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LIGHT COLOR ATTRACTION AND DIETARY SUGAR COMPOSITION FOR SEVERAL MOSQUITO (DIPTERA: CULICIDAE) SPECIES FOUND IN NORTH CENTRAL FLORIDA

By

DOUGLAS ARTHUR BURKETT

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1998

Dedicated to
Laura, Alexis, Sara, Betty and Bob

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctorate of Philosophy

LIGHT COLOR ATTRACTION AND DIETARY SUGAR COMPOSITION FOR SEVERAL MOSQUITO (DIPTERA: CULICIDAE) SPECIES FOUND IN NORTH CENTRAL FLORIDA

By

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August 1998

Chair: Daniel L. Kline
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The behavioral response of mosquitoes to different wavelengths of light and an evaluation of sugar meals was studied for several species of mosquito found in north central Florida. Gas chromatography (GC) was used to analyze dietary sugars of *Anopheles quadrimaculatus*, *Coquillettidia perturbans*, *Culex nigripalpus*, *Culiseta melanura* and *Psorophora ferox*. GC was also used to determined whether carbohydrases are present in the mosquito diverticula. A wide range of sugars was found in wild mosquitoes including fructose, glucose, sucrose, maltose, turanose, melezitose, raffinose, erlose, arabinose, rhamnose, and several unknowns. Laboratory time course studies with *Aedes albopictus* showed rapid hydrolysis of sucrose within 2 hours of ingestion.

Conversely, melezitose remained relatively unchanged after 8 hours. The frequency of wild mosquitoes containing sugars ranged from 10-11% in *An. quadrimaculatus* and *Ps. ferox* to 48% in *Cq. perturbans*. All species tested contained honeydew sugars including *An. quadrimaculatus* (55%), *Cs. melanura* (33%), *Cx. nigripalpus* (15%), *Cq. perturbans* (10%) and *Ps. ferox* (7%). Light trap capture numbers for woodland mosquitoes were evaluated using light emitting diodes (LEDs) of different colors. Analysis of data by species showed significant differences in color attraction (green and blue best) for *Ae. atlanticus*, *Ae. dupreei*, *Ae. infirmatus*, *An. crucians*, *Cs. melanura*, *Cx. nigripalpus*, *Ps. columbiae* and *Uranotaenia sapphirina*. Attraction was also evaluated using different orientations (light reflected of off lid, or 360° radius) of green/blue LEDs and found significant capture number differences for only *An. crucians*, *Cs. melanura*, *Cx. (Melanoconion spp.)* and *Ur. sapphirina*. In the lab, a ten port visualometer was constructed to evaluate the feeding response of 4 mosquito species over various wavelengths of light. Color preferences were based on feeding durations obtained electronically over 4 hour intervals and numbers of fecal specks after a 16 hour exposure. Feeding duration and fecal speck data were collected for female *Ae. aegypti*, *Ae. albopictus*, *An. quadrimaculatus* and *Cx. nigripalpus* in the visualometer on an artificial host illuminated from below with light of equal intensities in 50 nm increments (700-350 nm). Significant color preferences based on feeding durations were detected for *Ae. albopictus*, *An. quadrimaculatus* and *Cx. nigripalpus*. Fecal speck numbers significantly correlated with feeding durations for *An. quadrimaculatus* and *Cx. nigripalpus*.

CHAPTER 1
MOSQUITO SUGAR FEEDING AND VISUAL ATTRACTANTS REVIEW

Introduction To Sugar Feeding

An increased understanding of mosquito biology, improved surveillance and effective control practices are the main driving forces that has made Florida and other coastal states habitable. Blood loss, annoyance and their ability to transmit a variety of serious diseases have given mosquitoes dubious notoriety. Research on the sugar sources of mosquitoes can have great potential returns to biologists and mosquito control personnel. Determination of crop carbohydrate composition would allow researchers to make inferences about the identification of plants and plant-derived products being used to provide metabolic demands of wild mosquitoes. These inferences, however, are only possible if common plant-derived oligosaccharides remain unhydrolyzed and identifiable in the crop for a significant amount of time. A better understanding of these facets of mosquito ecology can be applied directly to the development of new attractants and improving control methods.

It is currently unknown as to whether natural sugars are an unlimited and universally available resource, or if suitable sugars are scarce and their lack can adversely affect mosquito survival and reproduction. In general, mosquitoes obtain sugars from a wide range of sources (Gadawski and Smith 1992, Grimstad and DeFoliart 1974,

Magnarelli 1977, 1979, 1983). Furthermore, not much is known about species-specific preferences for natural sugar hosts. Are mosquitoes generalists and opportunistic, obtaining sugar from sources that are readily available or do they use a narrow set of discrimination criteria to choose their natural sugar sources?

Plant taxa differ in the proportion and to a lesser extent, the composition of sugars present in nectar. If sugar meals remain unchanged upon ingestion and storage in the crop, it may be possible to identify specific plants or at least groups of plants with similar properties based on the presence or absence and relative concentrations of sugars present in the mosquito crop. It is currently unknown how many mosquito species contain salivary or crop enzymes which hydrolyze sucrose and other common plant oligosaccharides into simpler molecules.

As a precursor to determining sugar sources utilized by various wild mosquito species, one must determine if, how much, and how fast the plant-derived mono- di- and trisaccharides are broken down in the crop. If these ingested sugars remain unchanged in the crop for any length of time, inferences could be made as to their sugar source utilization of wild mosquitoes. Ultimately, we could determine if certain mosquito species preferentially utilize various plant families with certain nectar characteristics, or if they utilize sugary exudates from Homoptera (e.g. honeydew) or some other sugar source. For a given mosquito species, what proportion of the population feeds on nectars or other plant juices and what proportion feeds on honeydew?

Carbohydrate and Amino Acid Sources

Much of the evidence for mosquito nectar feeding comes from direct field observations rather than through indirect chemical qualitative analysis of crop contents. West and Jenkins (1951), Sandholm and Price (1962), Gadawski and Smith (1992), Breeland and Pickard (1961, 1967), and Magnarelli (1977) have observed some mosquito species feeding during the day. Most direct observations, however, have been made at night, dawn or dusk (Grimstad and DeFoliart 1974, Magnarelli 1983, Andersson and Jaenson 1987, Bowen 1992, Vargo and Foster 1984, Yee et al. 1992). Interestingly, not all common mosquito species have been observed feeding on sugar in the field. Bidlingmayer and Hem (1973) found evidence of fructose in the crops of *Culiseta melanura* (36%), *Culex nigripalpus* (17%), *Psorophora ferox* (20%) and *Anopheles quadrimaculatus* (15%). Likewise, Magnarelli (1978) obtained similar results for *Cq. perturbans* (57%). There have been no published accounts of field sugar feeding for *Aedes albopictus*, *An. quadrimaculatus*, or *Cs. melanura* and only a few for *Cx. nigripalpus* (see Nayar 1982). Regardless of species, most direct field observations of sugar feeding have occurred at ground level nectar sources on flowering herbs and low growing shrubs. These observations do not incorporate sugar feeding in trees or at the potentially numerous honeydew, extrafloral nectary or other plant exudates scattered up in the canopy and throughout the environment.

As indirect evidence for "nectar" feeding, dozens of mosquito species have been chemically analyzed for the presence or absence of fructose. Fructose is a ubiquitous

monosaccharide present in almost all plant sugars. Many authors (Andersson and Jaenson 1987, Edman et al. 1992, Van Handel et al. 1972, 1994, Bidlingmayer and Hem 1973, Magnarelli 1978, 1979, 1980, 1983, Reisen et al. 1986, Nasci and Edman 1984, Smith and Kurtz 1994) have used the cold anthrone test (Van Handel 1972) to qualitatively establish the presence or absence of fructose (or oligosaccharides containing fructose or other reducing sugars). According to Van Handel (1967), the cold anthrone reagent reacts with fructose, inulin, sucrose, melezitose, and raffinose. These sugars all contain the fructose moiety and give a positive or negative response based on a resulting color reaction. Unfortunately, the cold anthrone test does not provide information about the origin of crop sugars or the exact carbohydrate composition. Many other common mono-, di- and trisaccharides are present in potential natural sugar meals, but few attempts have been made to categorize the individual sugars and relate the meals back to the original source.

Although usually used as evidence for "nectar feeding," the cold anthrone test will also react positively for insects having fed on honeydew or other non-floral sugar sources. Few attempts have been made to determine the exact crop sugar concentration and composition for various Dipteran crops using modern chromatographic techniques. Thin Layer Chromatography (TLC) has been used by Magnarelli and Anderson (1977), Magnarelli (1980), and Van Handel (1984). High performance liquid chromatography (HPLC) was successfully used by MacVicker et al.(1990), to examine the contents of crops of five wild Italian sand fly species. Hoppe (1983), used HPTLC for determining the sugars found in horse flies. In spite of its common use in other systems, Moore et al.

(1987), Chang et al. (1977), and Alexander (1988), were among the first (and few) to use modern and sensitive gas liquid chromatography (GC o r GLC) for investigating dipteran diets.

The Need for Dietary Sugars

With few exceptions, mosquitoes generally require dietary sugars for survival, longevity, host finding and reproductive success. In terms of survival, female *Ae. aegypti*, *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* lived much longer (Briegel and Kaiser 1973, Harada et al. 1976) and laid more eggs (Nayar and Sauerman 1975a) in the laboratory when provided with both sugar and blood meals than when given blood alone. When fed individual sugars, Galun and Fraenkel (1957) reported that *Ae. aegypti* survived the longest (at least 25 days) on glucose, fructose, sucrose, maltose, trehalose, melibiose, raffinose or melezitose. With the exception of melibiose, similar results were found for *Cs. inornata* (Salama 1967). Other sugars reduce the longevity or have no effect on survival or fecundity (Nayar and Sauerman 1971a). The sugars enhancing survival are commonly found directly or indirectly with plants. Nayar and Sauerman (1975a) reported duration of mosquito survival to be directly related to the rate of disappearance of glucose which followed an exponential law of decline. Furthermore, the disappearance rate differed between mosquito species. *Ae. aegypti* and *An. quadrimaculatus*, for example, used glucose faster than *Ae. sollicitans* and *Ae. taeniorhynchus* under similar conditions.

Some species or populations of some species may require minimal or no sugar to survive and reproduce. An *Ae. aegypti* population from Thailand was found to rarely, if ever take sugar meals, apparently obtaining all their sugar requirements from multiple blood meals (Day et al. 1994, Edman et al. 1992). Interestingly, this *Ae. aegypti* population produced as many eggs as those that took sugar meals. In another similar case, a Puerto Rican *Ae. aegypti* population contained only 5% of the mosquitoes positive for fructose (Van Handel et al. 1994). Whereas, in a Florida population, 50-75% of the sampled mosquitoes were positive for fructose. Some authors find sugar vital for stimulating ovarian development. For example, Klowden (1986), found that laboratory reared *Ae. aegypti* did not develop a batch of eggs without obtaining a sugar meal before a blood meal. Much remains to be learned about sugar feeding. A lack of sugar feeding by some species or populations of some species may be more common than portrayed in the literature. Indeed, many species have only been rarely observed sugar feeding in the wild and of those species examined, some contain only a very low percentage of sugars in their crops. Foster (1995) summarizes that some species have only 1-4% of the population containing fructose. In most mosquito species, however, greater than 40% contain fructose and a few species have more than 80%.

In terms of host-finding, female sugar feeding mosquitoes may enhance their vectorial competence and improve their success of obtaining a blood meal. Walker and Edman (1985), reported that female *Ae. triseriatus* and *Ae. aegypti* that have had access to sugar sources were more persistent (thus more successful) in their attempts at blood feeding than were sugar-deprived females. However, in terms of frequency blood feeding

by *An. quadrimaculatus* was not affected by the availability of sugar (Foster and Eischen 1987).

Floral Nectaries

The most documented source of carbohydrate for mosquitoes is floral nectaries. Haeger (1955) ranked floral nectar as most important followed by honeydew and extrafloral nectars for natural sugar sources. In a recent review article, Foster (1995) agreed that floral/extrafloral nectars and honeydew are probably mosquitoes' main source of sugar for mosquitoes, but stated that their importance was difficult to determine. What exactly is nectar? Kevan and Baker (1983) state that nectar is a phloem-sap derivative produced in a series of complex physiological processes in special glands of plants. It is composed mostly of sugars, but also may contain free amino acids, proteins, lipids, antioxidants, alkaloids, vitamins, organic acids, allantoin and allantoic acid, dextrins and inorganic materials such as minerals. Crop sugar contents of mosquitoes were 20-50% w/v as reported by Hocking (1953, 1968). The majority of evidence indicates that mosquitoes are selective about the source of sugar meals and may not feed on whatever is present.

All authors agree that the most common nectar sugars consist of sucrose, glucose, and fructose (Wykes 1951, Van Handel 1972, Baker and Baker 1983a, 1983b, Kevan and Baker 1983, Percival 1961). There tends to be disagreement, however, as to the exact composition and relative abundance of some of the less common sugars. Wykes (1951), Percival (1961), Baker and Baker (1983b) state that the monosaccharides galactose and

arabinose, the disaccharides maltose and melibiose, and the trisaccharides melezitose and raffinose are present in some plant species. It is rare to find a nectar with only one detectable sugar and none contain only fructose, or sucrose and fructose in the absence of glucose (Baker and Baker 1983b). Some plant families may be characterized by the relative proportions of the three major sugars. For example, certain plant families such as Lamiaceae (mints) and Ranuculaceae (buttercups) are characterized by sucrose-rich nectars (Baker and Baker 1983a). Other families such as Brassicaceace (mustards) and Asteraceae (composites) have hexose-rich nectars. Wykes (1951), Percival (1961), and Van Handel (1972) examined many plant species in several families and concluded the constituent sugars and their relative proportions in nectar tend to remain constant from any one species, while their occurrence appears to be characteristic for certain families. Percival (1961) tabulates the major plant families likely to contain the less common nectar sugars such as raffinose, maltose, and melibiose. If salivary or crop enzymatic activity is not found in the mosquito species being tested, it may be possible to provide generalizations on the relative abundance of each of the dominant sugars present in wild sugar sources.

