether or not energy stores are available to successfully arrive at the host.

Mosquito upwind flight may also be influenced by the likelihood and payoff of a blood meal against the energy needed to get to the host. High concentrations of excitatory odors (CO_2, lactic acid, etc.) would tip the balance toward investing energy in host seeking, while inhibitory factors such as wind would make such efforts too costly (or even impossible) with respect to time and energetics.

How might a mosquito assess distance to a target that is unknown and unseen? I propose an *Integrated wind and odor concentration model* by which mosquitoes might determine the distance to the host. According to this model, one behavioral requirement and two physical abilities have to be integrated. First, a mosquito must have a programmed response to a particular host-odor release rate. Second, the mosquito must possess the ability to perceive in-air host odor concentrations. Finally, the mosquito must have the ability to determine wind velocity with respect to the ground. The integration of these elements is probably governed by across-fiber patterning (Chapman 1998).

There is evidence that mosquitoes possess all of the required sensory abilities to perform the required integration. Insects as a group are well known to have dosage-response curves to stimuli, indicating that they are able to quantitatively assess chemical concentration rather than mere presence or absence. Many species of mosquitoes have preferred hosts (Clements 1992) and may have selected responsiveness to a particular range of host odors. Mosquitoes also exhibit an acceptable range (either innate or learned) of wind-assessing flight behaviors. The capacity to determine wind velocity is evidenced by the behavioral response of lower flight altitude and/or landing in high wind conditions (Grimstad and DeFoliart 1975, Gillett 1979, Snow 1979). This adjustment in flight speed is likely associated with the optomotor response-linked, preferred rate at which objects are perceived as passing under the mosquito. At maximum flight output, the ground should be passing at a particular rate. If the ground is passing at a slower-than-preferred rate, the mosquito is likely encountering a headwind. I am not suggesting that a mosquito can determine the precise velocity of the wind. The requirement of this model is more of a general perception of ambient wind speeds and the ability to integrate that wind speed with the detected concentration of host odors in making some determination of distance to the odor source.

Such integration would hinge on some sort of dilution computation; however, no math is required. The mosquito is determining distance in the same way you or I determine how close you are to a barbecue without seeing it. We have an expectation of what a barbecue smells like at close range, and some impression for how the intensity of the odor decreases over distance. Thus you can assess how close you are to the barbecue without any mathematical equations. I suggest that incorporating the wind velocity component is not an exceptional feat, either. If it is a windy day, we could probably make some compensation for the dilution effect.

This is a time-averaged model, and focuses more on dilution than the favored models proposed for insect (primarily Lepidoptera) pheromone plumes. CO_2 has a much lower molecular mass than the typical insect sex-attractant pheromone, making it a more mobile molecule in air (Chapman 1998). Mosquitoes are also considered to orient at much shorter ranges (under 15m) than Lepidoptera (Edman 1979).

A simple equation for calculating in-air concentration of a host odor is:

$$[C] = \frac{R * f(d)}{V_w}$$

Where:

$[C]$ is the in-air concentration

R is the release rate ml/min (a constant)

$f(d)$ is some diffusion/dilution coefficient dependent on the distance from the point source

V_w is the wind velocity m/s (a constant)

V_w is in the denominator because concentration of a chemical from a constant point source is inversely related to the wind velocity- assuming no diffusion.

The practical application of this general concept would be arranging the equation to determine, "What 'd' corresponds to the measured concentration $[C]$ and wind velocities V_w given the expected release rate R?" For the mosquito seeking a blood meal or a human seeking a barbecue, the value need not be to the millimeter, but close enough as to make a determination of the amount of time required to get to the odor source.

Minimum time to the source is represented by the equation:

$$T = \frac{D}{V_m - V_w}$$

Where:

T is the time required to get from the current position to the odor source

D is the perceived distance to the odor source

V_m is the maximum ground speed of the mosquito

V_w is the ambient wind speed that the mosquito is flying against

This equation assumes that the mosquito is taking the most direct route to the odor source, and is affected only by direct headwinds. After making the determination of time to the odor source, the mosquito can assess the energy expenditures required for such a sustained flight at maximum velocity and the status of physiological reserves.

This model corresponds to the behavioral predictions illustrated in Figure 1D. For a given wind velocity (under any flight maximum), the mosquito response depends on what the instantaneous concentration of CO_2 is, and visa versa. Since the shape of the gain function is unknown, it is not possible to postulate the form of a response curve if mosquito trap catch were plotted against wind velocity. Mosquito mass would likely prove a significant factor in upwind success; the cost-benefit approach is closely tied to energy reserves.

While time-to-host is linked to energy consumption, it also extracts other costs from the insect not explored in this model. Exposure to predation and other mortalityinducing events is time-related, and an insect may become dehydrated during flight (Brantjes 1981).

Evolutionarily Stable Strategy - Host Odor Thresholds

Perhaps mosquitoes are not using energetics to determine whether or not to fly upwind towards an odor source at all. There may be a threshold concentration of host odors above which a mosquito will orient to a host regardless of the energetic cost (to exhaustion) until a) bloodfeeding occurs or b) the host odor is lost. This threshold would be above ambient odor concentrations and would imply a high probability that the mosquito would encounter the host before running out of energy. This approach is more evolutionary than the energetics model and places less of a burden on the mosquito to have a distance/concentration function imbedded in its neural circuitry.

Mosquitoes with a low threshold for stimulatory host odors are likely to orient to more distant hosts than mosquitoes with higher thresholds. Since they are flying farther to their hosts (or flying in higher headwinds), these less-selective mosquitoes use a higher proportion of their energy reserves for flight than the more selective elements of the population. With high levels of energy being devoted to host-orienting and upwind flight, less energy can go to egg production and maturation. While individual fitness may not be compromised by longer flights, fecundity in such individuals is almost certainly lower.

Individuals with a very high threshold for the appropriate host odors also suffer reproductive loss. Given the rapid drop in vapor concentration with distance from the source, a high-threshold mosquito has to be very close to a host to elicit the change from ranging to host-seeking behavior. High-threshold mosquitoes would spend most of their energy in ranging behavior (appetitive flight), searching for an odor plume. Actual host-orienting and upwind flight would be rare, since the volume of air containing the minimum odor concentrations is low. These individuals would be sacrificing fecundity for flight in much the same way that low-threshold individuals do.

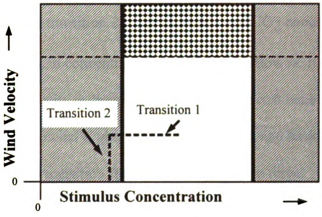
This evolutionary model suggests that a genetically stable odor concentration threshold exists such that, on average, reproductive success is maximized. Mutations for new thresholds would need to be accompanied by co-occurring mutations in other sensory abilities or flight capacity in order to comprise a stable portion of the population.

Implications and Applications - Research Questions

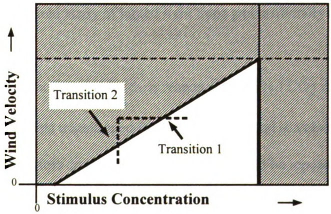
Efficient testing of the first three physical models would involve experiments where wind velocity, CO_2 release rate, and in-air CO_2 concentrations could be measured and manipulated and mosquito responses recorded. The fourth, evolutionary, approach suffers a common limitation of evolutionary theory- testability. It is nearly impossible to test an entire population of mosquitoes for a common CO_2 threshold, and tests of a limited number of individuals would be inappropriate. It would be impossible to discriminate whether individuals departing from the mean were refutations of the model, less-fit individuals, or a mosquito that has additional physiology (or behavior) to compensate for the higher or lower CO_2 thresholds.

The physical models could be first tested against their predicted responses to wind velocity and CO_2 concentration. The first two models fit figure 3A, while the cost-benefit approach is represented by figure 3B. A wind tunnel could be used to fly mosquitoes in various wind/CO_2 conditions. One could find an instantaneous CO_2 concentration at which a mosquito could orient and fly upwind. The CO_2 concentration

▨ No oriented flight □ Upwind flight ▦ Supercritical flight



A) Flight threshold- and stimulus– mediated host seeking models



B) Integrated wind and odor concentration model

Figure 3: Mosquito responses as CO_2 concentration decreases (transition 1) and as wind velocities are subsequently decreased (transition 2) for A) flight limit– and concentration-mediated models and B) the integrated wind and odor concentration model.

could then be dropped to the point where the mosquito no longer exhibited host-seeking behavior (Figure 3A and 3B, transition 1). While CO_2 concentration was held constant, wind velocity would then be reduced until the mosquito again began host-seeking behaviors (Figure 3A and 3B, transition 2). For all winds and CO_2 concentrations below physiological maxima, wind and concentration models would show no recovery of host-seeking behavior as wind velocity is dropped. Conversely, the cost benefit model predicts a changeover from upwind flight, to no oriented flight, and back to oriented flight as the wind/CO_2 levels transition from point one to two to three.

If it is determined that there is *no* recovery of host-seeking behavior with decreased wind velocity and a fixed CO_2 concentration, then it comes time to separate the concentration- and flight limited- mediated models. This could be done in the wind tunnel as well, although a field study of baited light traps and artificially applied wind would be effective.

Mosquitoes would be assayed for their response to a fixed CO_2 release rate (not in-air concentration) as winds transitioned from no applied wind to velocities at which mosquito orientation behaviors ceased. The experiment would be repeated for increasing CO_2 release rates up to the physiological maximum where CO_2 becomes repellent or anesthetic.

If the response curves showed a dramatic decrease at a particular wind velocity and no difference at varied CO_2 release rates, then the dilution model would be refuted. In contrast, the dilution model predicts an exponential-type decay curve with an asymptotic approach to zero as wind increases and increased responses to higher release rates at a given velocity.

In addition to exploring the practical possibilities for using wind to reduce mosquito attacks on humans in the backyard setting, the research of this thesis was also slanted toward differentiating between the various models whereby these effects are mediated.

CHAPTER III

Reduction of Mosquito Attacks on a Human Subject by Combination of Wind and Vapor-Phase DEET Repellent

Abstract

In a central Michigan wetland setting, electric fan-generated wind at 2 m/s plus DEET vapor at 800 μg/L air significantly reduced mosquito behaviors of orientation, landing, and probing in response to a human subject. The reductions relative to no applied wind and no DEET were 74, 75, and 70%, respectively. The DEET effect was significant as revealed by a wind/DEET interaction. We suggest a combination of directed wind and volatile repellent might be developed as a mosquito deterrent strategy for the backyard setting.

Introduction

It is well established that environmental factors significantly impact the ability of mosquitoes to find and feed upon their hosts. For example, humidities of 70-80 percent% and temperatures between 37-40°C promote peak activity in female *Anopheles quadrimaculatus* (Say) (Platt et al. 1957). Optimal flight temperatures for *Aedes aegypti* center around 21°C (Rowley and Graham 1968), while Takken et al. report that rising humidity is a key stimulus for *An. gambiae* host seeking (1997*b*). Wind velocity is also a major factor impacting mosquito attraction. Bidlingmayer et al. (1995) documented trap catch reductions of 50% at ambient wind velocities of 0.5 m/s, and further decreases as wind velocity approached or exceeded mosquito air speeds. As estimated mosquito flight speeds in still air range from 0.4-1.6 m/s (Bidlingmayer 1985), the potential for controlling mosquitoes by manipulating wind velocity appears real but perhaps underappreciated.

Currently, dermal application of chemical repellents is the mainstay of personal mosquito control. *N,N*-diethyl-m-toluamide (DEET) formulations remain the most effective and commonly used of the available repellents (Cockcroft et al. 1998, Fradin 1998). DEET has been extensively reviewed, and Osimitz and Grothaus (1995) evaluated its toxic risk as "very low." Despite high levels of effectiveness and strong safety record, many people still object to dermal application of (DEET) for protection from mosquito bites. Oily feel, chemical smell, and fear of toxicity are reasons cited by those who avoid the products (Fradin 1998). DEET's functionality as a vapor-phase repellent, rather than a topical contact repellent, has not been established.

Given the published data on mosquito flight and our experiences with mosquito behavior, this study was performed to determine whether: 1) artificially generated wind might effectively deter nuisance mosquitoes, and 2) adding repellent vapor to this moving air would augment a wind effect.