A highly relevant, but almost completely ignored aspect of mosquito sugar feeding, is that nectar also contains detectable quantities of amino acids. If indirect determination of sugar feeding origin is not possible by examining sugar composition alone, it may be possible that examining both the amino acid and composition sugar will provide valuable clues about where mosquitoes obtain their sugar meals. As listed in descending order of commonness, Baker and Baker (1983a), report floral nectary amino

acids as follows (percentage in ()): alanine (96), arginine (90), serine (89) proline (87), glycine (84), isoleucine (73), threonine (67), valine (66), leucine (66), glutamic acid (62), cysteine, etc. (55), phenylalanine (55), tyrosine (52), tryptophan (48) lysine (41), glutamine (41), aspartic acid (32), asparagine (27) methionine (20), histidine (19), and nonprotein amino acids (36). Baker and Baker (1973) report the vast majority of the plant species tested to have nectar amino acids in detectable quantities. Furthermore, they state that the amino acid compliments, as well as the concentration of these acids in the floral nectar, will help to determine the nectar's "taste" to the flower visitor in addition to whatever nutritional significance it may have. Similar to sugar concentration/composition, amino acids vary depending on the nutritional requirements of the pollinator. In general, the concentrations of nectar amino acids seem to be greater if nectar is the only or the predominate source of protein for the flower visitor (Baker and Baker 1983a). For example, ants can act as selective agents, and favor plants with particular amino acids in their nectar (Lanza and Krauss 1984). Amino acids also appear to extend life expectancy of insects. Eischen and Foster (1983) reported that nectar amino acids extend survival, but are of insufficient quantity to trigger ovarian development in mosquitoes. It remains unclear as to whether mosquitoes choose their plant sugar sources based on the amino acid contents.

Many species of mosquitoes have been observed feeding on a large variety of flower species. Flowers or some plant sugars may serve as attractants. Indeed, sugar-containing flower or honey extracts are attractants for *An. quadrimaculatus*, and *Cx. nigripalpus* (Kline et al. 1990, Hancock and Foster 1993, see Foster and Hancock 1994),

Breeland and Pickard (1961, 1967), observed several flood water species, *Ae. vexans, Ae. trivattatus. Ps. cyansens. Ps. ferox, Ps. ciliata* feeding on composites such as goldenrod (*Solidago*) and ageratum (*Eupatorium*), dock (*Rumex*) and ironweed (*Vernonia*). The composites, ox-eye daisy (*Chrysanthemum*), yarrow (*Achillea*), golden rod (*Solidago*), and the common milkweed (*Asclepias*) were the dominant feeding sources of *An. earlei. An. walkeri, Cq. perturbans, Ae. canadensis. Ae. communis, Ae. sticticus, Ae. stimulans, Ae. vexans. Cs. silvestris* and *Cx. restuans* (Grimstad and DeFoliart 1974). With the exception of milkweed, the dominant nectar sources all contained high levels of glucose (considered hexose-rich). The univoltine snow pool mosquito, *Ae. provocans,* preferred feeding on trees and shrubs (particularly Rosaceae) over herbaceous plants (Gadawski and Smith 1992). Magnarelli (1983) observed *Ae. canadensis* and *Ae. stimulans* commonly probing white baneberry (*Actaea pachypoda*), bird's rape (*Brassica rapa*), maple leaved viburnum (*Viburnum acerifolium)*, and less commonly on yarrow (*Achellea millefolium*), evening lychnis (*Lychinis alba*), wild lily-of-the valley (*Maianthemum canadense*) and false Solomon's-seal (*Smilacina racemosa*). Direct field observations made by Sandholm and Price (1962), found 10 species of Minnesota mosquitoes feeding on a wide variety of flowering species from a local arboretum. *Ae. vexans* was present feeding on 39 species of plant, the most common being common milkweed *(Asclepias syriaca)*, wild cucumber *(Echinocystis lobata)*, fall phlox *(Phlox paniculata)*, goldenrod *(Solidago latifolia)*, and meadowsweet *(Spiraea latifolia*). *Ae. vexans* and *Cx. resturans* were the common species of mosquito observed by Vargo and Foster (1984), on Canada goldenrod (*Solidago*) and white snakeroot (*Eupatorium rugosum*). The most common

floral nectaries visited by *Ae. vexans*, and *Ae. trivittatus* included Queen Anne's lace, (*Daucus carota)*, common milkweed (*Asclepias*), dogbane (*Apocynum medium*), and oxeye daisy (*Chrysanthemum*)Yee et al. (1992). Interestingly, although blooming yarrow was also common, mosquitoes were not observed feeding on it as in other aforementioned studies. Based on the large percentage of mosquitoes that contained crop sugars and the relatively few direct observations of flower sugar feeding, Magnarelli (1980) concluded that *Psorophora ferox* obtains most of its sugars from sources other than floral nectaries.

Both male and female mosquitoes seek a sugar meal shortly after emergence and continue to take nectars throughout adulthood (Yuval 1992). Males probably feed nearly every day (McCrae et al.1976, Magnarelli 1979, 1983, Reisen et al. 1986). Field observations and chemical analyses (cold anthrone test) indicate that females of mosquito species obtain sugar meals throughout the gonotrophic cycle (Magnarelli 1978, 1979, 1983, Andersson 1990, Nasci and Edman 1984, Vargo and Foster 1984, Reisen et al. 1986, Andersson and Jaenson 1987, Haramis and Foster 1990). According to Pappas and Larsen (1978), mosquitoes may remain feeding on a floral nectary for up to 18 minutes. Similarly, lab work by Jepson and Healy (1988), found that *Ae. aegypti* feed on flowers for an average of 21 minutes.

Extrafloral Nectaries

Plant nectaries located outside the flowers are termed extrafloral nectaries. Most extrafloral nectaries do not involve pollination, and their function is not as uniform as that of floral nectaries (Koptur 1992). Extrafloral nectaries may be present on virtually every

vegetative and reproductive structure. They occur on the petiole, rachis, upper and lower surfaces of the blade, leaf margin, stipules and on most external parts of the flower. Although inconspicuous and often widely scattered, extrafloral nectars are readily available on assorted ferns and about one quarter of all angiosperms (Koptur 1992). They are unknown in gymnosperms (Keeler and Kaul 1984). Koptur (1992) provides an extensive list of plant species and the locations of their extrafloral nectaries.

The sugar and amino acid contents of extrafloral nectars contain slightly different constituents compared with their floral counterparts. Extrafloral nectar has a wider range of sugars, usually being poor in sucrose (Koptur 1992). That sugar concentrations of extrafloral nectars vary over a much wider range than floral nectars of the same species. Similar to the sugar composition, the amino acid composition within a species usually also differs slightly from that of the floral nectaries (Baker et al. 1978). Most notably, the cysteine group (lysine, asparagine, and tyrosine) are more common in extrafloral nectar. Differences in amino acid composition presumably relate to attraction of different insects such as ant or wasp "guards" which may have different nutritional requirements than those of pollinators. Baker et al. (1978) reported that nonprotein amino acids are more frequent in extrafloral nectars.

There have been only a few direct observations of mosquitoes feeding on extrafloral nectaries. Of the >20 nectar sources reported by Gadawski and Smith (1992), for *Aedes provacans*, only one source was extrafloral (*Crataegus*, hawthorn). Haeger (1955) observed *Aedes taeniorhynchus* feeding on the tender branch tips of buttonwood (*Conocarpus erectus*) on Sanibel Island Florida. More recently, Taylor and Foster (1996)

observed *Cx. nigripalpus* feeding on an extrafloral nectary on caster beans as well as several mosquito species feeding on extrafloral nectars on cashew trees.

Honeydew

Perhaps the single most underrated and common sources of sugar in dipteran biology are honeydews. The sugary exudate, "honeydew," refers to the liquid excretions from the alimentary tract, as released through the anus by aphids, coccids, and many other plant sucking insects. Auclair (1963) reports that honeydew (a.k.a. mannas) are usually complex mixtures of a large variety of chemical compounds, including several sugars, amino acids, amides, organic acids, alcohols, auxins, and salts. Honeydew typically contains about 11% dry matter, of which 88 % are carbohydrates and 7 % nitrogenous compounds. Honeydew is probably extremely common and may be used by mosquitoes and other flies far more often than what the literature reports. Auclair (1963) also summarized that although honeydew production varies widely between Homoptera species, in general, aphids produced 1.7-20 drops per aphid every 10 hours with an average volume and weight of 0.5 mm^3 and 0.9 to 8.6 mg, respectively.

Although, undoubtedly varying by species, it is currently unknown what proportion of the medically important mosquitoes of Florida utilize honeydew regularly as a sugar source. It is possible that honeydew feeding may have at least partially influenced mosquito evolution. Downes and Dahlem (1987), convincingly argue that Diptera (Cyclorrhapha in particular) evolved ingesting honeydew. They conclude that honeydew-producing Homoptera (present in the Permian era) occurred prior to the first

undisputed fossil evidence of Diptera, (occurring later in the Triassic era). Honeydew feeding and/or perhaps the piercing and sucking of sugar-containing plant juices must have occurred. Flowering plants, with their associated nectaries, did not occur until much later in the Cretaceous era.

Only a few floral or extrafloral nectars contain sugars, amino acids, or other chemicals unique to specific plant families or genera. This can make generalizations about specific mosquito and plant relationships very difficult if these are based solely on comparative chemical analysis. Honeydew, however, contains oligosaccharides uncommon in plant nectars, and which are largely unique to honeydew and their presence may be indicative of feeding on honeydew. A review of the literature on the sugars commonly found in honeydews produced by Homoptera is summarized by Auclair (1963). Fructose, glucose and sucrose were present in all species tested, glucosucrose (a.k.a. fructomaltose, erlose, 4-alpha-glucosylsucrose, alpha-maltosylfructoside) and melezitose were present in most species, and maltose and maltotriosucrose were present in a few species. A melezitose hydrolysis product, turanose, is also indicative and uniquely associated with homopterous honeydew (Hudson 1946). Using several modern chromatographic techniques, Lombard et al. (1987), found fructose, glucose, sucrose, maltose, turanose, trehalose, melibiose, raffinose, melezitose and some other unknown oligosaccharides in the honeydews of several homopterous species. More recently, Bates et al. (1990) used N.M.R. and Byrne and Miller (1990) used HPLC to characterize trahalulose (a disaccharide) as the dominant honeydew sugar produced by white flies. Further analysis by Yee et al. (1996) ranked white fly honeydew sugar components as

follows: trehalulose > melezitose > sucrose > fructose > glucose. Some sugar alcohols are also commonly found in honeydew. Stachyose has been reported from honeydew but not in the host plant (Byrne and Miller 1990). Ribitol, dulcitol, and mannitol have also been reported from honeydew (Ewart and Metcalf 1956). These alcohols were not considered unique products of honeydew, however, but were thought to pass unchanged from the plant through the insects alimentary tract. Melezitose, glucosucrose, turanose, stachyose, and trehalulose are not, or only rarely found in plant nectar. By association, the presence of any of these sugars in the crops of mosquitoes is indicative of honeydew feeding.

Amino acids are also a common component of honeydew, comprising up to 3-15% of the total composition (Auclair 1963, Ewart and Metcalf 1956). The most common amino acids present in honeydew are alanine, asparagine, aspartic acid, glutamic acid, glutamine, leucine, phenylalanine, proline, serine, threonine and valine (summarized by Auclair 1963). Unfortunately, in most cases, the amino acids present in honeydew were also present in the host plant. There do not seem to be any amino acids uniquely associated with Homoptera excretion.

Mosquitoes can, and probably do, routinely ingest honeydew sugars. Honeydew probably ranges from liquid to a semi-liquid syrup to dry solid residues on stems and leaves. The latter may not pose a problem for ingestion by some species. Mosquitoes have been shown to ingest solid sugars by first liquefying with saliva. For example, Eliason (1963) found *Cx. tarsalis, Cx. pipiens, Cs. incidens* and *Cs. inornata* fed readily

on solid, crystalline sugar; however, *An. freeborni*, *Ae. sierrensis*, *Ae. aegypti* and *Ae taeniorhynchus* did not readily feed on solid sugars.

Just as mosquitoes appear to have preferences for certain species of plant nectars, there is no reason to believe they do not also discriminate between different insect/plant honeydews. There have been a few direct observational accounts or chemical analyze proving that mosquitoes ingesting honeydew. Nielsen and Greve (1950), observed *Ae. cantans* feeding on honeydew and concluded it was probably its main sugar source. They also observed *Ae. taeniorhynchus*, *Ae sollicitans*, *Cx. nigripalpus*, and *An. atropos* feeding on green aphid honeydew on *Bidens* spp.. Honeydew feeding by *Cx nigripalpus* was also observed by Haeger (1955) who found honeydew second in importance compared to nectar. Killick-Kendrick (1987) conducted laboratory experiments demomstrating that the sand fly, *Phlebotomus ariasi*, ingests honeydew from aphids on plants, and presented strong circumstantial evidence indicating that wild flies feed on honeydew. Using wild sand flies, MacVicker et al.(1990) categorized sugars in the crop using HPLC and found significant levels of melezitose in five sand flies species from different habitats.

Other Sugar Sources

The piercing-sucking mouthparts of mosquitoes are utilized for feeding on animals, and could possiblly be used for feeding on plant tissues. Patterson et al. (1969) showed plant tissue feeding by mosquitoes, but was insufficient to sustain the life of mosquitoes and thought to be a means of obtaining water. Joseph (1970) observed

mosquitoes feeding on fruit. Other researchers (McCrae et al. 1976), Mogi and Miyagi 1989) observed mosquitoes imbibing exudates from leaves damaged by feeding insects. By products of fermenting sugars may be attractive to some mosquitoes. Worth (1975) noted that a wide variety of mosquito species were attracted to a sugar-beer-rum bait painted onto the sides of trees. One interesting theory of plant juice ingestion was proposed by Schlein and Muller (1995), who found evidence of plant tissue feeding by sand flies and mosquitoes and concluded that although low in sugar content and questionable as an energy source, the plant juices may be a means of helping to control internal parasites.

Diel Sugar Feeding Activities

Sugar and blood feeding may not be mutually exclusive events. Field data from Grimstad and Defoliart (1975), Yee et al. (1992), and Yee and Foster (1992) reported that nectar feeding and blood feeding activities occur at or about the same time of day with respect to photoperiod. Although not directly observed, both Reisen et al. (1986), and Yuval et al. (1994), found the percentage of mosquitoes containing fructose changed depending on what time of night the mosquitoes were sampled. The highest percentage occurred around sunrise for *Cx. tarsalis* and male *An. freebornii.* Conversely, however, in laboratory studies by Jepson and Healy (1988), *Ae. aegypti* fed on flowers most frequently at dusk or dawn, slightly different from its normal blood-seeking times. Habitat and trap type may also bias sugar feeding results. A significantly higher percentage of *Cs. melanura* were positive for fructose at the perimeter of its swamp

breeding habitat than closer to the center (Nasci and Edman 1984). For *Cx. nigripalpus*, differences in fructose levels depended on the habitat collected, and the type of trap used in sampling (Bidlingmayer and Hem 1973). Conversely, MacVicker et al. (1990) reported that sand flies captured from different sites and habitats did not differ significantly in the crop sugar composition between any of the species examined.

Morphology and Physiology of Mosquito Alimentary Tract

Several factors appear to govern the destination of sugar meals. Nearly all of the mosquitoes investigated to date, store sugar solutions obtained from plant juices in the crop diverticula and not in the midgut (Clements 1992). The alimentary tract of the mosquito is typical of most Diptera. Near the posterior end of the foregut are two small dorsal and one large ventral diverticula or crop. Valves separate the three diverticula and the posterior foregut and midgut (see Clements 1992). In general, sugar meals, depending on their composition and concentration, initially go to the crop and are gradually released into the midgut (Friend 1981). The crop is considered a storage organ with no known secretory capabilities (Christophers 1960). Sugar meals may be partially converted into glycogen or fat, but unlike other insects and birds, mosquitoes do not use fat as fuel for flight. Mosquitoes can only use glycogen and simple sugars for flight fuel (Nayar and Van Handel 1971). Likewise, Yuval et al. (1994) showed that only simple sugars and glycogen are used during swarming. Friend et al. (1988, 1989) and Schmidt and Friend (1991), studied the effects of sugars on ingestion and diet destination in *Cs. inornata*. They found two response systems regulating the crop, one controlling the

amount ingested, the other controlling diet destination. Apparently, the destination depends on the molecular configuration of the meal. At a concentration of 0.5 M for example, common plant sugars like sucrose, maltose, fructose, alpha-glucose, and isomaltose induce crop valve opening and closing of the midgut valve. The valve closing results in the meal being shunted almost exclusively into the crop in the majority of the insects tested. Conversely, sugars not commonly found in nectars such as cellobiose, β-glucose, lactose and gentiobiose induce much less crop opening and almost no midgut closing. As for controlling the amount consumed, Friend et al. (1989), found ingesting large quantities of sucrose directed the meal to the crop, smaller sucrose meals were directed to both the crop and midgut. When sucrose concentrations were 0.4 M or more, most insects ingested large amounts and deposited it into the crop. At concentrations below 0.4 M the mosquitoes did not consume as much, and the meal went to both the crop and midgut. Contrary to popular belief, crop contents are not always strictly sugar meals. Trembly (1952) found crops of several species to contain remnants of blood meals.

In order to accurrately determine the natural sugar sources of the mosquitoes, samples must be taken as soon as possible after feeding. The crop volume varies depending on species. *Aedes communis* and *Ae. punctor* have volumes of about 0.91 and 3.39 microliters, respectively (Hocking 1953). Sugar content for a typical crop as in *Cs. melanura* contains about one microliter of liquid (Friend et al. 1988) and according to Schaefer and Miura (1972), typically contains about 0.087 to 0.45 mg of sugar per crop. Shortly after sugar feeding, the sugars may be wholly or partially broken down

enzymatically (see later section) and begin to slowly empty as needed energetically by the mosquito. In general, sugars appear to be digested in a species-specific manner and the half-life may depend on the ambient temperature and the individual energy requirement for the mosquito. As an example, Andersson and Jaenson (1987) found fructose in 90% of wild mosquitoes immediately after being caught, 30% in mosquitoes held and tested 10 hours later, and undetectable 20 hours after sampling. Likewise, Smith and Kurtz (1994) found *Aedes triseriatus* could empty its crop in as little as 12 hours when fed 10% glucose. If, as stated by Foster (1995), temperate and subtropical mosquitoes only ingest sugar every 2-5 days, collecting large enough samples to determine the relative proportion of the mosquito population feeding on preferential sugars sources (honeydew for example) or amino acids may prove difficult indeed.

Clements (1992) states that nearly all pentoses, hexoses, and di- and trisaccharides stimulated feeding; however, the acceptance thresholds varied between sugars or mosquito species. Conversely, trioses, tetroses and heptoses failed to stimulate feeding. Clements (1992), also states that the common nectar sugars, fructose and sucrose had low median acceptance thresholds of 0.020 and 0.023 M for *Ae. aegypti*, but for glucose was considerably higher at 0.11 M. The most potent phagostimulant for *Cs. inornata* was sucrose followed by a 1:1 mixture of glucose and fructose, maltose, fructose, and alpha-glucose (Friend et al.1988). The trisaccharides, raffinose and melezitose also stimulated gorging (Clements 1992).

Crop Enzymatic Activity

It is often assumed that the crop is simply a storage organ with little digestion of sugars or amino acids occurs there. Enzymatic activity in the saliva and/or crop may greatly and quickly alter the sugar and amino acid composition of a nectar or honeydew meal making it impossible to determine the exact sugar meal source based on composition and concentrations. To complicate matters further, crop and salivary enzymatic activity may vary by species. Several hematophagous Diptera contain carbohydrases in their saliva (Gooding 1975) which are presumably shunted to the crop upon ingestion of sugar meals. Marinotti and James (1990) and Marinotti et al. (1990) show *Ae. aegypti* to possess an alpha-glucosidase in the saliva which breaks down some sugars in the crop. Of the sugars tested, Marinotti and James (1990) indicated that sucrose was broken down the fastest, followed by maltotriose, maltopentaose and maltose. Melezitose, trehalose, raffinose, starch and others were only minimally affected. Schaefer and Miura (1972) also found carbohydrate enzymes in the saliva of *Cx. tarsalis* as well as invertase, maltase, melezitase, amylase and lactase. Indirect evidence for salivary and/or crop enzymes also exists. Crops of sucrose fed sand flies were analyzed with a GC and found to contain the hydrolysis products of fructose and glucose (Moore et al. 1987, Alexander 1988). However, there was no evidence of hydrolysis products for melezitose-fed flies (Alexander 1988). Interestingly, the problem of crop microbes digesting and altering a sugar meal may be reduced. Mosquito saliva contains a

bacteriolytic factor in the glands of both sexes and this substance is present in the crop after a sugar meal (Pimentel and Rossignol 1990, Rossignol and Lueders 1986).

Introduction To Mosquito Visual Attractants Review

Insects have long been known to have an attraction to certain wavelengths of light (Herms and Ellsworth 1934, Gui et al. 1942, Pfrimmer 1957) The attraction of many haematophageous Diptera to light is thought to be a host-seeking response. Indeed, the majority of insects haematophageous insects attracted to light traps are host-seeking females (see Service 1995). Host-seeking responses of female mosquitoes or other blood feeding Nematocera is largely due to the physiological requirement for a blood meal required to stimulate and complete ovarian development. The initiation of mosquito host-seeking is a least partially due to visual cues which are used to detect illumination levels and perceive objects (Laarman 1955, 1958). Mating, dispersal, appetitive flight, location of sugars, hosts, resting, oviposition and overwintering sites are all governed to some degree by vision (Allan et al. 1987). Many authors' have examined the important visual components of host finding and divided them into shape, color (reflected and transmitted), size, contrast, light intensity, texture and movement. All of these factors alone or in combination appear to play an important role in the ability of a blood-feeding fly to discern preferences and successfully find a suitable host or other resource.

Reflected Light

Although this paper is concerned mostly with host-seeking preference as it relates to various wavelengths of filtered transmitted light, much of the work done with mosquitoes and other dipteran color vision has focused on host-seeking behavior toward various wavelengths of reflected light. In general, research on reflected light research has found flies to be most attractive to darker, less reflective colors. Brown (1951, 1954) reported attractiveness to vary inversely with reflectivity or brightness between 475 and 625 nm wavelengths and found darker colors (those with lower reflectivity) more attractive than lighter colors for a variety of wild Canadian mosquito species. Browne and Bennett (1981) reported host-seeking female *Aedes spp.* and *Mansonia perturbans* were most attracted to black followed by red, blue, white and yellow. Similar results for *An. maculipennis* and *Ae. aegypti* respectively (Brett 1938). O'Gower (1963) reported black moist surfaces more attractive than gray moist surfaces for *Ae. aegypti* during host-seeking. Gilbert and Gouck (1957) also found darker shades to attract the most *Ae. aegypti*, and lighter shades more attractive to *Ae. taeniorhynchus*. Bracken et al. (1962), Granger (1970), Bradbury and Bennett (1974), Browne and Bennett (1980), and Allan and Stoffolano (1986) all found similar results for tabanids or black flies where low reflective colors like blue, black and red were more attractive than white, yellow and ultraviolet. For nocturnal mosquito species, Barr et al. (1963) reported light trap color to have some effect on catches, but concluded intensity was the most important factor.

Transmitted Light

Most dipteran visual research, has focused on diurnal species host-seeking preferences as a function of reflected light color. Significantly less has been written about day or night blood feeding responses to various wavelengths of transmitted light. Ultraviolet lamps have long been known to increase the numbers of host or resource seeking mosquitoes captured at light traps (Headlee 1937, Weiss 1943, Williams et al. 1955, and Breyev 1963). Electroretinograph (ERG) studies consistently show most flies possess a bimodal spectral response. ERG's conducted by Muir et al. (1992) found *Ae. aegypti* to have spectral sensitivities ranging from ultraviolet (323 nm) to orange-red (621 nm) with sensitivity peaks in the ultraviolet (323-345 nm) and green (523) wavelengths. Similarly, Smith (1986) reported that several tabanid species to have a peak activity of 400-600 nm and a smaller peak between 330-400 nm. No ERG references could be found for nocturnal mosquitoes. Using a different approach, Browne and Bennett (1981) tested filtered light of known wavelengths to equate host preference with landing rates for *Mansonia perturbans*. Shorter wavelengths (400-600 nm or blue-green) attracted significantly more mosquitoes than longer wavelengths, particularly those above 600 nm (infrared). In fact, for all wavelengths tested, the greatest numbers of mosquitoes landed on colored filters with the infrared wavelengths removed. In Georgia, Bargren and Nibley (1956) found several local species to have varying levels of attractiveness to New Jersey light traps using different color bulbs of similar intensities. *Aedes vexans* and *Cx. salinarius* were most attracted to blue (447 nm) lamps followed by red (670 nm), yellow

(570 nm) and white. *Culex pipiens fatigans*, however, was attracted to red, followed by yellow, white and blue. *Culex nigripalpus* showed no preference for any of the four colors tested. In field tests, Vavra et al. (1974a,b) tested several types and colors of light and with no significant difference in the numbers of mosquitoes attracted to each of the colors. Similarly, in a lab test using *Culex tarsalis*, *Culex pipiens fatigans* and *Anopheles sierrensis*, Gjullin et al. (1973) tested New Jersey light traps with ceramic dipped bulbs colored red, green, blue, orange and white incandescent bulbs and an ultraviolet light. No convincing difference in attraction between any of the colored lights tested were observed. Conversely, Wilton and Fay (1972) tested *Anopheles stephensi*, a nocturnally active mosquito, against a clear incandescent bulb and monochromatic light of various wavelengths. This species was highly attracted to 290 and 365 nm in the ultraviolet region and 690 nm in the infrared. Blue, green and yellow (490, 540, and 590 nms) were found to be repellent compared to the clear bulb.

CHAPTER 2
ANALYSIS OF SUGAR MEAL COMPOSITION OF WILD MOSQUITOES BY GAS CHROMATOGRAPHY

Introduction

Adequate dietary sugar is a critically important aspect of mosquito and other dipteran survival that is often overlooked. Some species have been commonly observed feeding on natural sugar sources including floral nectars (Haeger 1955, Breeland and Pickard 1961,1967, Sandholm and Price 1962, Grimstad and DeFoliart 1974, Magnarelli 1980, 1983, Vargo and Foster 1984, Yee et al. 1992, Yuval 1992, Foster 1995), extrafloral nectars (Haeger 1955, McCrae et al. 1976, Gadawski and Smith 1992, Taylor and Foster 1996), honeydew (Nielsen and Greve 1950, Haeger 1955), fruit (Joseph 1970), and other sources (Worth 1975, Patterson et al. 1969, Mogi and Miyagi 1989). Several aspects of mosquito sugar feeding have been reviewed by Yuval (1992) and Foster (1995).

Few attempts have been made to determine the exact sugar composition within dipteran crops using modern chromatography. Thin layer chromatography (TLC) has been used for pooled samples of tabanids by Magnarelli and Anderson (1977) and of mosquitoes by Magnarelli (1980). Hoppe (1983) and Burgin and Hunter (1997) used high performance thin layer chromatography (HPTLC) for qualitative determination of

sugars found in tabanids and black flies, respectively. High performance liquid chromatography (HPLC) was successfully used by MacVicker et al. (1990) to examine the crops of five wild Italian sand fly species. Despite its common use in other systems, only 4 studies have used gas chromatography (GC) for investigating dipteran diets, including Schaefer and Miura (1972) for *Culex tarsalis*; Moore et al. (1987) and Alexander (1988) for phlebotomine sand flies and Chang et al. (1977) in tephritid fruit flies. All of these studies analyzed pooled crop samples.

As indirect evidence for "nectar" feeding, thousands of mosquitoes and other Diptera have been tested using the cold anthrone method that determines the presence or absence of reducing sugars (e.g. fructose) found in almost all plant nectars. Many authors (Van Handel et al. 1972, Bidlingmayer and Hem 1973, Magnarelli 1978, 1979, 1980, Reisen et al. 1986, Andersson and Jaenson 1987, Nasci and Edman 1984, Smith and Kurtz 1994 and Edman et al. 1992) have used the cold anthrone test developed by Van Handel (1972) to qualitatively establish the presence or absence of crop "fructose." According to Van Handel (1967), the cold anthrone reagent reacts with fructose, inulin, sucrose, melezitose, raffinose and others. These sugars all contain the fructose moiety and give positive or negative responses based on a resulting color reaction. Unfortunately, the cold anthrone test does not provide any information about the origin or exact composition of sugars found in the dipteran crop and provides no quantitative information. Indeed, providing there is no enzymatic breakdown of sugars in the crop, it would be possible to determine if flies have preferences for certain families of plants or types of nectar (sucrose-rich for example). Furthermore, we suspect that many of the

reports for "nectar" feeding due to the presence of "fructose" in the crop, may actually have been a positive anthrone reaction to melezitose (a common trisaccharide found in honeydew) or other reducing sugar not commonly found in floral nectaries.