Materials and Methods

Study site

All trials were conducted in a 1,000 m^2 meadow on the Rose Lake Wildlife Research Area in Clinton County, Michigan (Peacock Rd, 1 km north of I-69). The test site was surrounded by trees and shrubs and was less than 100 m from standing and flowing water sources. Mosquitoes were present and abundant; the most common species collected during this study were *An. punctipennis, Ae. vexans, Ae. stimulans,* and *Ae. trivittatus*.

Spatial layout of test components

The layout of the experiment is illustrated in Figure 4. A 40.6 cm x 50.8 cm air filter was placed behind a variable-speed electric floor fan and the wind generated by the fan was aimed at a human subject seated 1.6 m downwind of the fan/filter combination. An observer/assistant was present but seated outside of the plume area to assist with data collection and to monitor for overt reactions to the chemical plume or overexposure to mosquito bites. Both the human subject and observer wore protective clothing including mosquito netting headgear to conform to Michigan State University human subjects committee approval guidelines. The approval also stipulated that the number of human subjects be highly limited until the value of this approach is assessed by peer review.

Wind generation

Wind was generated using one of two identical Cyclone 25 cm radius, 3-speed electric floor fan (Lasko, West Chester, PA). An Onan 4-cycle gasoline-powered generator (Briggs & Stratten; Milwaukee, WI) delivered electrical power (120V, 12.5 amps), and was situated at least 15 m from the test subject. The "low" fan setting was used; at 1.6 m downwind the velocity was 0.9 ± 0.2 m/s SD as determined by a hot-wire anemometer (Series 471, Dwyer Instruments, Inc.; Michigan City, IN). Separate fans were used for DEET treatment and the no-repellent "controls."

Chemical plume generation

N,N-diethyl-*m*-toluamide (DEET) was applied to the E-Z Flow II air filter (Flanders Precisionaire, Inc; St. Petersburg, FL) at 2.4 mg/cm^2, for a total of 4.75 g per filter. Lower dosages were tried initially, but appeared to offer little benefit. The formulation used was Ben's 100 95% DEET (Tender Corporation; Littleton, New

Figure 4. Experimental setup for human subjects-based wind velocity and vapor-phase DEET experiments.

Hampshire) applied via a finger pump. DEET evaporation rates were calculated gravimetrically to be 470 μg/cm^2/hr. The filter area was 1,981 cm^2, yielding a maximum loss of 0.91 g/h of DEET. This information in combination with approximations of plume volume yields an estimation of DEET vapor concentration at 800 μg/L air. This concentration was detectable by human smell, though it was not objectionable.

Experimental design, data collected, and analysis

A 2 x 2 factorial design was used, with the factors being fan on vs. off and repellent present vs. absent. Each of the four treatment combinations was tested for 10 min. intervals separated by a 5-min. setup period between treatments. Treatments were blocked by one-hour periods; no more than two blocks were completed per day. Data collected were visual counts of numbers of mosquitoes orienting to, landing on, and probing the subject's exposed forearms.

Mosquitoes were judged *Orienting* when <1 m from the subject and directing flight towards or station-keeping near the subject. Random flight through the test area occurred rarely. *Landing* was scored when a mosquito alighted on the subject's exposed forearms, and *Probing* required lowering the proboscis as if to pierce the skin. Mosquitoes were allowed to freely orient and land, but as soon as they initiated probing behavior, and usually before biting, they were aspirated into a vial for subsequent identification. Nine complete blocks of this experiment were accumulated for analysis between July 1-29, 1999. Data were transformed by ($\log_{10}$ + 0.5) and analyzed by 1-way ANOVA using PROC GLM (SAS Institute 1996). Both main effects and interactions were evaluated.

Results

Mosquito pressure was high for most of the blocks and provided adequate data for analysis. A maximum of 46 probing mosquitoes was counted in 10 min and the average was 6.3 ± 9.4 SD. Averages for orienting and landing were 13.1 ± 18.3 and 8.6 ± 12.9, respectively. With both DEET-present and DEET-absent treatments included in the analysis, turning the fan on significantly reduced the number of mosquitoes orienting (F = 8.29; d.f. = 1; $P = 0.008$), landing (F = 9.66; d.f. = 1; $P = 0.005$), and probing (F = 14.45; d.f. = 1; $P = 0.001$) (Figure 5). There was no significant effect of repellent alone on mosquito orienting ($P = 0.18$), landing ($P = 0.26$), or probing ($P = 0.48$). DEET was not expected to have a statistical main effect due its inability to envelope and protect the human subject without the fan creating a vapor plume.

However, there was a significant interaction between the fan and repellent for orienting ($P = 0.050$) and landing ($P = 0.027$) behaviors. The interaction was not pronounced for the probing behavior ($P = 0.061$). The mean number of mosquitoes was lower for the fan-on, repellent-present combination than for the fan-on, repellent-absent combination for orienting, landing and probing.

Discussion

Administration of DEET repellent to the wind stream generated by an electric fan was relatively simple to accomplish. While it did not completely eliminate visits from mosquitoes, fan-generated wind plus DEET did confer appreciable protection at a wind flow judged "comfortable" by the subject. The significant interaction between the Fan and DEET factors is attributable to the action of the fan delivering DEET vapor to the human subject. This action also explains the fan's main effect, which is only apparent

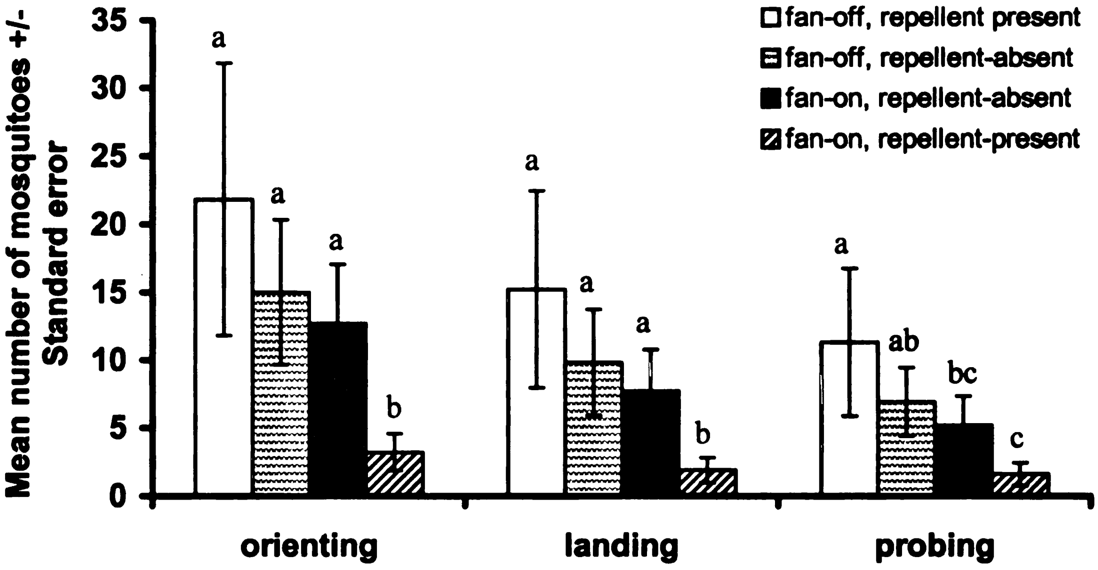


Figure 5: Number of mosquitoes observed orienting, landing, and probing under four fan and repellent combinations. Means followed by the same letter within a behavioral category are not significantly different at $P < 0.05$.

when there is DEET in the wind stream (Figure 5). Although the fan-on, DEET-absent treatment was not significantly different from the fan-off, repellent-absent treatment in this experiment, preliminary tests of higher fan velocities strongly suggested that higher winds without DEET conferred substantial protection from mosquitoes.

Wind likely has at least three impacts on mosquito host seeking efficiency. First, increased wind velocity reduces the absolute rate of mosquito recruitment to an upwind host (Bidlingmayer et al. 1995). A second impact is based on optomotor theory (Kennedy 1939, Klassen and Hocking 1964, Snow 1980, Gillies and Wilkes 1981) and suggests that mosquitoes have preferred rates of ground passage and will cease flying if perceived ground speed drops below tolerable rates; wind has a direct effect. A third possible impact of wind is dilution of host-stimulus chemicals. A given rate of emanating host volatiles (such as CO_2 and lactic acid) would become less stimulatory as wind velocity increased because the concentration, and thus dosage delivered to chemosensory organs, would fall. This experiment did not seek to quantify the contribution of each of these impacts, and these models are addressed experimentally in Chapter IV.

Chemical suitability is an issue to be addressed for this as well as any repellent studies. Suitability is based on several factors, not least of which is the toxicity profile of the chemical. While DEET has an admirable safety record (Robbins and Cherniak 1986, Osimitz and Grothaus 1995, Goodyer and Behrens 1998), careful attention must be paid to dosage. We estimated maximum uptake for a 10-minute exposure in this study could have reached 100 mg if the subject, inhaling 13 liters of plume air per minute, absorbed *all* of the volatized chemical inhaled. By comparison, Robbins and Cherniak (1986) analyzed available DEET application data and estimated that an Everglades park worker averages 4.25 g of dermally applied DEET per day, while the average DEET user in the general population was found to apply 1.65 g/d. Given a mean cutaneous absorption rate of 5.6% (Osimitz and Grothaus 1995), the worker would be absorbing 238 mg of DEET per day and an average user 92 mg/d. The rate of DEET used in the current study was high compared to these measures. If this wind-vaporized DEET use were to be pursued, the actual absorbed dosage would need to be quantified beyond these conservative estimates; it is judged likely that the subject of this study absorbed only a fraction of the inhaled DEET. The toxicity profile for inhaled chemical should also be determined. It is possible that there are different physiological effects of DEET vapor compared to dermal applications.

While DEET may not be the most suitable fan-propelled vapor-phase repellent, we suggest that this strategy has a place in personal protection from mosquitoes. Electric fans are readily available, comfortable, and could serve a dual purpose of cooling and pest deterrence on warm evenings. Tests of this nature should be expanded to other repellents, including the piperidine compound Autan® (Bayer AG) and some of the essential oils. Benefits of fan-delivered chemicals must be carefully scrutinized in the light of toxicity profiles.

CHAPTER IV

Artificially Manipulated Wind and Vapor–Phase DEET Reduce Mosquito (Diptera: Culicidae) Catches in CDC Light Traps

Abstract:

In a central Michigan wetland setting, artificially manipulated wind velocities reduced mosquito catches in CDC light traps with CO_2 released at 650 or 1,950 ml/min. This function was negatively logarithmic over the range of velocities tested (0 m/s to 3.7 m/s or 0 to 8.3 mph). This reduction closely matches expected the dilution function as wind increases over a constant release source. Tripling the CO_2 release rate consistently doubled the number of mosquitoes caught across the range of wind velocities tested. Addition of DEET vapor (910 mg/h) at 650 ml/min CO_2 significantly ($P < 0.001$) reduced the number of mosquitoes caught relative to equivalently moving air without repellent. Analysis of responses to wind by *Aedes vexans, Anopheles walkeri,* and *Coquillettidia perturbans* does not support the hypothesis that different species respond differently to wind. There was no relationship between mosquito body mass and either applied wind velocity ($P = 0.25$) or CO_2 release rate ($P = 0.7836$) across the range of wind velocities tested. Collectively, our data suggest that applied wind diminishes mosquito response primarily by diluting attractants rather than by exceeding the flight capability of mosquitoes. Outdoor fans as well as horizontal or vertical wind curtains are proposed as local deterrent tactics for nuisance mosquitoes in the backyard setting.

Introduction

Environmental factors such as temperature, humidity, and wind strongly influence mosquito activity levels (Thomson 1938, Kennedy 1939, Platt et al. 1957, Lewis and Taylor 1967). Temperature and humidity effects have received considerably more research attention than has wind. Mosquitoes rely heavily on wind-generated, host-odor plumes to find blood meals, but velocities greater than 0.3-1.0 m/s can reduce successful orientations supposedly because of mosquitoes' weak flight capabilities (Gillies and Wilkes 1981, Bidlingmayer 1985, Bidlingmayer et al. 1985, Bidlingmayer et al. 1995). The highest wind velocities at which orientation to hosts or flowers has been documented range from 0.8 to 2.8 m/s for subarctic species (Grimstad and DeFoliart 1975, Bidlingmayer et al. 1995). An arctic species, *Aedes cantans*, has been observed station-keeping in winds up to a remarkable 8 m/s (Nielsen 1953). When wind velocities increase to levels above optimal ground speeds (Kennedy 1939), mosquitoes cease flying or fly closer to the ground and take advantage of the *boundary layer*, where wind velocities are substantially lower (Vogel 1981, Snow 1982).

Information is scant for mosquito flight responses to manipulated wind velocities under field conditions. Using a live calf in an open-ended wind tunnel, Gillies and Wilkes (1981) found that catches of *Mansonia* and *Anopheles* mosquitoes on the African savannah began to decline at wind speeds greater than 0.5 m/s and nearly ceased above 1.4 m/s. This study neither precisely quantified the relationship between wind velocity and mosquito response, nor fully explored the potential of artificially-generated wind as a tool for deterring or otherwise manipulating mosquito behavior.

The intent of this study was to use electric fans to manipulate wind velocity and assess the practical potential of wind as a protectant from mosquitoes. These studies used carbon dioxide (CO_2) as an attractant and, in one test series, DEET was added to the wind. CO_2 is one of the best-known chemical cues mediating mosquito host-finding (Gillies 1980, Takken 1991, Takken and Knols 1999). It is suspected to be both a behavioral activator and a true kairomone (Gillies 1980). CO_2 alone is effective as bait in behavioral experiments and for population monitoring (Reeves 1953, McIver and McElligott 1989, Mboera et al. 1997); human equivalencies (ca. 250 ml/min CO_2) are relatively easy to obtain. The effective active space (linear distance from release point) of CO_2 increases linearly with increasing release rates up to 1000 ml/min (Gillies 1980).

N,N-diethyl-*m*-toluamide (DEET) is a widely used and potent topical chemical used in the management of the biting behaviors of mosquitoes and other human-biting insects. In addition to its repellent value in topical applications, there is evidence that it functions in the vapor phase. DEET-impregnated gauze in an olfactometer was sufficient to significantly inhibit mosquitoes from orienting to a lactic acid bait (Dogan et al. 1999).

DEET inhibits neurons sensitive to lactic acid, another important attractant chemical for mosquitoes (Davis and Sokolove 1976). By inhibiting lactic acid perception, DEET may break the suggested chain of chemically-mediated behaviors that lead to a successful blood meal (Davis 1985, Dogan et al. 1999). It is unclear whether DEET acts on other sensory structures in addition to lactic acid sensors.

While mosquitoes as a group are clearly influenced by environmental factors and physiological status, it is thought likely that there is intraspecific variation in preferred flight speeds (Snow 1980), responses to temperature (Wright and Knight 1966), and CO_2

dosages (Reeves 1953, McIver and McElligott 1989, Constantini et al. 1996, Dekker and Takken 1998).

The specific objectives of the current study were to: 1) quantify mosquito response to fan-manipulated wind velocity and two release rates of CO_2, 2) determine whether wind-reduction of mosquito catch could be enhanced by vapor-phase DEET, 3) determine whether different species of mosquito have different response profiles to CO_2 and wind velocity and, 4) attempt to differentiate between the flight-limit and dilution-based models as explanations for the mode of wind action upon mosquito orientation.

Materials and Methods.

Study site and trapping method

Experiments were conducted between June 3 and September 9, 2000, in the center of an open grass field (ca. 5 ha) at the Rose Lake Wildlife Research Area in Clinton County, Michigan. The experimental area, adjacent to two remote storage buildings, was bordered by woodland and less than 1,600 m from standing water habitats. These habitats ranged from small woodland pools less than 3 m in diameter to a large lake in excess of 2.5 km^2. Mosquito populations were high throughout the season. Moderate temperatures and consistent rains kept mosquito breeding sites productive. Adults were actively host seeking throughout the experimental area, as evidenced by persistent attempts to bloodfeed from the investigators during all tests.

Because of the impracticality of deploying equivalently attractive humans (Price et al. 1979, Schreck et al. 1990) for prolonged field tests, we used Centers for Disease Control (CDC) light traps (John W. Hock; Gainesville, FL) with and without CO_2 bait to quantify mosquito orientation success. Steel support poles held the traps 1.5 m above ground height in two parallel rows with approximately 12 m between each trap. Each trap intake fan and 1.4 W light (when used) was powered by a 12-volt rechargeable gel-cell battery.

Carbon dioxide release

For treatments using host odors, a thermos (ca. 2L) containing 600 g dry ice was inverted above the CDC light trap. As the dry ice sublimed, CO_2 gas spilled from the spigot. The release rate of CO_2 was determined gravimetrically after placing approximately 600 g of dry ice into each of three different thermoses for six h, the planned field experimental interval.

Wind generation and velocity determination

Artificially-generated wind was produced by 51 cm-diam, three-speed floor fans (Model WC-2000, Lakewood Engineering & Mfg. Co.; Chicago, IL). Desired at-trap wind velocities were obtained by aiming fans at differing power settings from various distances. Fans powered from 120-volt outlets in the adjacent storage buildings were mounted on 0.6 m stepladders for stability and support. 16-Gauge, outdoor-use, extension cords carried power from multiple outlets to the fans with little voltage drop.

Average wind velocities for various power settings and distances were determined by aiming the fan at a circular (43 cm radius) string grid with nine intersections (Figure 6). Wind velocities were taken at each intersection and the four cardinal points with a hot-wire anemometer (Model HHF51, Omega Engineering, Inc.; Stamford, CT) mounted on a heavy video tripod for stability and averaged to obtain the mean velocity for a given distance and power setting.

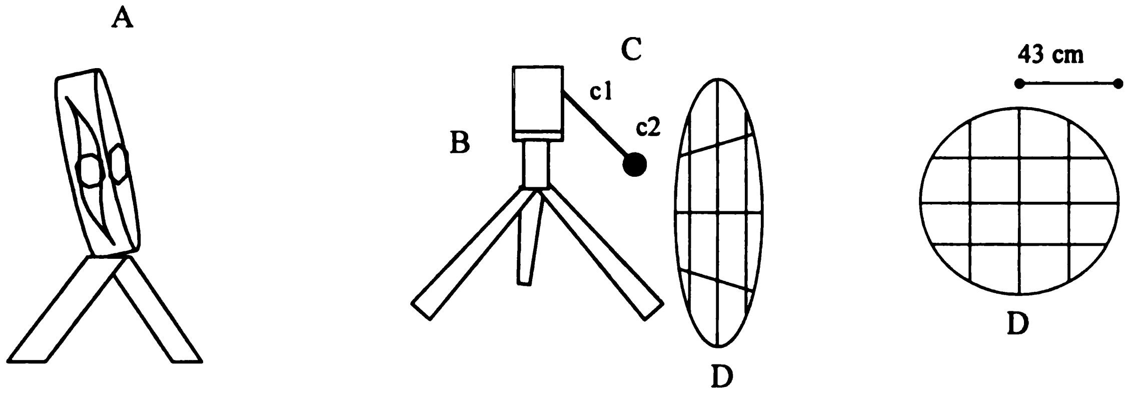


Figure 6: Experimental setup for wind velocity determination. A) Three-speed fan on step ladder mount, B) Heavy-duty video tripod, C) Hot-wire anemometer c1) extension arm and c2) sensor, D) Circular target with string grid.

DEET Generation

DEET vapor was generated by spraying 4.3 g technical grade DEET (Fluka; Milwaukee, WI) onto a 38 cm x 51 cm E-Z Flow furnace filter (Flanders Precisionaire, Inc; St. Petersburg, FL) and affixing it to the back of fans aimed at CDC traps. DEET was vaporized as a fan drew air through the impregnated filter and propelled it toward the trap. The combination of fan, light trap, thermos, and DEET screen (when used) was termed a trapping station.

Each nightly trap run (block) lasted from ca.1730- 2330 EDT. Runs were set up only when ambient winds were non-existent to <1 m/s and predicted to remain so. It was accepted that transient breezes above 1 m/s might occur. These were viewed as affecting the performance of those traps with the lowest applied velocities for only short times during an otherwise acceptable experimental period. Temperatures were above 10°C for all trapping periods. During experimental periods, I was attentive to weather conditions in the test vicinity to assure appropriate experimental parameters were maintained. On three occasions, tests were abandoned and results disqualified because of excessive wind and/or rain. Trap collection bags were immediately sealed when tests were terminated and trapped mosquitoes frozen at – 40°C within 8 h of collection. Counting and taxonomic determination were performed under a dissecting microscope.

Statistical Analysis and Experimental Designs

Quantification of mosquito response to wind, CO_2, and DEET

To correct for variance-mean dependence, count data were transformed using a $\log_{10}$ (number of mosquitoes caught + 0.5) before analysis. ANOVA was performed using PROC GLM in SAS (V8) and regression statistics were generated by ANCOVA using PROC GLM (SAS Institute 1996).

Four wind velocities with and without CO_2 (Experiment 1)

Eight trapping stations were arranged in two parallel rows of four. The experimental design was a blocked, randomized 4 x 2 factorial design with applied wind velocity and CO_2 release rate as the factors. The experiment was blocked by night to account for uncontrolled effects of varying temperature, humidity, and mosquito population density. Applied wind velocities were 0, 1.3, 1.6, or 2.4 m/s. CO_2 release rates were 0 or 650 ml/min. Trapping stations were in the same location each night, but the treatment combination assignments to individual stations were randomized before each block. Eight blocks were completed.

Assessment of mosquito background densities and high velocity response (Experiment 2)

The spatial arrangement was similar to Experiment 1 except that only seven stations were used. Instead of a factorial design, a randomized complete block design was employed with five applied wind velocities at 650 ml/min CO_2 and two wind velocities with unbaited (no light or odor stimulus) traps. Baited wind velocities were 0, 1.1, 2.0, 2.5, or 3.7 m/s, and unbaited velocities were 0 or 3.7 m/s. Eight blocks were completed. Unbaited traps were used as negative controls to measure the background level of mosquitoes flying through the experimental area. While the unbaited traps caught non-orienting mosquitoes, the baited traps sampled actively orienting females. Baited trapping stations with mosquito catches at or below those of the unbaited traps imply that the applied wind treatments for these traps negated the attractants. This is a reasonable measure of the degree of personal protection afforded by a particular treatment, since it establishes the number of mosquitoes in the environment that would be encountered by chance alone.

Five wind velocities and two CO_2 release rates (Experiment 3)

This experiment used 10 stations in two parallel rows of five. The design was a blocked 5 x 2 factorial with applied wind velocity and CO_2 release rate as factors. The wind velocities applied were 0, 1.0, 1.1, 2.1, or 2.7 m/s. CO_2 release rates were 650 or 1,950 ml/min. The higher release rate was achieved by hanging three thermoses from the support pole. Nine blocks were completed.

Five wind velocities with and without DEET vapor (Experiment 4)

The test of DEET vapor used 10 stations in two parallel rows of five. A blocked, 5 x 2 factorial design was used with applied wind velocity and DEET vapor as factors. The wind velocities applied were 0, 0.3, 0.4, 1.4, or 2.4 m/s. The slight difference between these velocities and those of Experiment 2 resulted from addition of the furnace filters to the back of the fan. DEET was either applied to the filter attached to the back of the fan, or a filter was left untreated. Fans used for DEET dispersion were never used for non-DEET treatments. DEET-present and no applied wind treatment was created by deploying a DEET-impregnated filter 2 m from the CDC light trap. All of the trapping stations were baited with light and 650 ml/min CO_2. Seven blocks of this experiment were completed.

Comparison of species responses to wind velocity and CO_2

Variation in mosquito responses was assessed using $\log_{10}$- transformed counts from Experiment 3 after species identifications. Regressed responses to wind were compared using PROC GLM ANCOVA in SAS. Only slopes were compared, since the intercepts would vary with species' population densities, which were unknown and not likely to be equivalent.

Relationship between mosquito mass and wind velocity

Using mean mosquito mass/ trapping station samples from Experiments 2 and 3, ANOVA procedures in PROC GLM of SAS (V8) (SAS Institute 1996) were used to determine whether mosquito responses to wind velocity and CO_2 were influenced by mosquito mass. Regressed responses to wind were compared using PROC GLM ANCOVA in SAS.