GC is an excellent and powerful tool for qualifying and quantifying the exact dietary sugar preferences, occurrence and composition of wild flies. Processing crop contents for GC is only slightly more labor intensive than the methods used for the cold anthrone test. A quantity of less than a microliter is all that is required. The technique is relatively rapid, employing silylation (using a TSMI derivitizing agent) of crude crop contents and subsequent GC analysis. We describe here a method using gas chromatography allowing researchers to determine the individual sugar meal components for mosquitoes and other flies, or the apparent presence or absence of carbohydrases in the crops of mosquitoes and/or other flies by monitoring the decline in parent sugar concentrations, and the increase in the metabolites over time.

Methods and Materials

Specimen Preparation

Representative samples of wild mosquitoes were vacuumed from resting sites shortly after sunrise in a ca. 1 hectare cypress swamp surrounded by pine flat woods located north of Gainesville, FL. Mosquitoes were kept alive, chilled, and processed within four hours of capture. Specimens were sacrificed by laterally inserting a # 0 or smaller insect pin just above the mesothoracic spiracle. Legs and wings were removed

using fine forceps. The crop (ventral diverticulum) was exposed by grasping third or fourth abdominal segment and pulling back slowly so that the crop would emerge between the abdominal segments. For crops containing liquid contents, 1 µl or less of material was sampled using a fine-tipped 10 µl capillary tube made from heated and pulled glass tubing. The capillary tube contents were transferred to a 200 µl glass insert tube inserted into a 3 ml glass GC sampling vial (National Scientific Company, Lawrenceville, GA) secured with a teflon lined cap. A separate vial was used for each specimen.

Sample Preparation for GC Analysis

Sugars are highly polar compounds and their analysis by gas chromatography requires silylation to derivitize the polar carboxyl and hydroxyl groups. The derivitizing agent Tri-Sil Z,® (Pierce Chemical Company, Rockford IL) composed of TMSI (N-(TMS) imidazole), in dry pyridine was used to process crop contents. Tri-Sil Z® (100 µl) was added to each vial containing one crop extract and each vial was vortexed, heated at 60-70°C for 15 minutes and frozen until analysis. Using a 1.0/100 µl aliquot sample, GC was performed using an Hewlett Packard 6890 instrument with an on-column auto injector, flame ionization detector, and equipped with a DB-5 fused silica capillary column (30 m X 0.25 µm, J & W Co., Folsum, CA). The column was heated from 60 to 300°C at a ramp of 20°C/min for 20 minutes. Pyridine and acetonitrile were used as solvents to clean the syringe between samples. The following sugars (Aldrich Chemicals, Milwaukee, WI) were made up as 0.01% standards in distilled water: D(-)fructose, D-

glucose, sucrose, maltose, D(+) melezitose, L-arabinose, L-rhamnose, D(+)melibiose, D(+)raffinose, turanose (a hydrolysis product of melezitose), and trehalose. Trehalose is a sugar present in insect hemolymph (Friedman 1985); the others have all been found associated directly or indirectly with plants (Percival 1961, Van Handel et al. 1972). Melezitose and turanose are known to be associated with honeydew (Auclair, 1963). The data and resulting chromatograms and integrations were recorded and processed using a PE Nelson 900 Series (970A) interface with Turbochrome® software (Ver 4.1, 1995 [Perkin-Elmer Corp., Cupertino, CA]).

Crop Sucrose and Melezitose Hydrolysis

Thirty to fifty laboratory reared *Aedes albopictus* (July 1995, USDA, Gainesville FL) pupae were placed in clean 35 mm film canister lids and allowed to emerge in new 200 ml urine cups with fitted fine mesh screen lids. A total of 25 cups with pupae were assembled. The adult mosquitoes were deprived of sugar and water for two days and kept in a rearing chamber at 28°C, 80% RH, and 14:10 L:D photoperiod. The mosquitoes then were provided with two hour unlimited access to 10% standard solutions of sucrose and melezitose. Blue food coloring was added to the sugar solutions to aid detection in the crop. Mosquitoes were fed by placing 10 drops of the standard sugar solutions atop the screens of each cup. Mosquitoes from each cup were sacrificed until several samples of males and females were found to have contents in their crops. Individual mosquito samples were taken at 2, 4, 8, and 20 hours after ingesting one of the sugar solutions. A

sample of the dyed standard sugar solutions was used as a control (time 0). Mosquitoes were otherwise processed and analyzed as discussed above.

Results and Discussion

Our preliminary analyses show a wide variation in the types of sugars present in wild mosquitoes. Our chromatographic analysis of combined standard sugars indicate that these common plant mono-, di-, and trisaccharides have unique retention times (Fig. 2-1). Some of the sugars, such as fructose, glucose and others, are anomeric molecules and thus display one peak for each form. Qualitative analysis of crude crop contents of wild *Culiseta melanura* (Coquillett) (Figs. 2-2) and *Anopheles quadrimaculatus s.l.* Say (Figs 2-3), indicate that most of the peaks are identifiable by GC. Figures 2-2 and 2-3 show that these female mosquitoes had recently fed on sugar sources containing fructose, glucose, sucrose, turnanose, melibiose, melezitose, raffinose, and a few unknowns. Later GC runs identified the unknown trisaccharide as erlose (glucosucrose). Honeydew has been found to contain oligosaccharides (e.g. melezitose, erlose, turanose, trehalulose) largely unique to homopteran exudates and are uncommon in plant nectars (Auclair 1963, Hudson 1946, Lombard et al. 1987, Bates et al. 1990, Byrne and Miller 1990, Yee et al. 1996). The presence of a large sucrose peak in *Cs. melanura* (Fig. 2-2), suggests that either salivary sucrase is not present in this species, or more likely, was not secreted at the time of feeding. Another possibility is that the sugar source contains carbohydrase-

inhibiting compounds that prevents the immediate hydrolysis of sucrose into glucose and fructose.

Much of the evidence for mosquito nectar feeding has come from direct field observations rather than through specific chemical qualitative crop content analysis. West and Jenkins (1951), Sandholm and Price (1962), Gadawski and Smith (1992), Breeland and Pickard (1961, 1967), and Magnarelli (1977) have observed some mosquito species feeding at sugar sources during the day. Others have observed them feeding on sugar sources at night, dawn or dusk (Grimstad and DeFoliart 1974, Magnarelli 1983, Andersson and Jaenson 1987, Bowen 1992, Vargo and Foster 1984, Yee et al. 1992). Many mosquito species have been found to contain "fructose" in their crops (Bidlingmayer and Hem 1973), yet some of these species have not been observed sugar feeding.

Sucrose and Melezitose Fed Mosquitoes

Extensive work has been done characterizing protein acquisition and the enzymatic processes of protein (blood meal) digestion in the mosquito midgut. Relatively little, however, has been done to assess the relative importance of sugar acquisition and its subsequent digestion. GC analysis provides a rapid method of indirectly determining the presence or absence of carbohydrases in the crop (from saliva shunted to the crop).

A total of 45 samples of sucrose and melezitose-fed *Ae. albopictus* were processed. Figure 2-4 shows the time course of crop sucrose and melezitose,

respectively, for males and females following ingestion. Almost complete hydrolysis (>90%) of sucrose occurred within 2 hours of ingestion, while melezitose remained relatively unchanged even 8 hours after ingestion. There was no significant difference between the response of the males and females fed sucrose or melezitose ($p = 0.61$ and 0.65 respectively). In general, the crops were largely empty 16 hours after ingestion and the number of unidentified peaks increased after 4 hours in the crop. Figure 2-5 shows a representative chromatogram of crude crop sucrose extract 2 hours after ingestion. This chromatogram shows sucrose broken down into the anomers of fructose and glucose. Conversely, melezitose-fed *Ae. albopictus* remained relatively unaltered 2 hours after ingestion (Figure 2-6) and was found to remain largely unhydrolyzed even 20 hours after ingestion.

Crop and salivary enzymatic activity undoubtably varies by species. Depending on the species and type of sugar, GC analysis can reveal important primary sugar meal preferences. Only a few hematophagous Diptera have been reported to contain salivary carbohydrases (Gooding 1975), which are presumably shunted to the crop upon ingestion of sugar meals. Marinotti and James (1990) and Marinotti et al. (1990) demonstrated that *Ae. aegypti* possessed an alpha-glucosidase in the saliva which works to break down some sugars in the crop. Of the sugars tested, they found that sucrose was broken down the fastest, followed by maltotriose, maltopentaose and maltose. Melezitose, trehalose, raffinose, starch and others were only minimally affected. Indirect evidence for salivary and/or crop enzymes also exists. Schaefer and Miura (1972) found carbohydrases in the saliva of *Culex tarsalis* including invertase, maltase, melezitase, amylase and lactase.

Crops of sucrose-fed sand flies were analyzed with GC and found to contain the hydrolysis products fructose and glucose (Moore et al. 1987, Alexander 1988). Likewise, in preliminary GC runs, there was no evidence of hydrolysis products for melezitose-fed flies (Alexander 1988). At least for some species, it is possible to use GC to determine the proportions and sugars present in the crops of wild mosquitoes and the relative importance that melezitose or other unhydrolyzed sugars play in the diets of medically important Diptera.

In most cases, sugar-fed female mosquitoes should be able to enhance their survival, host finding ability, and ultimately improve their chances of vectoring pathogens. A few studies have shown that female sugar-fed mosquitoes of 3 species have lower host avidity than starved or water-fed females (Foster and Eischen 1987, Xue and Barnard 1997). Other authors, however, have found that females with access to sugar sources are more persistent in their attempts at blood feeding than sugar and water starved females (Walker and Edman 1985). Likewise, Kelly and Edman (1996) found higher parasite transmission rates in sugar-fed *Ae. aegypti*.

This paper proposes a technique allowing an accurate representation of sugars present in individual mosquito meals. Unlike other studies which typically use pooled samples, our GC technique, when applied to sufficient sample sizes, can provide valuable insight into the occurrence, composition, and importance of certain life-sustaining sugar sources for wild mosquito populations. From this information, attractants or other methods could be developed to help control or improve sampling of mosquito populations.

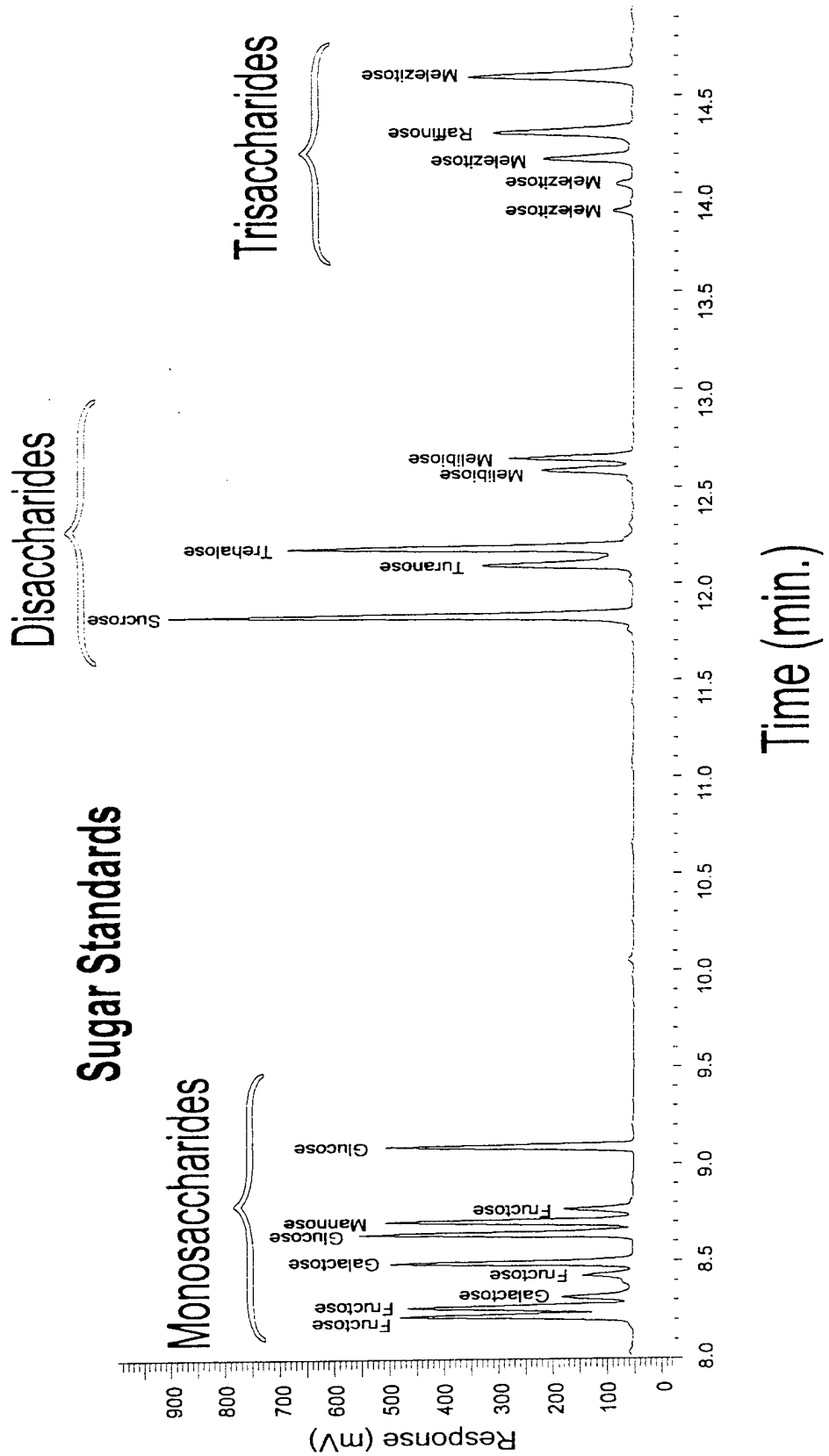


Figure 2-1. Chromatogram of combined standards (ca. 0.1%) for common sugars associated with plants. Multiple peaks of the same sugar are anomeric forms.