Results

Carbon dioxide release

The average loss of CO_2 was 74.4 g/h (range of 67.3 to 81.8 g/h) (Figure 7). R^2-values exceeded 0.99 for each thermos, confirming a linear (thus constant) release rate over the chosen time interval. Meeting the criterion of constant release rate vastly simplified interpretations of experimental results.

The volume of CO_2 corresponding to this loss can be calculated with the relationship $PV = nRT$: where P is the air pressure (atm), V is the volume of CO_2 (L), n is the moles of CO_2 (1.69 mol), R is the constant 0.0821 L atm/ K mol, and T is the temperature (K). The air temperature in the laboratory was measured as 22°C (294K) and the pressure was approximated at 1 atm. The calculated mean volume of CO_2 lost was 680 ml/min for the three containers (range 615 to 742 ml/min CO_2). Since the atmospheric pressure was approximated, it was deemed appropriate to approximate the release rate of an individual thermos as 650 ml/min CO_2.

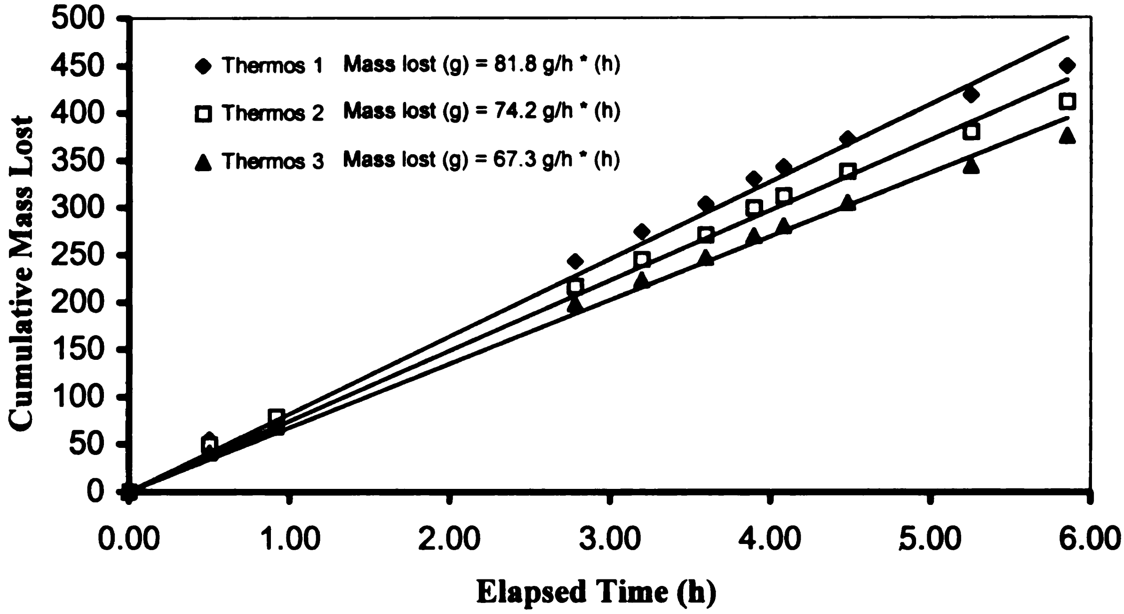


Figure 7: Cumulative mass loss of CO_2 due to sublimation in three thermos containers at 22°C.

Mosquito Responses to Wind, CO_2, and DEET

Four wind velocities with and without CO_2: Experiment 1

Addition of CO_2 significantly increased the number of mosquitoes caught in the light traps (Figure 8, Figure 9) ($F = 289.43$; d.f. = 1; $P < 0.0001$). Conversely, applied wind velocity significantly reduced the number of mosquitoes caught ($F = 5.76$; d.f. = 3; $P = 0.020$). The log-transformed regression equation (Figure 8) for the CO_2-baited traps was $\log_{10}$ (catch + 0.5) = - 0.44 (wind velocity (m/s)) + 2.93 and there was a significant interaction between wind velocity and CO_2 ($F = 3.80$; d.f. = 3; $P = 0.016$) on the number of mosquitoes captured. The slope of the linear relationship between wind velocity and log-transformed trap catch was significant ($t = -5.46$; d.f. = 1; $P = 0.001$) for the CO_2-baited data but not for the unbaited traps ($P = 0.691$). Untransformed nightly means with a best-fit logarithmic curve are shown in Figure 9.

Assessment of mosquito background densities and high velocity response (Experiment 2)

The slope for log-transformed mosquito trap catch vs. wind velocity was significantly different from zero ($t = -9.63$; d.f. = 1; $P = 0.001$) for those traps employing 650 ml/min CO_2 as a stimulatory odor (Figure 10). The equation for this relationship is $\log_{10}$ (catch + 0.5) = - 0.67 (wind velocity (m/s)) + 2.45 with an x-intercept of 3.7. There was no significant difference between the baited and unbaited traps at 3.75 m/s (Tukey's HSD: $P = 0.05$); at this velocity, mosquito catch was reduced to background levels. Unbaited traps (0 and 3.75 m/s) were also not significantly different from one another, which supports the assumption that higher wind velocity did not measurably impair trap efficacy. Untransformed nightly means with a best-fit logarithmic curve are shown in Figure 11.

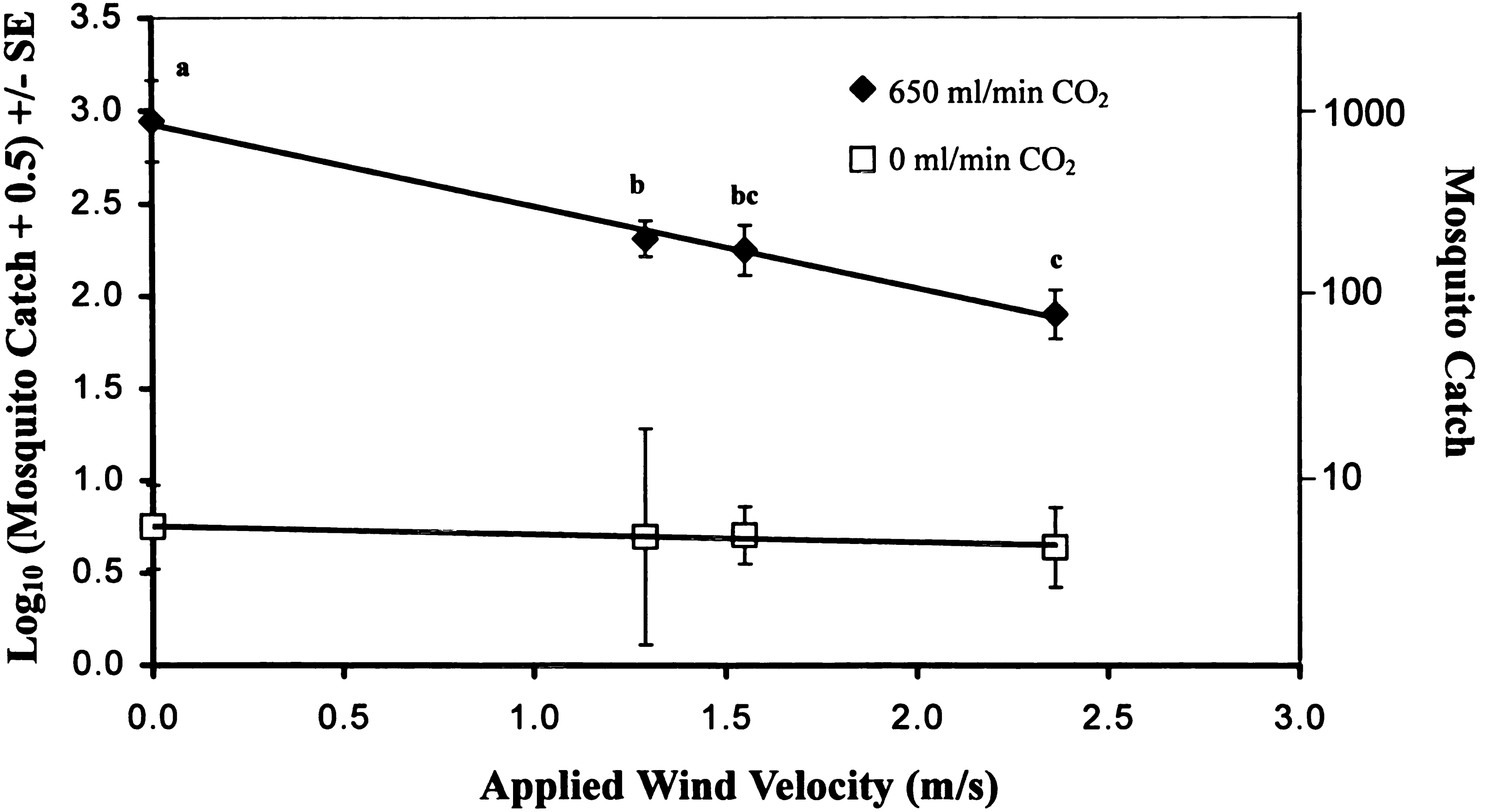


Figure 8: Number of female mosquitoes (combined species) captured with and without CO_2 stimulus (Experiment 1) as a function of wind velocity. Average nightly catch was 1,721 individuals. Individual means comprising the top line and sharing a common letter are not different at $P < 0.05$

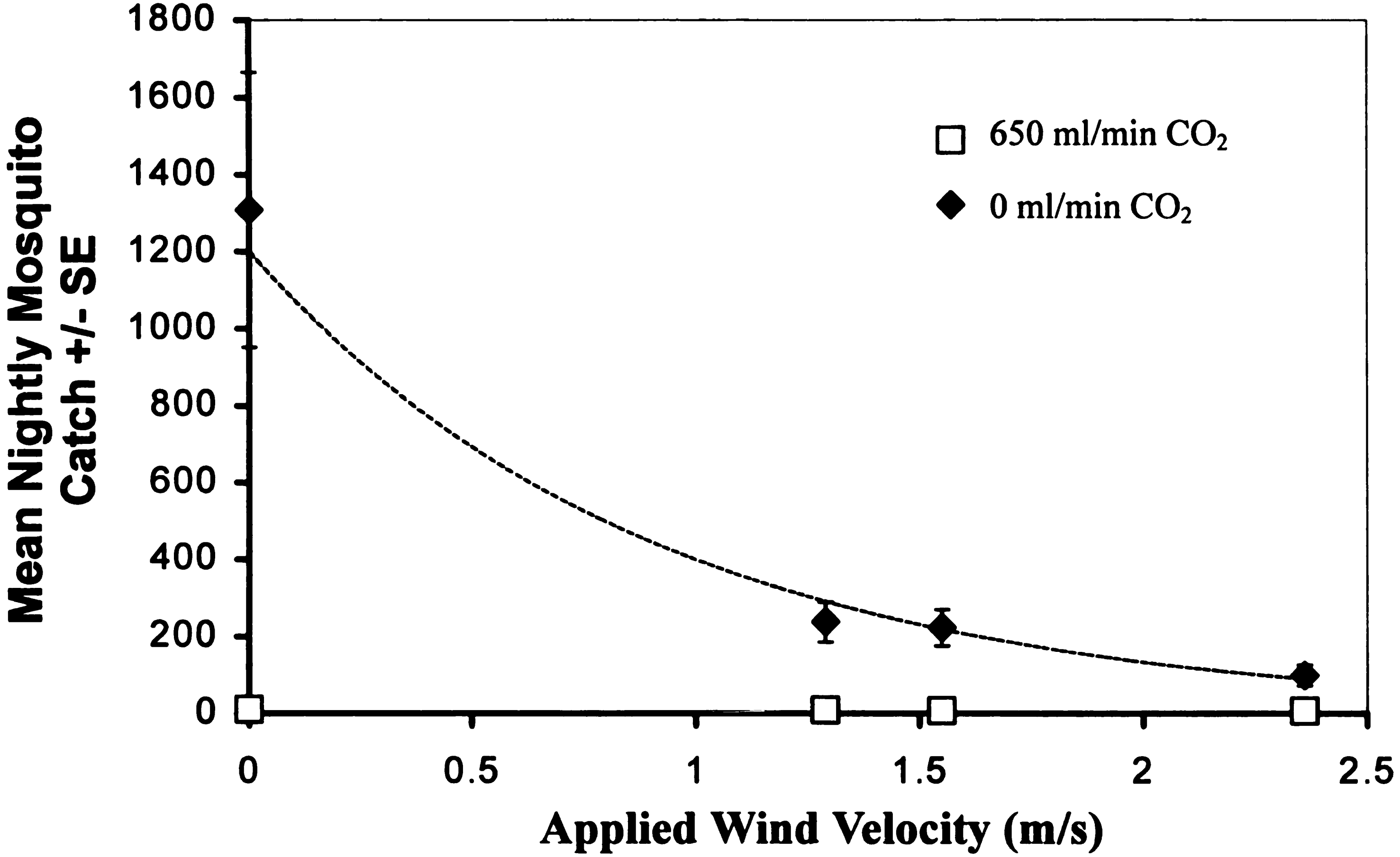


Figure 9: Number of female mosquitoes (combined species) captured with and without CO_2 stimulus (Experiment 1) as a function of wind velocity. The dotted line illustrates a general decay function.