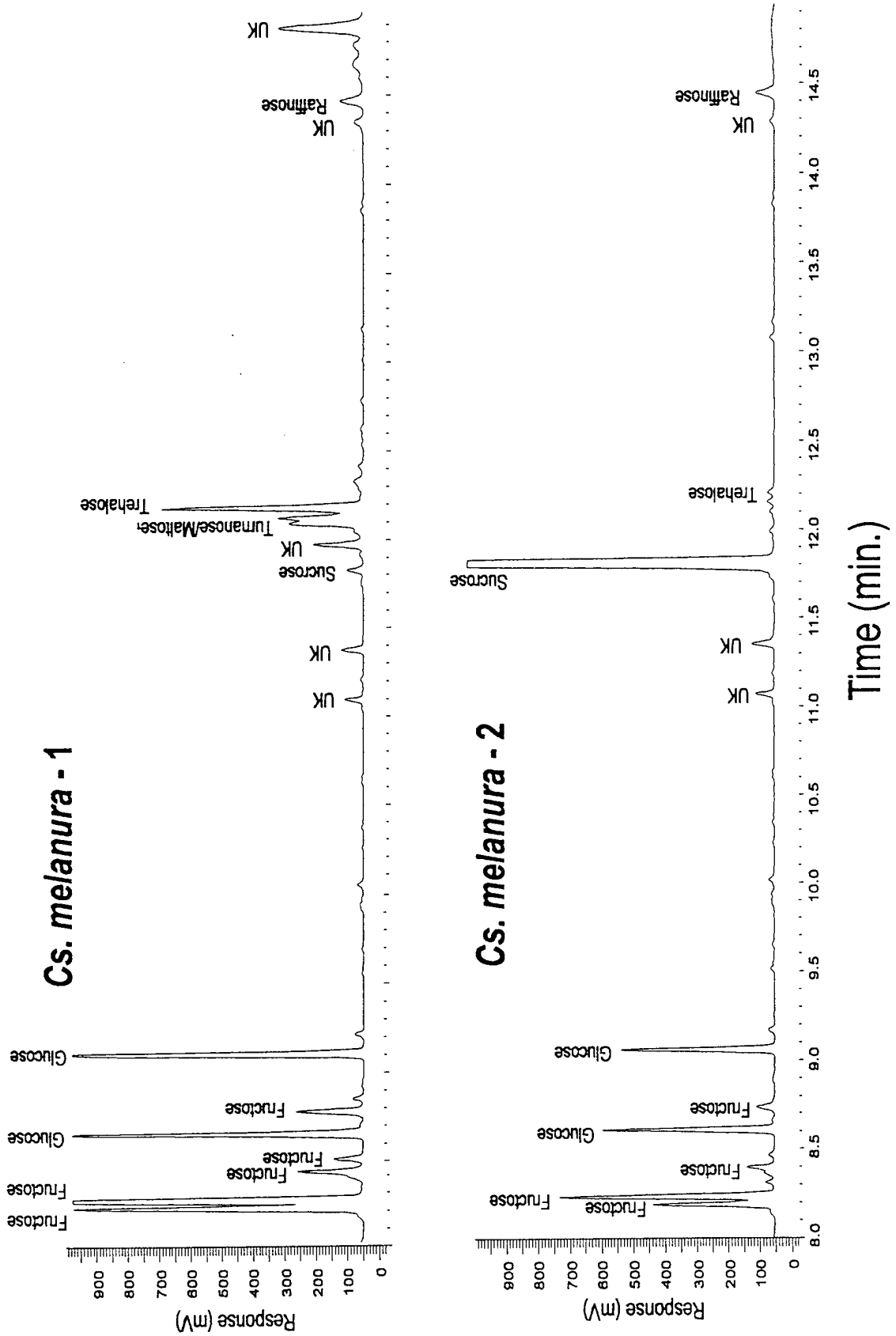


Figure 2-2. Representative chromatograms of crude crop extracts from two wild individuals of *Cs. melanura*. Multiple peaks of the same sugar are anomeric forms.

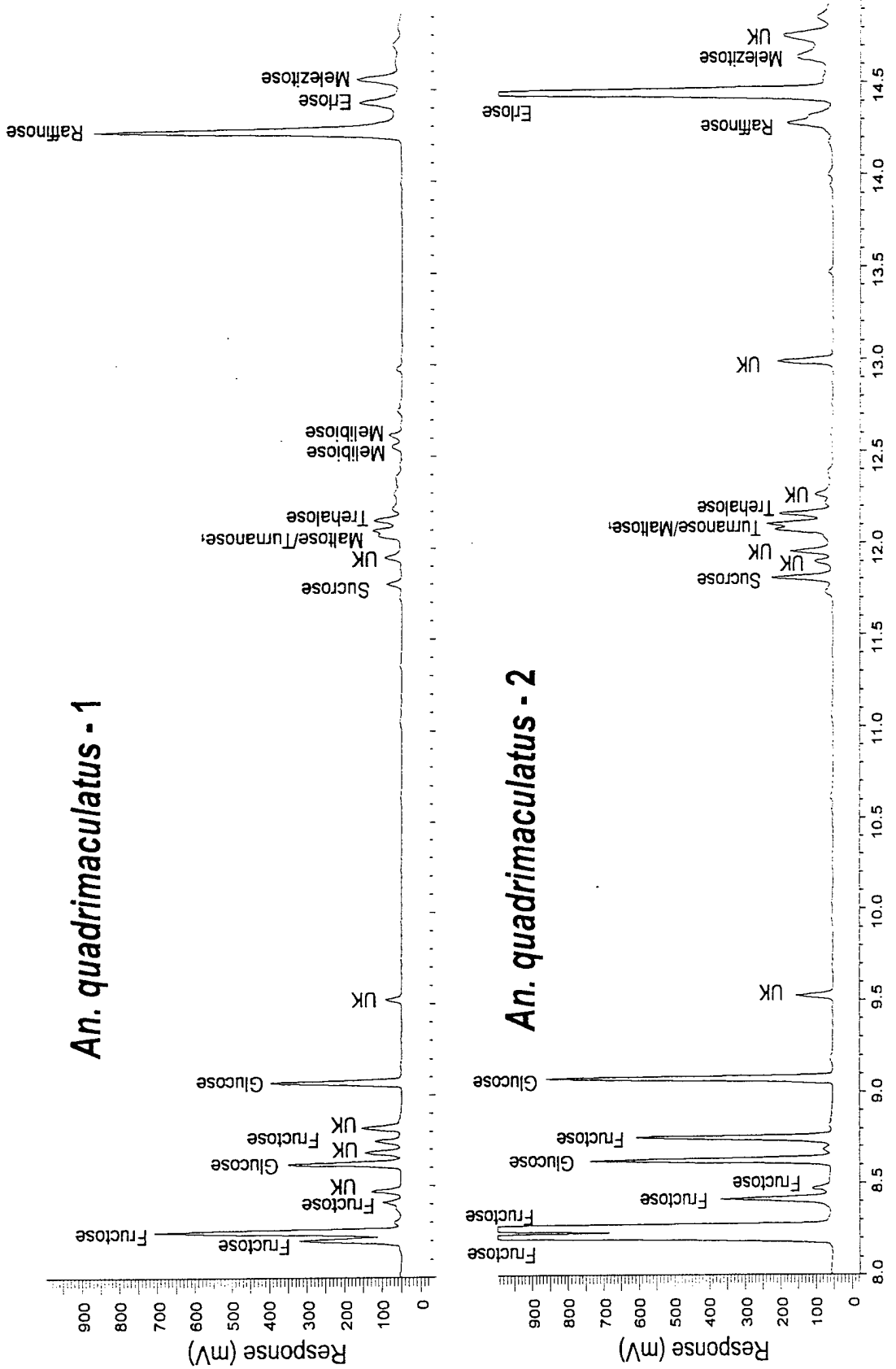


Figure 2-3. Representative chromatograms of crude crop extracts from two wild individuals of *An. quadrimaculatus*. Multiple peaks of the same sugar are anomeric forms.

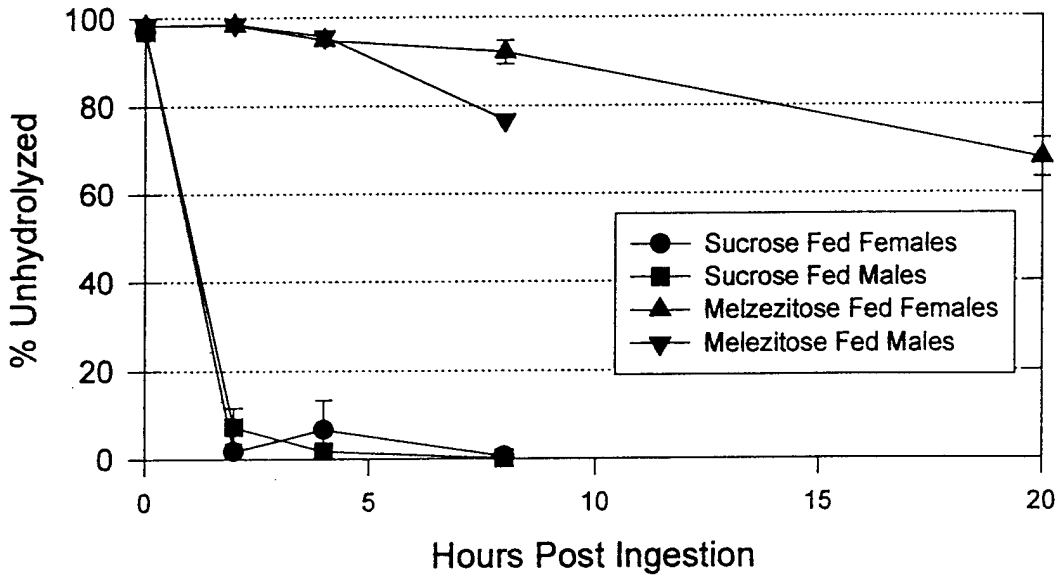


Figure 2-4. Timed feeding trials for male and female *Ae. albopictus* showing the hydrolysis of melezitose and sucrose occurring in the crop (n = 2 to 5).

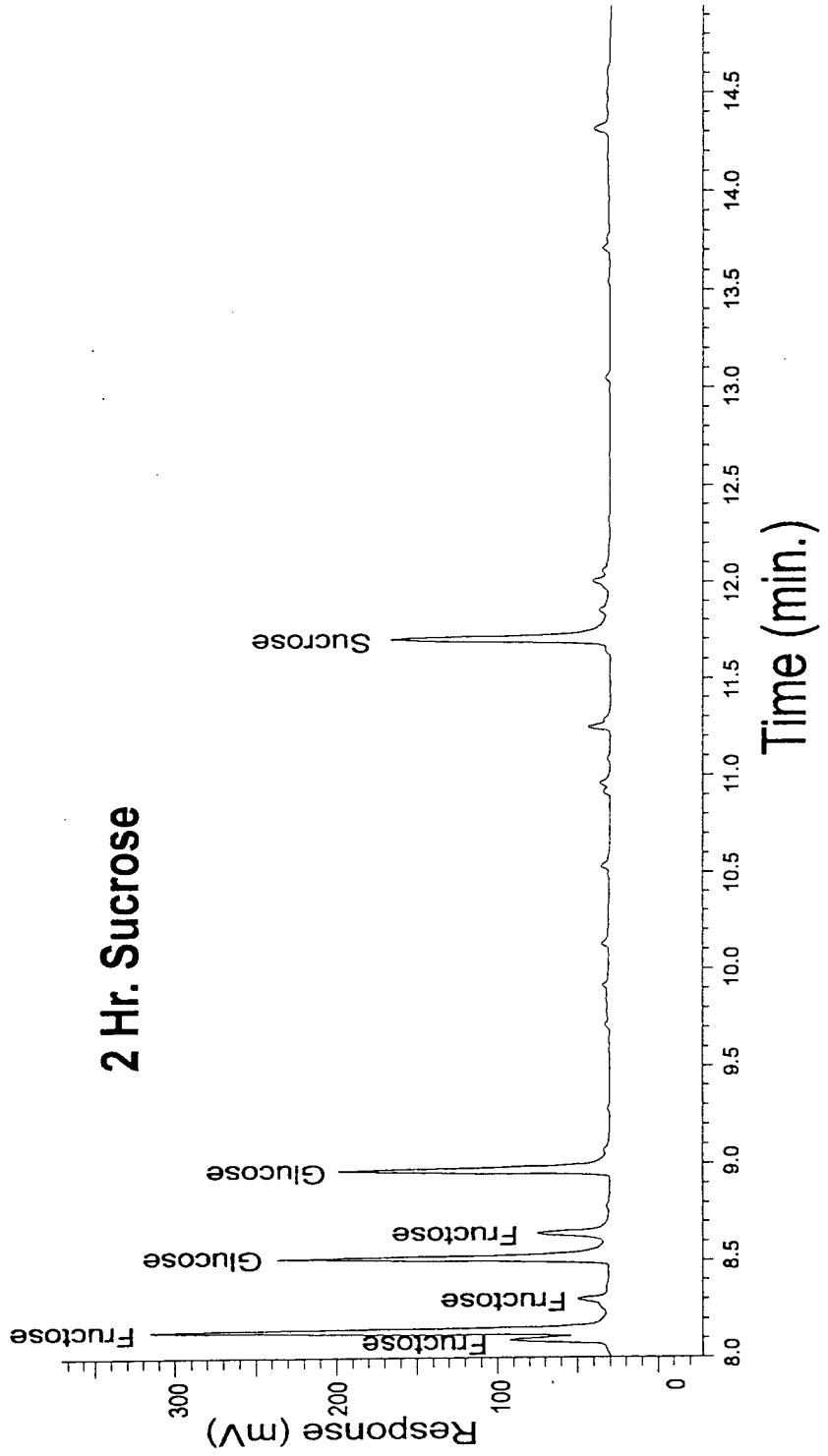


Figure 2-5. Representative chromatogram showing hydrolysis of sucrose into fructose and glucose 2 hours following ingestion in *Ae. albopictus*. Multiple peaks of the same sugar are anomeric forms.

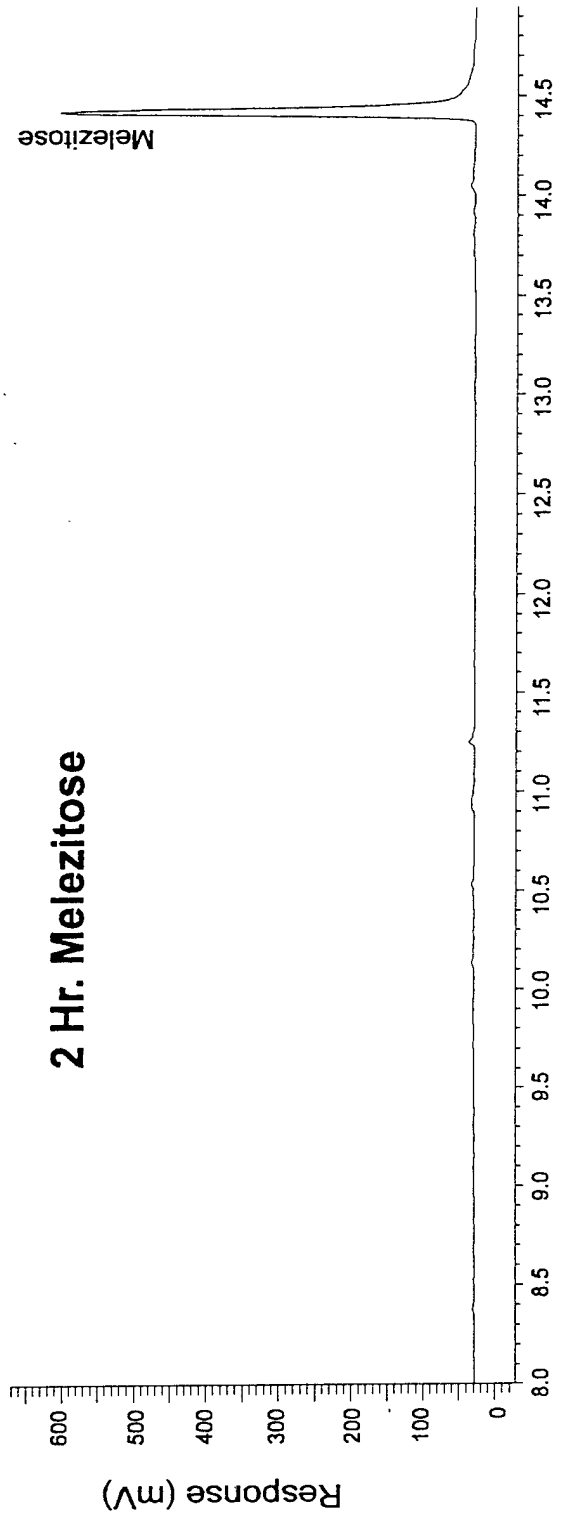


Figure 2-6. Representative chromatogram showing hydrolysis of melezitose 2 hours following ingestion in *Ae. albopictus*.

CHAPTER 3
SUGAR MEAL COMPOSITION OF FIVE NORTH CENTRAL FLORIDA MOSQUITO SPECIES AS DETERMINED BY GAS CHROMATOGRAPHY

Introduction

That male and female mosquitoes and other medically important Diptera feed on a variety of sugar sources of plant origin has long been established. For many species, however, their sugar sources remain speculative. Direct evidence of sugar feeding is documented by observing mosquitoes feeding on a variety of natural sources. These sugar sources include floral nectars (Haeger 1955, Breeland and Pickard 1961,1967, Sandholm and Price 1962, Grimstad and DeFoliart 1974, Magnarelli 1980, 1983, Vargo and Foster 1984, Yee et al.1992), extrafloral nectars (Haeger 1955, McCrae et al.1976, Gadawski and Smith 1992, Taylor and Foster 1996), honeydew (Nielsen and Greve 1950, Haeger 1955), fruit (Joseph 1970), and other sources (Worth 1975, Patterson et al.1969, Mogi and Miyagi 1989). As indirect evidence of sugar feeding, most researchers have used the cold anthrone test (Van Handel 1972), to determine the occurrence of crop fructose (or other reducing sugars) in wild populations. According to Van Handel (1967), the cold anthrone test reacts with sugars containing the fructose moiety (sucrose, melezitose, raffinose, etc.) giving positive responses based on a color reaction. Many authors (Magnarelli 1978, 1979, 1980, Reisen et al.1986, Andersson and Jaenson 1987,

Nasci and Edman 1984, Smith and Kurtz 1994, Edman et al.1992, Holliday-Hanson et al.1997) have used the cold anthrone test to qualitatively establish the presence of crop "fructose." However, the cold anthrone test does not provide any ecological information about the origin, distribution or exact composition of sugars found in the dipteran crop and provides little quantitative information. Recent reviews by Yuval (1992) and Foster (1995) covered much of what is known about mosquito sugar-feeding ecology.

Few attempts have been made to use modern chromatography to determine the composition of sugars found in dipteran crops. Thin layer chromatography has been used for pooled samples of tabanids (Magnarelli and Anderson 1977, Hoppe 1983), mosquitoes (Magnarelli 1980) and individual black flies (Burgin and Hunter 1997). High performance liquid chromatography was used by MacVicker et al. (1990) to examine Italian sand fly crops. Despite gas chromatography's (GC) common use in other systems, only 4 studies have used GC for investigating dipteran diets, including Schaefer and Miura (1972) for *Culex tarsalis*; Moore et al. (1987) and Alexander (1988) for phlebotomine sand flies and Chang et al. (1977) in tephritid fruit flies. All of these GC studies analyzed pooled crop samples.

Burkett et al. (1998a) presented a method and included representative chromatograms for analyzing sugar meal composition and hydrolysis in individual mosquitoes and found that GC can be used to show honeydew feeding based on crop sugar contents (e.g. melezitose, erlose, turanose). However, they did not assess the importance of honeydew in the diet in different mosquito species. Honeydew is speculated to be a common sugar source for mosquitoes, but its relative importance

among different taxa has not been established. This study identifies many of the mono-, di-, and trisaccharides found in field collected individuals of 5 mosquito species and shows that honeydew feeding is an important dietary component of wild mosquito populations.

Methods and Materials

Specimen Preparation

Wild adult mosquitoes (*Anopheles quadrimaculatus* s.l. (Say), *Coquillettidia perturbans* (Walker), *Culiseta melanura* (Coquillett), *Culex nigripalpus* Theobald, and *Psorophora ferox* (von Humboldt)) were collected from several locations in and around Gainesville, FL over the course of 2 seasons (April-October). *Ps. ferox* were aspirated while attempting to take a blood meal. All other species were vacuumed from typical resting sites (i.e., under bridges, in tree holes, bases of trees, etc.). All collections were made from 0700-0900 and mosquitoes were kept alive, chilled, identified, and processed within four hours of capture. Individuals were sacrificed by laterally inserting a #0 insect pin just above the mesothoracic spiracle. Legs and wings were removed using fine forceps. The crop (ventral diverticulum) was exposed by grasping the third or fourth abdominal segment and pulling back slowly so that it would emerge between the segments. For crops containing liquid contents, 1 µl or less of material was sampled using a fine-tipped 10 µl capillary tube made from heated and pulled glass tubing. Capillary tube contents were transferred to a 200 µl glass insert tube inserted into a 3 ml

glass GC sampling vial (National Scientific Company, Lawrenceville, GA) secured with a teflon lined cap. A separate vial was used for each specimen.

Sample Preparation for GC Analysis

Sugars are highly polar compounds. Their analysis by GC requires silylation to derivitize the carboxyl and hydroxyl groups. The derivitizing agent, Tri-Sil Z® (100 µl) (Pierce Chemical Company, Rockford IL), was added to each vial containing one crop extract. Each vial was vortexed, heated at 60-70°C for 15 minutes and frozen until analysis. Using a 1.0/100 µl aliquot sample, GC was performed on one of the following instruments. Some samples were run using an Hewlett Packard 6890 instrument with an on-column auto injector, flame ionization detector (FID), and equipped with a DB-5 fused silica capillary column (30 m X 0.25 mm, 0.25 µm , J & W Co., Folsum, CA). The column was heated from 60 to 300°C at a ramp of 20°C/min. Pyridine and acetonitrile were used as solvents to clean the syringe between samples. Depending on availability, other sample runs were made using either an HP 5890 GC, split-splitless injector (250°C), FID, J&W DB-5 (30m X 0.32 mm, 0.25 µm), heated from 125 to 300°C at 5°C/min., 15 min. hold or a Varian 3700 GC, split-splitless injector (250°C), J&W DB-1 (30 m X 0.32 mm, 0.32 µm) heated from 125-285°C at 5°C/min., 18 min hold. The resulting chromatograms and integrations were recorded and processed using a PE Nelson 900 Series (970A) interfaced with Turbochrome® software (Ver 4.1, 1995 [Perkin-Elmer Corp., Cupertino, CA]).

Standards

The following sugars (Aldrich Chemical, Milwaukee, WI) were made up as 0.01% standards derivatized as above: D(-)fructose, D(-)glucose, sucrose, maltose, D(+) melezitose, L-arabinose, L-rhamnose, D(+)melibiose, erlose (glucosucrose), D(+)raffinose, turanose (a hydrolysis product of melezitose (Hudson 1946)), and trehalose. Trehalose is a sugar present in insect hemolymph (Friedman, 1985), but also found in some honeydews (Hendrix and Wei, 1994). Turanose, melezitose and erlose are known to be associated with honeydew (Hudson 1946, Auclair 1963, Wolf and Ewart 1955). The other sugars have been found associated directly or indirectly with plants (Percival 1961, Van Handel et al. 1972).

Results and Discussion

The sugars reported here were unambiguously identified by comparison of retention times on 2 GC columns of slightly different polarities. Crop samples contained fructose, glucose, sucrose, maltose, turanose, melezitose, raffinose, erlose, and traces of arabinose, melibiose and rhamnose (Figures 3-1, 3-2, 3-3, 3-4 and 3-5). Fructose and glucose were found in all samples that contained sugars. All species tested contained trehalose, a sugar not found in nectar, that could have originated from hemolymph (Friedman 1985) or honeydew (Hendrix and Wei 1994). Maltose and turanose had similar retention times and could not be distinguished with confidence. A total of thirty unknown peaks were observed across all samples. Most of these unknown compounds

eluted during times atypical for mono-, di- or trisaccharides and were probably either incompletely silyated sugars or other miscellaneous crop components. Rarely were the unknown peaks consistent between species, and few constituted a significant percentage of any of the samples.

The percentage of mosquitoes containing detectable crop sugars ranged from 10-11% in *An. quadrimaculatus* and *Ps. ferox* to 47.7% in *Cq. perturbans* (Table 3-1). Crop sugar occurrence was significantly greater in females than males in *An. quadrimaculatus* (chi-square, p=0.0006) and *Cs. melanura* (p=0.00004). These findings agree with those for other Florida mosquitoes. Bidlingmayer and Hem (1973), for example, found similar sugar positivity results using the cold anthrone test for *An. quadrimaculatus* (♀=15%); *Cs. melanura* (♀=36%, ♂=29%); *Ps. ferox* (♀=20%); and *Cx. nigripalpus* (♀ and ♂=17%). Moreover, Magnarelli (1978) obtained similarly high values for *Cq. perturbans* (♀=57%). Our results differed only in that more female *An. quadrimaculatus* contained sugars than males. The diverse sugar composition, proportion, and occurrence found in the crops of all species tested show that these mosquitoes tend to be opportunistic and that a variety of sugar sources are used.

Ecological Significance of Honeydew in Mosquito Diet

The widespread and often invisible nature of honeydew complicates direct feeding observations (Foster 1995). Chromatography has been used to identify honeydew sugars in mosquitoes (Schaefer and Miura 1971, Burkett et al. 1998b) and other Diptera (Hoppe 1983, MacVicker et al. 1990, Moore et al. 1987, Burgin and Hunter 1997. However, until

now the proportion of mosquito populations feeding on honeydew and its ecological significance has only been speculative. All species tested (Figs. 3-1, 3-2, 3-3, 3-4 and 3-5) contained melezitose and/or erlose (*An. quadrimaculatus* (55%), *Cs. melanura* (33%), *Cx. nigripalpus* (15%), *Cq. perturbans* (10%), and *Ps. ferox* (7%)). These data show honeydew to be an extremely important resource for both *Cs. melanura* and *An. quadrimaculatus* comprising 1/3 and 1/2 of the sugar meals, respectively. These observations make sense, because unlike *Cq. perturbans* (Grimstad and DeFoliart 1974, 1975), *Ps. ferox* (Magnarelli 1980) and *Cx. nigripalpus* (see Nayar 1982), which have been observed feeding on a variety of natural sugar sources, *An. quadrimaculatus* and *Cs. melanura* have not been reported to feed on floral/extrafloral nectars. Of the mosquitoes investigated in this study, only *Cx. nigripalpus* had been previously observed feeding on honeydew (Haeger 1955).

Chromatography on a relatively small number of Homoptera has found honeydew to be a diverse complex of 20 or more sugars (Hendrix and Wei 1994), many of which are not found in plant tissues (Byrne and Miller 1990, Tarczynski et al. 1992). A comparison of honeydews among different Homoptera shows that unique sugars are found among various families (Gray and Fraenkel 1953, Wolf and Ewart 1955, Ewart and Metcalf 1956, Lombard et al. 1987, Bates et al. 1990, Hendrix and Wei 1994, Wei et al. 1996,1997, Yee et al. 1996). It is reasonable to expect that different Homoptera produce different sugars and ratios of sugar components that would be identifiable using GC or other modern chromatographic techniques such as HPLC. Although yet to be determined, mosquitoes may prefer honeydew from certain groups of Homoptera.

Percival (1961), and Van Handel et al. (1972) characterized nectar sugars and ratios for many plant species. We initially hoped that GC could be used for determining which plant(s) or even family of plants that mosquitoes use for sugar meals based on direct comparisons with published reports. Unfortunately, unlike melezitose and other trisaccharides, sucrose is rapidly hydrolyzed into fructose and glucose in insect crops (Schaefer and Miura 1972, Burkett et al. 1998b) negating any possibility of determining from which plant a sugar meal came. One interesting observation, however, is that some crop samples from *An. quadrimaculatus*, *Cs. melanura* and *Cx. nigripalpus* contained relatively large amounts of sucrose in their crops, showing that either some sugar sources contain carbohydrase (sucrase) inhibiting compounds, or that salivary sucrase is not always produced and shunted to the crop with sugar meals.

Sugar Feeding Field Observations

From 1900-1100 hrs in July and August 1997, two species of plants were commonly observed as hosts for sugar-feeding mosquitoes. *Ps. ferox*, *Ae. albopictus*, *Cx. nigripalpus*, *Cx. (melanoconion)* spp., and *Uranotaenia sapphirina* were observed feeding on the extrafloral nectaries of partridge pea (*Cassia fasciculata* Michx.). More interesting from a public health perspective was the observation of many insects including mosquitoes such as *Cx. nigripalpus* and *Ae. albopictus* feeding on the sugary exudates from the developing fruits of bahiagrass (*Paspalum notatum* Fluegge). Analyses of sugars from samples of bahiagrass exudates matched those from the crops of *Cx. nigripalpus* sampled while feeding on bahiagrass, showing raffinose to be one of the

primary sugars. Bahiagrass is a commonly planted turf and pasture grass whose characteristic "V" shaped seed heads are a familiar sight in residential areas in north central Florida. The presence of bahiagrass around human domiciles, and the absence of other suitable sugar sources may influence epidemiological factors for some of Florida's medically important mosquitoes.