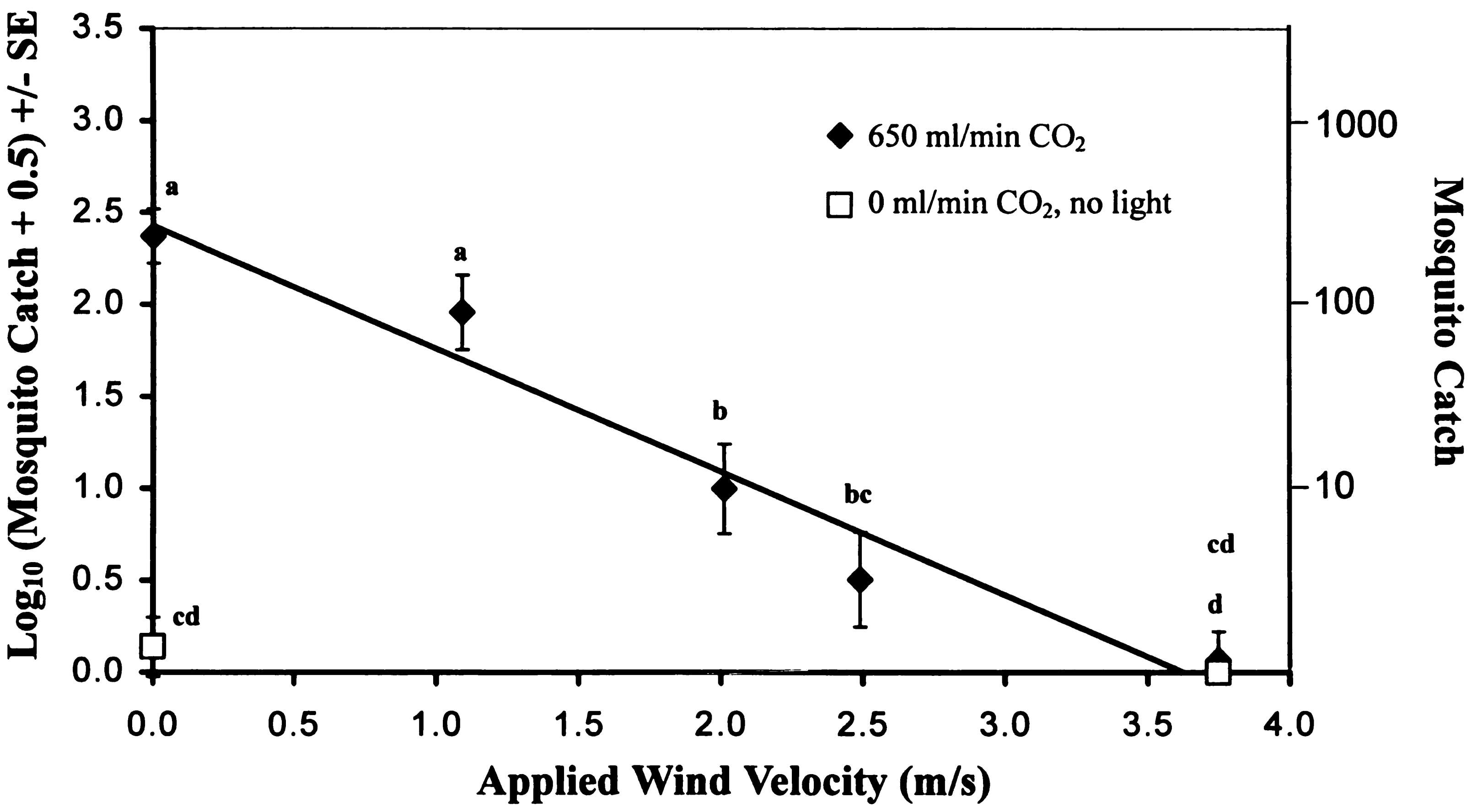


Figure 10: Number of female mosquitoes (combined species) captured with CO_2 stimulus or with no odor or light bait (Experiment 2) as a function of wind velocity. Average nightly catch was 500 individuals. Individual means sharing a common letter were not different at $P < 0.05$.

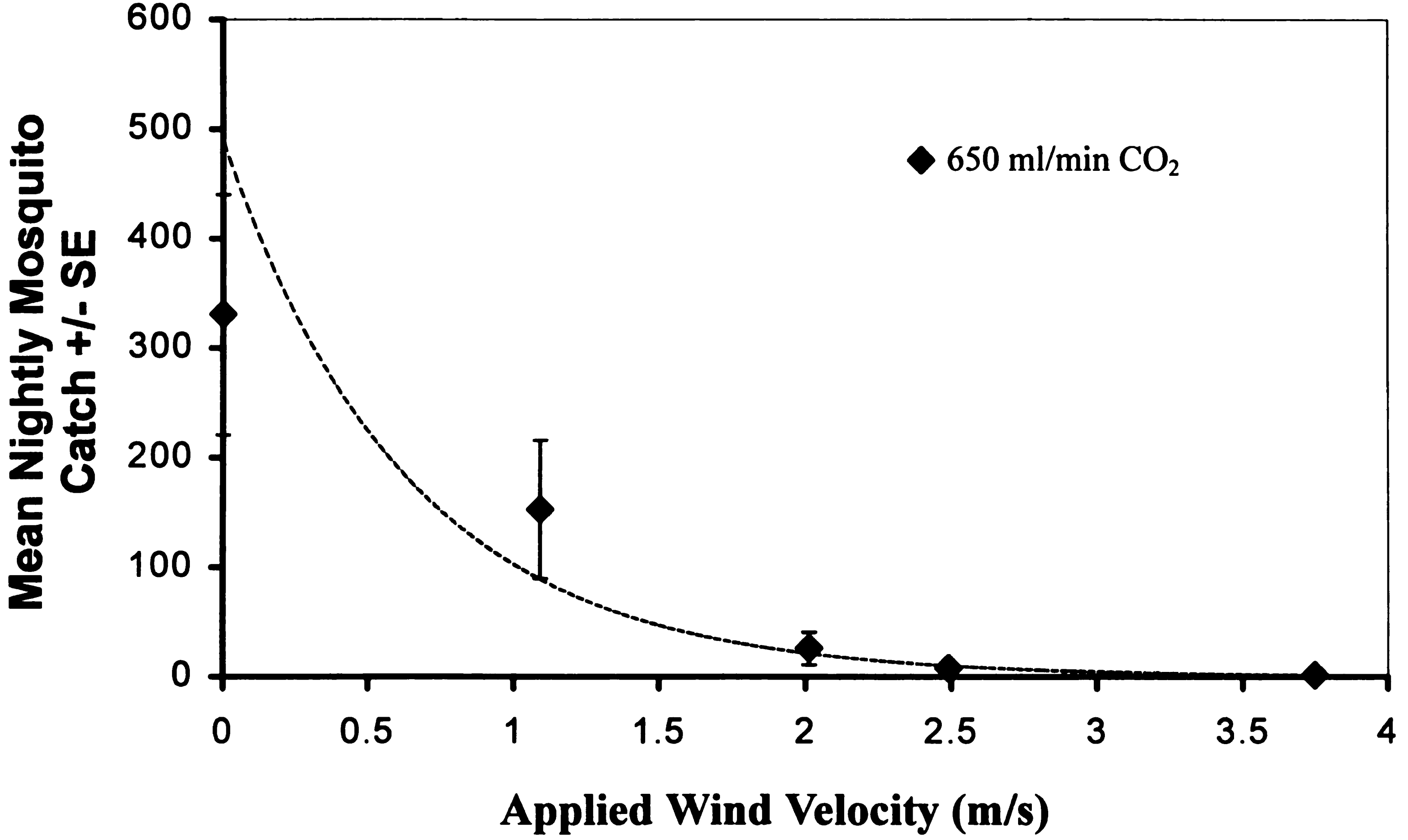


Figure 11: Number of female mosquitoes (combined species) captured with CO_2 stimulus (Experiment 2) as a function of wind velocity. The dotted line represents a general decay function.

Five wind velocities and two CO_2 release rates (Experiment 3)

Tripling the CO_2 release rate significantly and consistently increased (F = 15.79; d.f. = 1; *P* < 0.0002) while increasing wind velocity significantly decreased (F = 65.56; d.f. = 4; *P* < 0.0001) the number of mosquitoes caught under either CO_2 regime (Figure 12). There was no significant interaction between these two factors. Regression analysis yielded no significant difference in either the slope (*P* = 0.21) or y-intercept (P = 0.98) for the two CO_2 release rates. The equation for the 650 ml/min CO_2 release rate was: $\log_{10}$ (catch + 0.5) = - 0.67 (wind velocity (m/s)) + 2.13. This equation yields an x-intercept of 3.2, which is interpreted as the wind velocity (m/s) at which the trap catch is reduced to zero. The 1,950 ml/min CO_2 release rate yielded an equation of $\log_{10}$ (catch + 0.5) = - 0.67 (wind velocity (m/s)) + 2.45 with an x-intercept of 3.7.

Five wind velocities with and without DEET vapor (Experiment 4)

As in the previous experiments, increasing wind velocity significantly reduced the number of mosquitoes caught (F = 39.81; d.f. = 4; *P* < 0.0001), as did the addition of DEET vapor (F = 12.15; d.f. = 1; *P* = 0.001). The interaction between the two factors was nearly significant (*P* = 0.0563). The slopes for each of the treatment groups (Figure 13) were significantly different from zero (t = -5.91; d.f. = 1; P < 0.0001) but not one another (*P* = 0.1595). The y-intercept of the DEET treatment regression was not significantly different from that of the no-DEET line (*P* = 0.16). The regression equation for the no-DEET response is: $\log_{10}$ (catch + 0.5) = - 0.49 (wind velocity (m/s)) + 1.96 with an x-intercept of 4.0. When DEET was present, the equation was: $\log_{10}$ (catch + 0.5) = - 0.73 (wind velocity (m/s)) + 1.88 with an x-intercept of 2.6.