GC has proved to be an excellent and powerful tool for identifying and quantifying the occurrence and composition of sugar feeding in Diptera. In some cases, the sugar source can be determine (i.e., honeydew). Extracting/analyzing crop contents is not difficult, requiring only small quantities (0.1-1 µl) of crude crop content. Future efforts could address/utilize larger sample sizes, test for seasonal differences, run a variety of different honeydews, and incorporate mass spectrometry to determine unknowns. From this information, attractants or other methods could be developed to help control or improve sampling of mosquito populations.

Table 3-1. Summary of percent wild mosquitoes containing identifiable sugars using gas chromatography.

Species	n	Males n	Females n	% Total With Sugars	% Males With Sugars	% Females With Sugars
An. quadrimaculatus	370	119	251	11	3.4	15.9
Cq. perturbans	44	17	27	47.7	47.1	48.1
Cs. melanura	471	129	342	26.7	10.1	35.5
Cx. nigripalpus	184	41	143	27.5	26.8	27.8
Ps. ferox	134	8	126	10.4	12.5	11.1

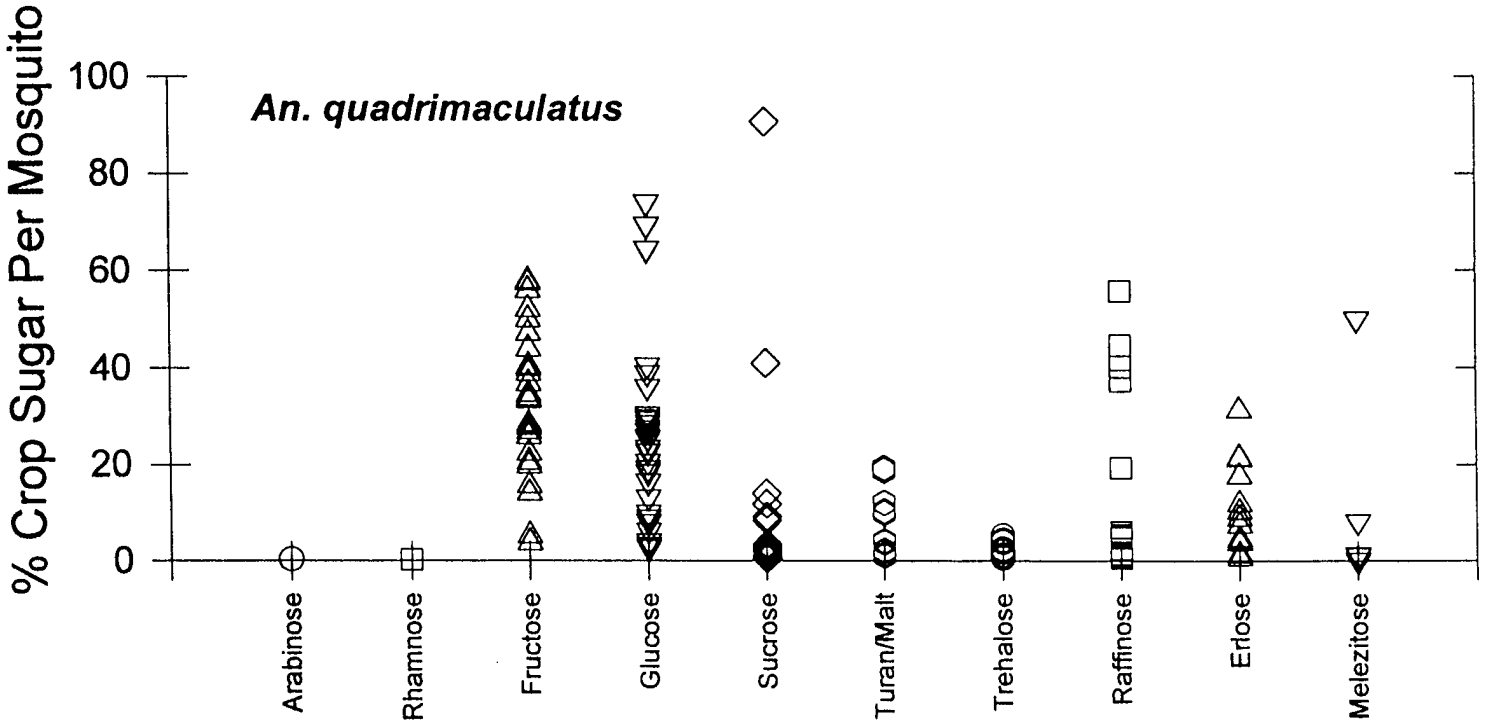


Figure 3-1. Representation of crop sugar distributions and proportions of wild *An. quadrimaculatus* (n = 33). The presence of the trisaccharides, erlose and melezitose, is evidence of honeydew feeding. [1]Maltose and turanose have identical retention times.

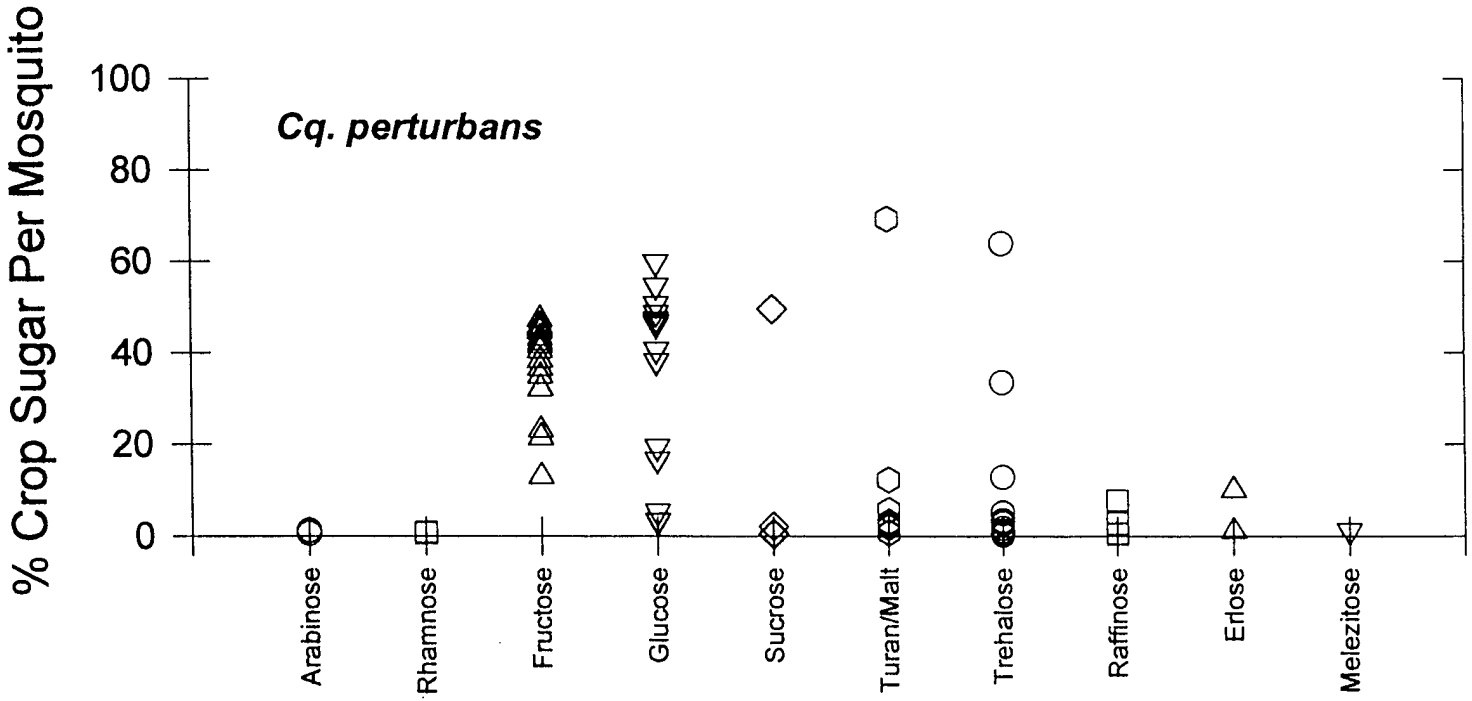


Figure 3-2. Representation of crop sugar distributions and proportions of wild *Cq. perturbans* (n = 20). The presence of the trisaccharides, erlose and melezitose, is evidence of honeydew feeding. [1]Maltose and turanose have identical retention times.

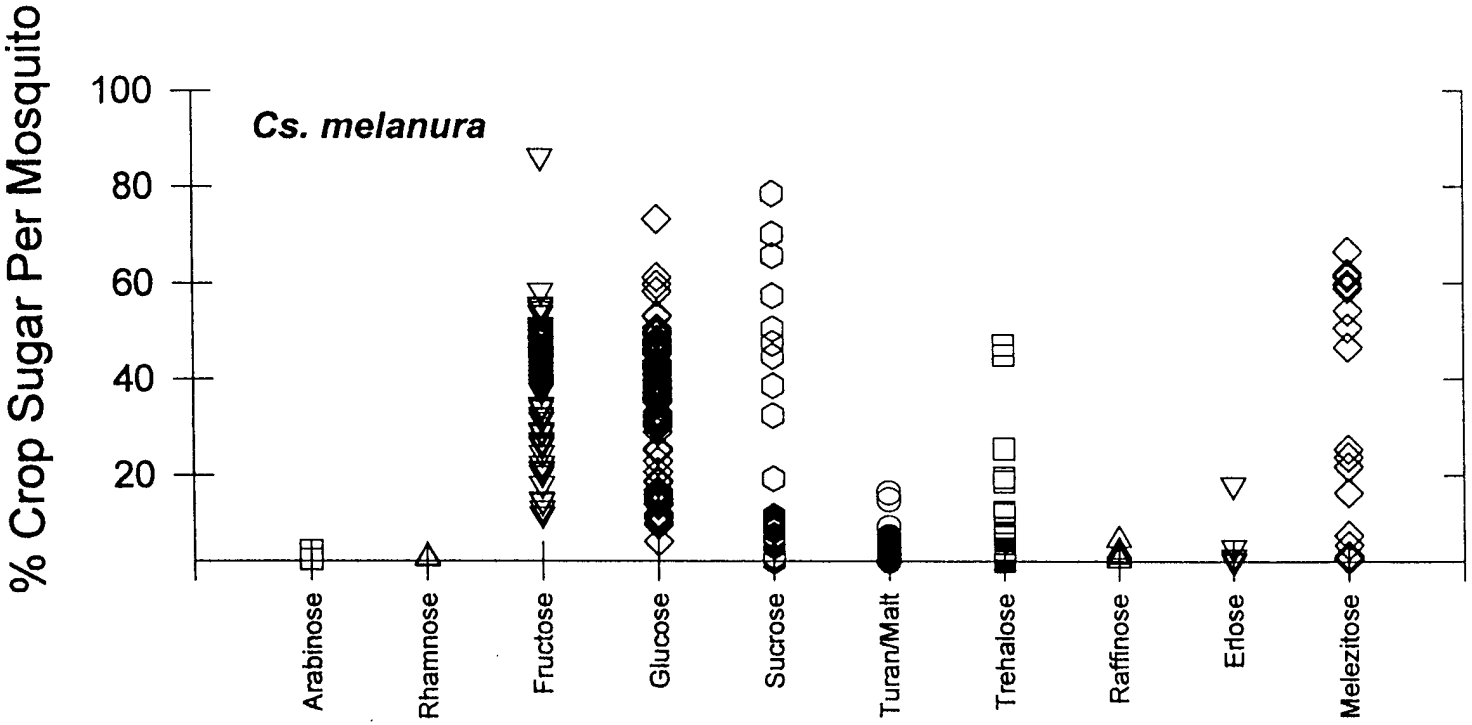


Figure 3-3. Representation of crop sugar distributions and proportions of wild *Cs. melanura* (n = 99). The presence of the trisaccharides, erlose and melezitose, is evidence of honeydew feeding. [1]Maltose and turanose have identical retention times.

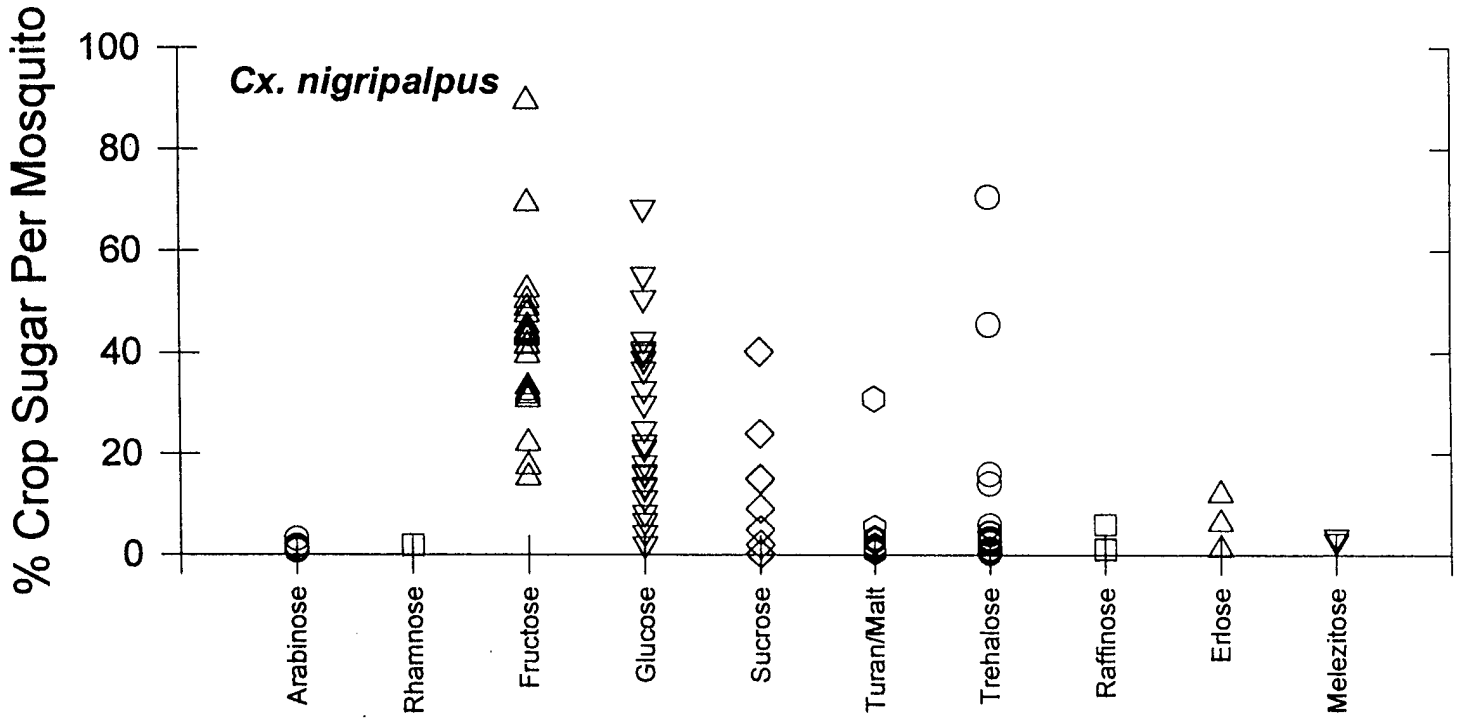


Figure 3-4. Representation of crop sugar distributions and proportions of wild *Cx. nigripalpus* (n = 26). The presence of the trisaccharides, erlose and melezitose, is evidence of honeydew feeding. [1]Maltose and turanose have identical retention times.