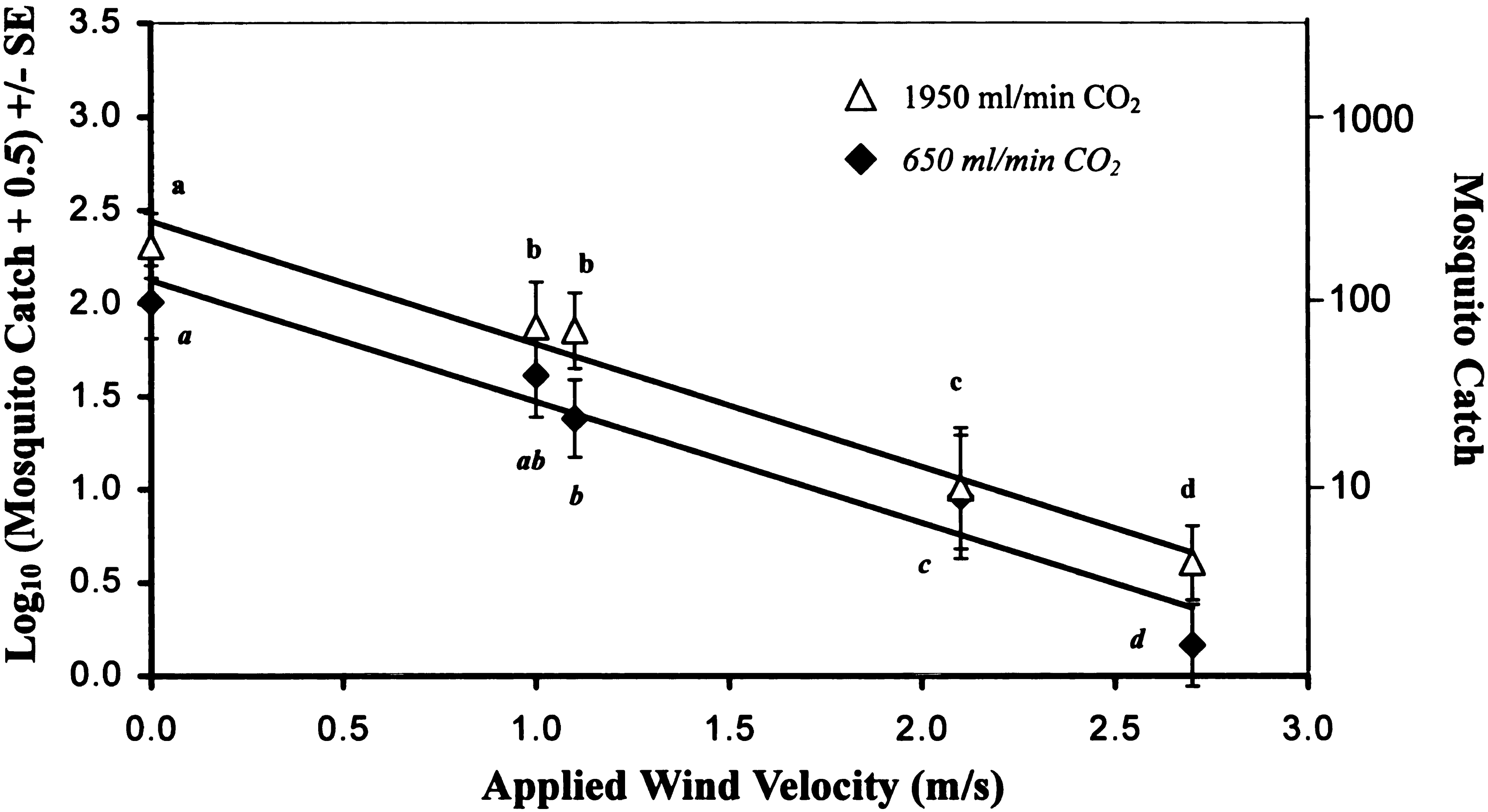


Figure 12: Number of female mosquitoes (combined species) captured at two different CO_2 release rates (Experiment 3) as a function of wind velocity. Average nightly catch was 875 individuals. Individual means comprising a given line and sharing a common letter are not significantly different at $P < 0.05$.

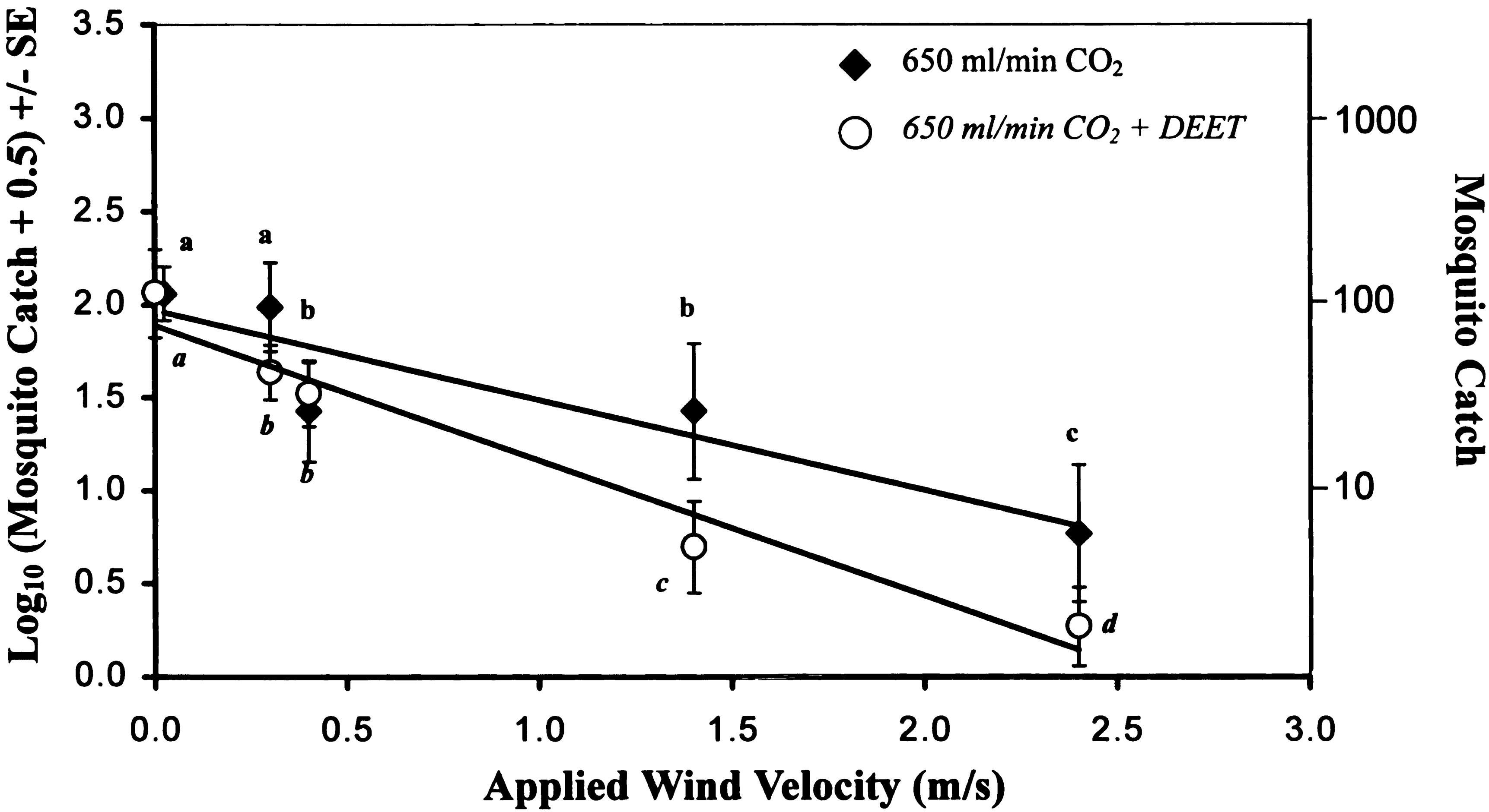


Figure 13: Number of female mosquitoes (combined species) captured with and without vapor-phase DEET (Experiment 4) as a function of wind velocity. Average nightly catch was 900 individuals. Individual means comprising a given line sharing a common letter are not significantly different at $P < 0.05$.

Comparison of Species Responses to Wind Velocity and CO_2

Sixteen species were present in the light trap samples for Experiment 3 (Table 1). Voucher specimens are deposited in the Entomology Museum, Michigan State University (Appendix 1). Collectively, *An. walkeri, Ae. vexans* and *Coquillettidia perturbans* made up over 92% of the identified individuals. I considered these three species sufficiently abundant to permit statistical analysis of response patterns on an individual species basis. The other species had either very low counts (1-5 per block) or were present in too few blocks for appropriate analysis. Figures 14-16 illustrate the responses to applied wind and CO_2 for each of these three species. Within-species comparisons of the slope relationships for the two CO_2 release rates were not significantly different for *An. walkeri* ($P = 0.577$), *Ae. vexans* ($P = 0.746$), or *Cq. perturbans* ($P = 0.275$). Between-species comparisons of linear-response slopes at each CO_2 release rate also yielded no significant differences.

Mosquito Mass and Wind Velocity Relationship

In Experiment 3, mass did not significantly influence (Figure 17A) mosquito response to CO_2 ($P = 0.783$) or applied wind velocity ($P = 0.22$). The slopes of the regressions (not shown in Figure 17) for either CO_2 release rate were not significantly different from zero ($P = 0.2545$) (Figure 17A). Likewise, There was no significant relationship between DEET and mosquito mass ($P = 0.13$) or applied wind velocity and mosquito mass ($P = 0.4409$) in Experiment 3 (Figure 17B). The slopes of the regression were not significantly different from zero ($P = 0.437$). Some error may have been introduced by damage to the mosquitoes in the handling stages of counting and

Table 1. Mosquito species composition for Experiment 3

Mosquito Genus/ Species	% of Total Mosquitoes Captured [1]
Aedes	
canadensis (Theobald)	0.09
cinareus (Meigen)	0.01
sticticus (Meigen)	0.03
stimulans (Walker)	0.48
trivittatus (Coquillett)	0.20
vexans (Meigen)	35.35
Anopheles	
punctipennis (Say)	1.70
quadrimaculatus (Say)	2.78
walkeri (Theobald)	39.10
Culex	
pip/rest [2]	0.28
salinarius (Coquillett)	0.34
Culiseta	
inornata (Williston)	0.15
melanura (Coquillett)	0.01
morsitans (Theobald)	0.29
Coquillettidia pertubans (Walker)	17.34
Damaged	1.85

1. Total number of mosquitoes was 7,919.
2. *Culex pipiens* (Linneus) and *Cx. restuans* (Theobald) are best distinguished by egg raft type. We lacked this information, and these species were grouped together.

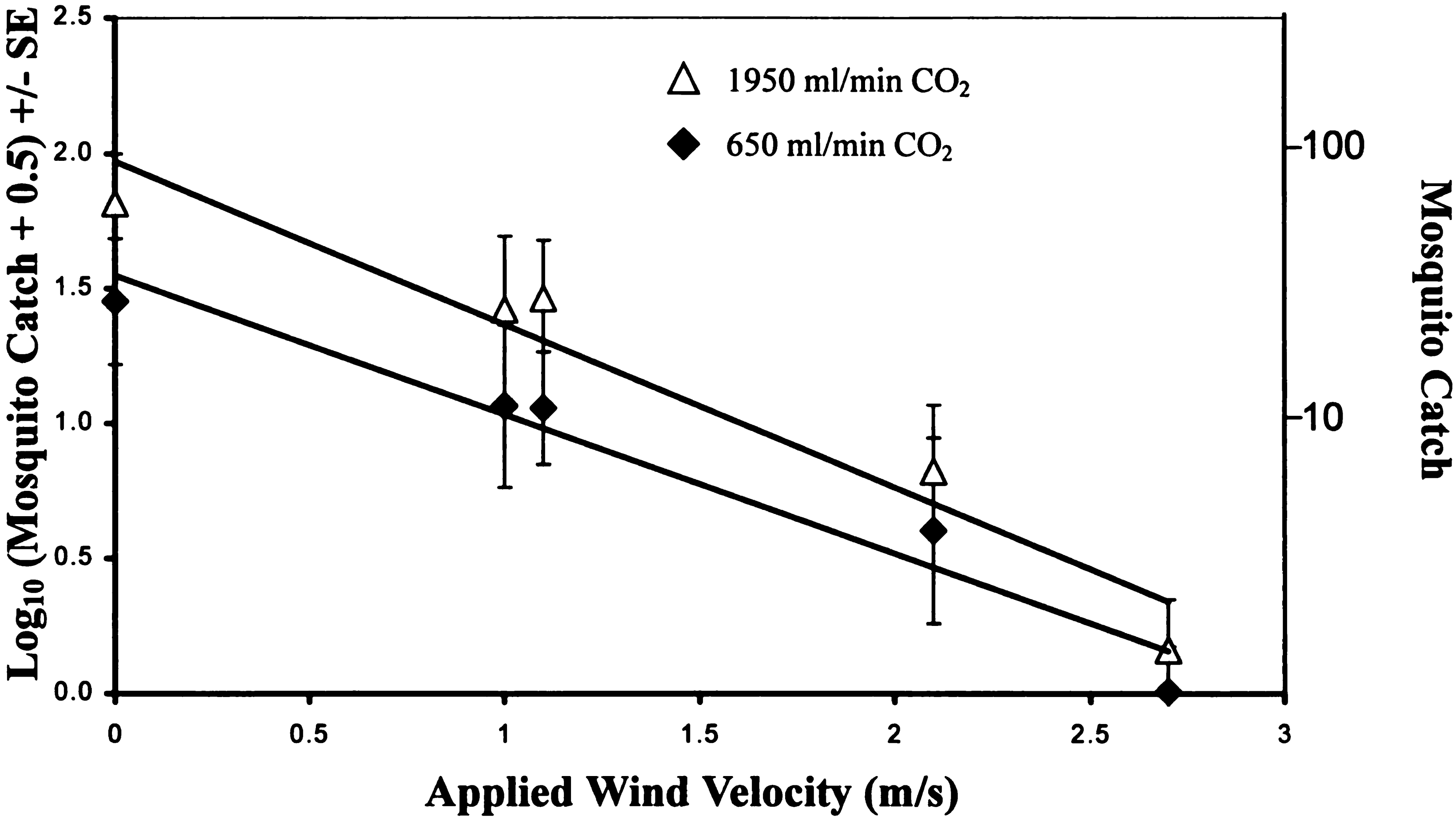


Figure 14: Number of female mosquitoes of *An. walkeri* captured at two different CO_2 release rates (Experiment 3) as a function of wind velocity.

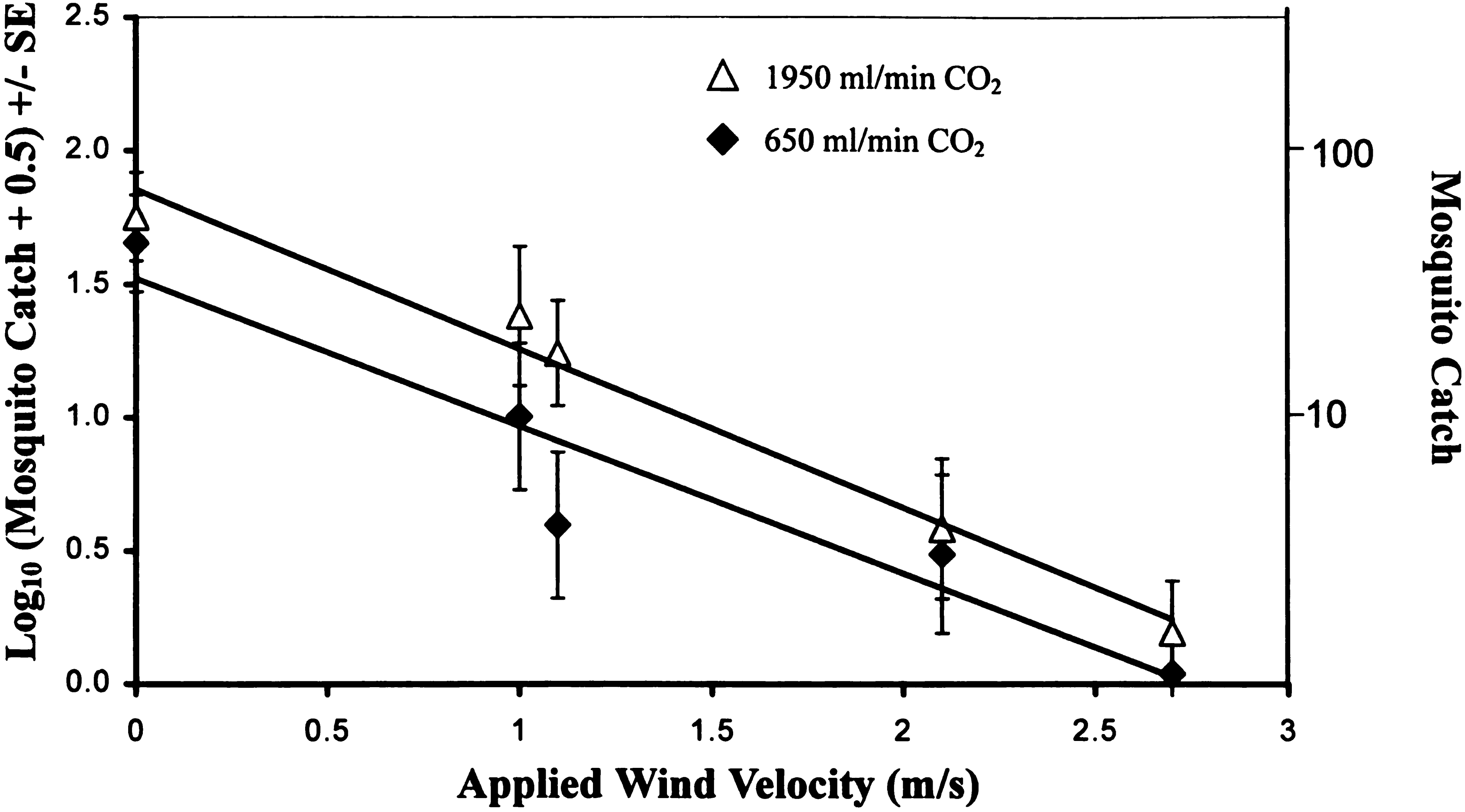


Figure 15: Number of female mosquitoes of *Ae. vexans* captured at two different CO$_2$ release rates (Experiment 3) as a function of wind velocity.

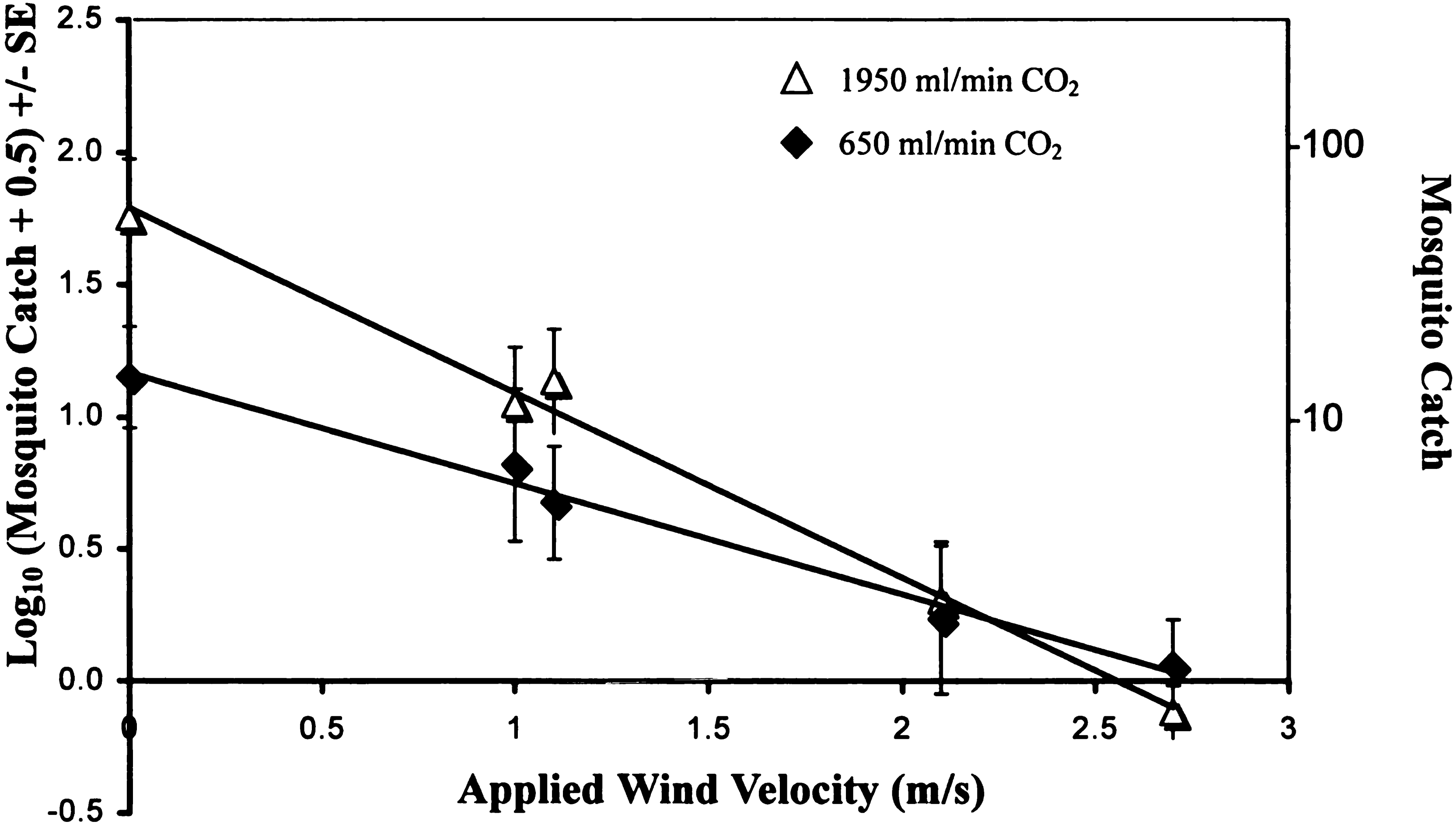


Figure 16: Number of female mosquitoes of *Cq. perturbans* captured at two different CO_2 release rates (Experiment 3) as a function of wind velocity.

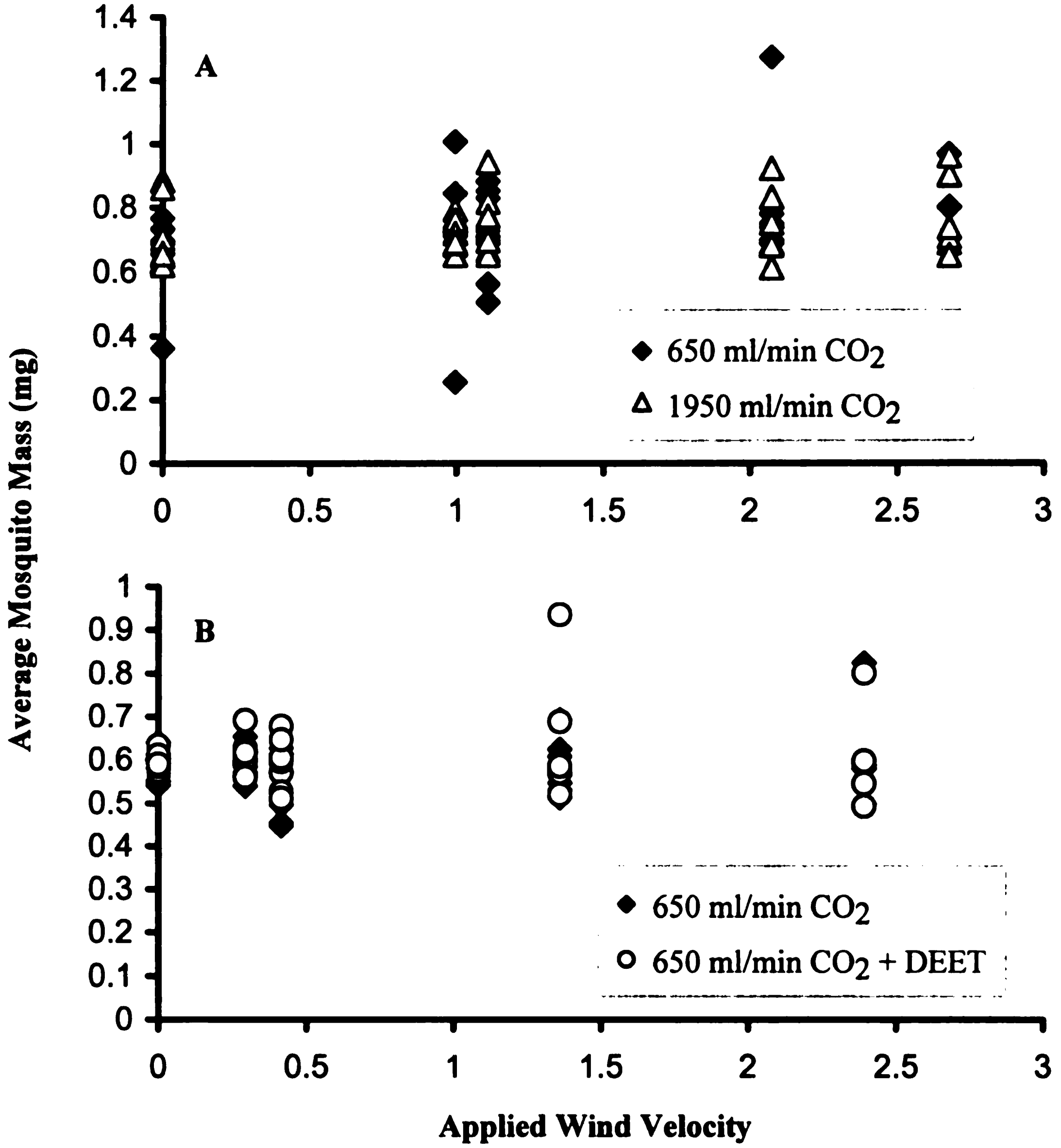


Figure 17 A and B: Scatter plots of mosquito masses as a function of applied wind velocity A) with two CO_2 release rates (Experiment 3), B) with two DEET release rates (Experiment 4).

transferring to storage containers. However, there did not appear to be a difference in damage intensity between larger and smaller specimens that would negate an authentic relationship between body mass and flight capacity.

Discussion

As previously established for ambient winds (Wright and Knight 1966, Snow 1980, Bidlingmayer 1985, Bidlingmayer et al. 1995), wind velocity as manipulated in the current study strongly reduced mosquito orientation. Moreover, my collective data allow differentiation between two possible modes of wind action: dilution vs. flight limitation. Despite the broad acceptance that mosquito orientation is limited by flight capacity, the data herein support only dilution, based on the following lines of evidence:

Mosquito Mass and Wind Velocity Relationship

If the flight-limit model were in play under my test conditions, a positive correlation of body mass would be expected vs. wind velocity, as supported by Lewis and Taylor's (1967) analysis for flying insects generally. As a robust 14,173 mosquitoes generated this outcome, it seems unlikely that a real effect was missed. The lack of mass effect is an outcome consistent with the stimulus dilution model, where response would be shaped by stimulatory factors, which are independent of body size.

Negative Logarithmic Response

The relationship between trap catch and wind velocity was negatively logarithmic for all baited treatments (Figures 8 - 12). This is to be expected when viewed in the light of a dilution-mediated model of mosquito host seeking. The concentration of a chemical emitted into air from a constantly-releasing point source is readily calculable. It diminishes geometrically with wind velocity; each doubling of wind velocity halves the concentration of the chemical. The general form of this equation is $y = 1/x$ where y is the stimulus concentration at a given wind velocity (x). Additional dispersion of the chemical due to turbulence and diffusion would cause the concentration to fall faster than the theoretical values of this equation, and the resulting relationship would appear more like a logarithmic function.

Patterns in my data seem completely explained by stimulus dilution. Trap catches consistently decayed more than geometrically over all applied wind velocities, and, there was no evidence of response truncation at the highest wind velocities. Such a truncation would be expected if mosquitoes were approaching their flight limit. Likewise, dilution effects appear to explain a preponderance of the negative logarithmic response documented in previous studies involving ambient winds (Bidlingmayer et al. 1995).

Parallel Responses for Two CO_2 release rates

The remarkably parallel responses to wind velocity with two CO_2 release rates (Figure 12) further support the dilution model. Tripling the release rate of CO_2 consistently doubled the number of mosquitoes caught for all applied winds (up to 2.7 m/s) with no evidence of truncation at the highest velocity. If these velocities approached a flight limit, the responses for the high and low release rates should have converged at the higher wind velocities.

Comparison of Species Responses

As evidenced by the uniformity of the log-transformed plots of trap catch, most of the mosquito species in this study responded similarly to applied wind velocity. However, the general response pattern of *Cq. perturbans* did differ from that of either *Ae. vexans* or *An. walkeri*. Whereas the latter two species had very linear (and remarkably parallel) responses to the two CO_2 release rates, *Cq. perturbans*' responses for the two release rates converged between 2 and 2.5 m/s.

This response could be viewed as evidence for a physical flight limit for this species at ca. 2.5 m/s with responses below this limit being driven by stimulus concentration.

These data for mosquito mass, logarithmic response, and response to elevated release rates of CO_2 lead us to conclude that general mosquito response to hosts is *not* driven by flight limits and preferred optomotor response, but rather by the stimulus concentration that results from wind- and distance-mediated dilution. It is quite possible that the mosquito species encountered here could successfully host seek at much higher velocities than those experimentally applied, provided that the instantaneous concentrations of CO_2 remained elevated and in the physiologically active range. Previous flight estimates of mosquitoes have all been taken from unbaited suction traps or experiments with one release rate. Maximum flight estimates in the literature of 1-2 m/s may widely underestimate these insects' flight capacity. More accurate mosquito flight capacities could be experimentally determined using protocols similar to those employed in this study, but using higher CO_2 release rates and correspondingly increased wind velocities.

DEET and Lactic Acid

Since lactic acid was not included in my experiments, DEET's effect in reducing trap catch (Figure 13) contradicts previous conclusions that DEET's repellent effect is derived solely from inhibition of lactic acid detection (Davis and Bowen 1994, Dogan et al. 1999). DEET is known to have physiological effects on lactic acid-stimulated neurons

(Davis and Sokolove 1976, Davis 1985, Davis et al. 1987), but this chemical's action appears to be more complex than eliciting host-seeking insensitivity to lactic acid. The physiological point of action may remain the lactic acid sensor (and/or other sensory organs), but the behavioral response might be decreased upwind displacement or inhibition of landing behavior. These effects are consistent with the increased turn angles for mosquito flight paths quantified for DEET vs. DEET-free stimulus plumes (Davis and Bowen 1994).

Potential Practical Applications of These Findings

Artificial wind either alone or in conjunction with vapor-phase DEET shows promise as a means of personal protection from mosquitoes. Chapter 3 established this effect for a human subject, and the current study demonstrated the reductive potential of wind as a function of the applied wind velocity. For all mosquito species encountered in this study, wind velocity alone uniformly and dramatically reduced captures of mosquitoes in traps. Such generalized effect across species is encouraging since, in the backyard setting, some 10 different species could be present at a given time in some locations. Fortunately, wind velocities substantially reducing mosquito orientation are comfortable for people. Thus, wind alone from an out-door fan could be a useful tool in non-chemical protection from these pests.

By employing a coordinated array of fans at ground height, it may be possible to establish a zone of protection (Figure 18) larger and thus more functional than that offered by a single fan. Arrangements like that of Figure 18, where the wind stream would not be aimed directly at the target of protection, would permit use of wind velocities in excess of those judged comfortable by humans. Deflection and dilution of

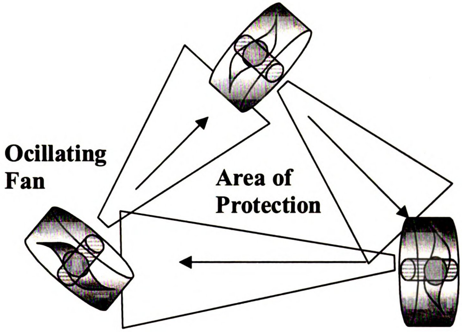


Figure 18: Vertical perspective of an area of protection generated by multiple fans collectively forming a horizontal wind curtain.

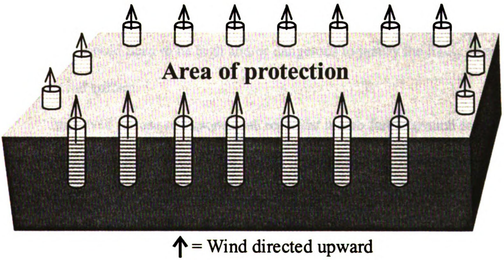


Figure 19: Side projection of an area of protection generated by a border of vertically oriented wind ports forming a vertical wind curtain.

odor plumes by these crosswinds from appropriate fans would likely present a strong challenge to orienting mosquitoes. It has been suggested that mosquitoes host seek primarily within the boundary layer (Snow 1979), and that they reduce flight altitude in the face of high wind velocities (Gillett 1979). Both of these behaviors would be exploited by a horizontal wind curtain, as mosquitoes would naturally be drawn to *lower* altitudes rather than flying over the barrier. With host-odor directionality disrupted, a mosquito at the barrier threshold would probably lose contact with the plume entirely. A horizontal wind curtain might be effective without vapor-phase repellent.

Vertical wind curtains might also be feasible. These could be generated using multiple fans aimed upward or upward-vented air from a single large fan (Figure 19). Mosquito reduction via this method would stem largely from the wall-like nature of the wind curtain, dilution of host stimuli, and the loss of the odor plume's directionality as attractant chemicals pass into the upward air currents. Admittedly, the mosquito populations would need to be high and/or dangerous to justify the likely expense of a vertical wind curtain.

Improved release of vapor-phase repellent is also fertile ground for additional investigation. An automated-release system that dosed vapor into the wind plume would be far more efficient than the primitive release method described in this study. I did attempt to use Michigan State University's Microsprayer^{TM} (Isaacs et al. 1999) technology to deploy vapor phase DEET, but the dispensed aerosol was not sufficiently fine to stay suspended and envelope a target area in vapor. However, Microsprayer-type devices could be improved to function as automated repellent delivery systems for area repellency when combined with fans. Special care should be taken to protect foliage from repellent aerosol droplets, though; preliminary experiments with DEET and Microsprayers resulted in significant phytotoxicity on grass and garden plants that were under or downwind of the release device. Alternative repellents such as Autan® or active botanicals should be tested for their effectiveness as wind driven vapors in addition to dermal applications.

Irrespective of whether these particular wind- or wind/chemical-based proposals to protect humans in the back-yard setting prove feasible, the data reported here will hopefully stimulate and anchor additional exploration of the potential for mosquito manipulation by air -- a medium so common and available it may have long been overlooked.

APPENDIX

APPENDIX 1

Record of Deposition of Voucher Specimens

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2001-07

Title of thesis or dissertation (or other research projects):

Behavioral Manipulation of Mosquito (Diptera : Culicidae) Host Seeking Using Artificially Applied Wind and Vapor-Phase Repellent: Potential for Personal Protection

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Investigator's Name(s) (typed)
Eric James Hoffmann

Date 8/16/01

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:
Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1

Table 2: Voucher specimen data

Page 1 of 4 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Aedes canadensis	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	June 19, 2000					1			MSU
	July 17, 2000					1			MSU
	August 1, 2000					2			MSU
Aedes cinareus	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	June 19, 2000					1			MSU
	July 17, 2000					2			MSU
Aedes sticticus	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	June 7, 2000					2			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Eric James Hoffmann

Date 8/16/01

Voucher No. 2001-07

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

21 AUG 2001

Curator Date

Appendix 1

Table 2 (cont'd).

Page 2 of 4 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Aedes stimulans	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	June 7, 2000					2			MSU
	July 12, 2000					2			MSU
Aedes trivittatus	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Peacock Rd 1km N of I-69								
	July 14, 1999					8			MSU
Aedes vexans	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	June 7, 2000					10			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Eric James Hoffmann

Date 8/16/01

Voucher No. 2001-07
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

Appendix 1

Table 2 (cont'd).

Page 3 of 4 Pages

		Number of:							
Species or other taxon	Label data for specimens collected or used and deposited	Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Anopheles punctipennis	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Peacock Rd 1km N of I-69								
	July 12, 1999					1			MSU
	July 16, 1999					2			MSU
	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	July 7, 2000					3			MSU
	July 17, 2000					2			MSU
Anopheles quadrimaculatus	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	August 7, 2000					1			MSU
	August 14, 2000					3			MSU
Anopheles walkeri	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	July 17, 2000					10			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

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Curator Date

Appendix 1

Table 2 (cont'd).

Page 4 of 4 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults	Adults	Other	Museum where deposited
Culex salinarius	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Peacock Rd 1km N of I-69								
	July 27, 2000					1			MSU
Culiseta inornata	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	June 7, 2000					3			MSU
	July 27, 2000					3			MSU
Culiseta morsitans	MICHIGAN Clinton Co. Rose Lake Wildlife Res. Area. Stoll Rd 1km E of Upton Rd								
	July 27, 2000					3			MSU
	August 1, 2000					2			MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Eric James Hoffmann

Date 8/16/01

Voucher No. 2001-07

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

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