Figure 3-5. Representation of crop sugar distributions and proportions of wild *Ps. ferox* (n = 14). The presence of the trisaccharides, erlose and melezitose, is evidence of honeydew feeding. [1]Maltose and turanose have identical retention times.

CHAPTER 4
FIELD EVALUATION OF COLORED LIGHT EMITTING DIODES AS ATTRACTANTS FOR WOODLAND MOSQUITOES AND OTHER DIPTERA IN NORTH CENTRAL FLORIDA

Introduction

Light emitting diodes (LED) were evaluated as an alternative light source for use as an adult mosquito attractant. Much of the research on Dipteran color preference is based on reflected light (Brett 1938, Bracken et al. 1962, O'Gower 1963, Granger 1970, Bradbury and Bennett 1974, Browne and Bennett 1980, 1981, Allan and Stoffolano 1986). Many authors have shown that mosquitoes are attracted to transmitted light (Headlee 1937, Weiss 1943, Williams et al. 1955, Breyev 1963, Bargren and Nibley 1956, Gjullin et al. 1973, Wilton and Fay 1972, Browne and Bennett 1981). Few reports detail the response of individual species. Several colors (100 nm width) of highly efficient, low cost, "super bright" LEDs have recently been developed. These colored LEDs when used in CDC traps have a greater intensity and require significantly lower amounts of energy (ca. 0.125 ma/hr vs. 150 ma/hr for standard CM-47 bulb). We evaluated the relationship between transmitted light color and its attractiveness to woodland mosquito and other dipteran species.

Methods and Materials

Three field trials were conducted using standard CDC-type traps (John W. Hock Company, Model 512, Gainesville FL) modified by replacing the standard bulbs with the LEDs. The LED was secured into a piece of 2 X 2 cm plexiglass and fastened to the "screen" atop the lid assembly 3 cm below the aluminum trap lid (Fig. 4-1). Trial 1 used 9 kg compressed-gas (carbon dioxide) cylinders equipped with double-stage (Victor Equipment Company, Model VTS453B-320, Denton TX) and microregulators (Nupro Inc., Series M, Wiloughby OH) to maintain a constant gas flow of 200 ml per minute. Carbon dioxide was delivered to the trap through a 3 m piece of 8 mm clear plastic tubing secured with a rubber band so the top of the tubing was even with the top of the trap opening. Gas flow was checked each morning and evening using an in-line flowmeter (Gilmont Instruments, Great Neck NY, no. 12). Mosquitoes attracted near the trap intake were drawn in by the trap fan, blown through a screen funnel and into a quart polypropylene jar containing a 3 X 6 cm piece of dichlorvos impregnated vinyl strip used as a killing agent. Batteries consisted of 6V, 10 ampHrs rechargeable gel cell batteries (Powersonic Corp., San Diego CA) which were used to run the fan motor and standard incandescent light.

Six Colors With and Without Carbon Dioxide (Trials 1 and 2)

From July 15-20 (Trial 1) and July 22-27 (Trial 2), 1996, different colored lights were used as attractants in standard CDC-type surveillance traps using a 6 X 6 Latin

square design. Light, day, and position effects were evaluated using a three-way ANOVA (SAS Institute, 1995) for the total number and most common species represented in the traps. Multiple comparisons were made using the Ryan-Einot-Gabriel-Welsh multiple range test (alpha = 0.05). Trial 1 used carbon dioxide as an additional attractant and Trial 2 used the same randomization, but did not use carbon dioxide. Four different colored "super bright" LEDs (Toshiba Tosbright,® Martech Optoelectronics, Latham, NY) were compared to no light and a standard incandescent bulb (John W. Hock Company, CM-47, 6.3 V, 520 millicandela [mcd], Gainesville, FL) used as controls. The diodes tested were red (613 ± 50 nm, 1600 mcd, 22°); orange (605 ± 50 nm, 2000 mcd, 22°); yellow (587 ± 50 nm, 2300 mcd, 22°) and green (567 ± 50 nm, 2400 mcd, 8°). Each LED was powered by 2 alkaline D cell batteries at 2.8 ± 0.2 volts and 18 ± 2 ma. A 10 ohm resistor was placed in series to prevent over driving the LED.

Trials 1 and 2 were conducted at the University of Florida's Austin Cary Memorial Forest, a research area located north of Gainesville, FL. The habitat consisted of a cypress swamp surrounded by pine flatwoods. Traps were hung 165 cm above ground level and placed every 30 meters near the banks of a seasonal forest stream that runs through the middle of the swamp. Due to the thick vegetation, none of the traps were visible to each other. The trapping period followed a 24.5 and 6.83 cm rainfall on July 5 and 9, respectively.

These trials ran from 1800 to 0800 hr for six days in a row using a 6 X 6 Latin square design. The trap and motor assembly remained stationary, but the lights or diodes were changed nightly so each light would occupy each position during the six-day period.

After each trap night, the captured mosquito adults were identified and counted. All *Culex (Melanoconion)* Theobald, *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann and *Aedes atlanticus* Dyar and Knab/*Ae. tormenter* Dyar and Knab species were pooled, as these taxa could not be distinguished with confidence.

Eight Colors With Carbon Dioxide (Trial 3)

Trial 3 was conducted from August 12-21 (Trial 3), 1996. Due to the fluctuating water levels and mosquito populations at the Austin Cary Forest site, a similar, but more permanent cypress swamp habitat was chosen north of Gainesville. In addition to the four LED colors and two controls previously discussed, two additional LED wavelengths, infrared (940 ±50 nm, 22°[Martech Optoelectronics, Latham NY, model MTE1080]) and blue (450 ± 50 nm, 800 mcd, 22° [Panasonic,® Digikey Corp, Thief River Falls, MN]]) were evaluated also. Using an 8 X 8 Latin square design, traps were placed around the perimeter of the swamp. Each trap was baited with 200 ml/min carbon dioxide as in trial 1 and otherwise treated as before.

Results

Six Colors With and Without Carbon Dioxide (Trials 1 and 2)

During the six trap-nights of trials 1 and 2, 32,059 and 1,916 specimens of mosquitoes were collected respectively. The mosquito species composition attracted to the incandescent light trap agree with those found by Mann (1993). Response of the most numerous mosquito species are shown in Figures 4-2, 4-3, 4-4 and 4-5. Means, standard

errors, p-values, and significant differences for species represented in large enough numbers are shown in Tables 4-1 and 4-2. As noted in the tables, there were significant trap-position and day effects for some species.

No significant differences were observed for the total number of mosquitoes captured at different colors in either the CO2 baited or unbaited trials ($p = 0.08$, $p = 0.24$, respectively). Differences were observed for individual species however. In trial 1, *Aedes dupreei* (Coquillett), *Ae. infirmatus* (Dyar and Knab), *Anopheles crucians s.l.*, *Culiseta melanura* ((Coquillett), and *Uranotaenia sapphirina* (Osten Sacken) showed significant color preferences. In trial 2, only *Ae. atlanticus*, *An. crucians s.l.*, and *Ur. sapphirina* showed significant preferences. *Aedes dupreei was* the predominant species and was the only species preferring the carbon dioxide baited trap using no light. This species was also abundant in the no light control. Three female *Lutzomyia shannoni* (Dyar) and 1 *Lutzomyia vexator* (Coquillett) were collected during trial 1. None were collected in trial 2.

Eight Colors With Carbon Dioxide (Trial 3)

During the eight trap-nights, 4,668 specimens of mosquitoes, 1,189 tabanids (*Diachlorus ferrugatus* Osten Sacken), 3,667 chaoborids (*Corethrella spp.*), and 3 phlebotomine sand fly specimens were collected. Response of the most numerous mosquito species is shown in Figures 4-6 and 4-7. There was a highly significant difference in the total numbers of mosquitoes captured for the different colors ($p = 0.0001$). Means, standard errors, p-values, and significant differences for species

represented in large enough numbers are shown in Table 4-3. As seen in trials 1 and 2, there were significant trap-position and day effects for some species. Overall capture of mosquitoes was significantly greatest with the standard white broad spectrum incandescent, followed by blue, green, orange, yellow, red, no light control, and infrared respectively. When collections were classified by mosquito species, clear preferences were seen between species. *Anopheles crucians s.l.*, *Cs. melanura*, *Cx. nigripalpus* Theobald, *Ps. columbiae* (Dyar and Knab) and *Ur. sapphirina* showed significant color preferences. White light captured the most *An. crucians s.l.*. The greatest numbers of *Cs. melanura* were captured in traps with white, green, and orange. The most *Ps. columbiae* were collected in traps with blue and significantly more *Ur. sapphirina* were captured in traps with standard white or blue. No colors were found to be repellent to mosquitoes when compared to the no light controls. No significant difference ($p = 0.26$) for color attraction were obtained for the tabanid, *Diachlorus ferrugatus*. Conversely, the chaoborids, *Corethrella spp.*, showed significant color attraction ($p = 0.002$), preferring white and blue over the other colors.

Discussion

Many common Florida woodland mosquitoes are medically important. Although one of the primary means of evaluating the presence/absence and relative abundance of certain mosquitoes is through the use of light traps, few studies have evaluated mosquito response to different wavelengths of transmitted monochromatic light. Even fewer studies have detailed the response of individual species. Browne and Bennett (1981) tested

filtered light of known wavelengths to equate host preference with landing rates for *Coquillettidia perturbans* (Walker). They found shorter wavelengths (400-600 nm or blue-green) attracted significantly more mosquitoes than longer wavelengths. Their results correspond well to ours in Trial 3. In Georgia, Bargren and Nibley (1956) found *Ae. vexans*, *Cx. salinarius*, and *Cx. quinquefasciatus* to have varying levels of attractiveness to New Jersey traps using different color bulbs of similar intensities. Other species, such as *Cx. nigripalpus*, showed no preference for any of the four colors (447, 570, 659, 670 nm) tested. This finding agrees with ours in Trials 1 and 2, but differs from those of Trial 3 that found significant color preferences (blue, green, white) for *Cx. nigripalpus*. Vavra et al. (1974a) tested several types and colors of light and found no significant differences in the total numbers of mosquitoes attracted to each of the colors. Attraction of individual species of mosquitoes was not examined. In a laboratory test using *Cx. tarsalis*, *Cx. quinquefasciatus* and *An. sierrenis*, Gjullin et al. (1973) tested New Jersey light traps equipped with either ultraviolet light , or ceramic dipped bulbs colored red, green, blue, orange, and white. They found no significant differences in attraction between any of the colors tested. Wilton and Fay (1972) tested *An. stephensi* against a clear incandescent bulb and monochromatic light of various wavelengths. They found this mosquito highly attracted to 290 and 365 nm in the ultraviolet region and 690 nm, but that blues, greens and yellows (490, 540, and 590 nms) were found not as attractive as the clear bulb.

Allan et al. (1987) stated that crepuscular and nocturnal biting flies are unlikely to have well-developed color vision, but their abilities to detect differences in intensity

and perhaps greatly increase the efficiency of the trap. Several LEDs in series would be many times brighter and still use significantly less battery power than a single incandescent bulb. Several of the ERG studies previously mentioned have shown peak dipteran spectral activity from 450 to 550 nm. Based on the numbers of certain mosquito species that were attracted to the blue and green wavelengths, an LED producing wavelengths between 450 and 550 nm may produce excellent results. Currently technology limits production of LEDs producing these wavelengths. Super-bright blues (450 nm) have only recently become available, and perhaps future technology will produce a blue-green diode peaking at about 500 nm.

LEDs run on significantly lower amounts of energy (ca. 1 ma/8 hrs) than incandescent bulbs resulting in substantial savings in battery life and expense. Hours of use (means ± SEM) with the no light control (69±7.5), blue (63±9) and green (63±5.7) were found to last significantly more hours ($n= 4$, $p=0.02$) than the standard white (36±0) bulb. For convenient use, LEDs can be soldered in series directly into the existing trap circuitry. Best results for all colored LEDs except blue (100 ohm) were obtained using 200 ohm resistors connected to the light motor assembly. Future studies should focus on combinations of colors oriented in different directions. The use of "super-bright" LEDs warrants serious consideration as a mosquito replacement for standard incandescent bulbs used in light traps. These results have potential for use in population dynamics studies or for enhancing the attractivity of certain species.

Table 4-1. CDC Trap counts using colored LEDs or incandescent light with 200 ml/min CO2 (Means ± SEM). Means within each row having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). n = 6 nights.

Species	Red	Orange	Yellow	Green	No Light	Incandescent	P-Value
Ae. dupreei[2,3]	549.7±108.9ab	619.7±163.1ab	411.7±98.5b	502.5±117.1b	825.8±205.9a	499.8±112.5b	0.02
Ae. fulvus pallens	0.3±0.3a	0.2±0.2a	0.5±0.3a	0.0±0.0a	0.5±0.3a	0.7±0.2a	0.57
Ae. atlanticus[1,2,3]	306.2±64.0a	232.0±31.4a	227.5±57.8a	198.7±40.2a	251.2±51.2a	269.7±64.9a	0.34
Ae. canadensis	1.2±1.0a	0.2±0.2a	0.2±0.2a	0.5±0.5a	0.0±0.0a	0.0±0.0a	0.37
Ae. infirmatus[2,3]	60.2±15.1a	33.8±5.2ab	27.3±4.8b	31.8±5.4b	34.7±9.0ab	35.2±7.8ab	0.01
An. crucians s.l.	6.2±1.2ab	5.7±2.1ab	9.2±2.4ab	5.3±1.4ab	0.8±0.4b	13.8±4.8a	0.03
An. quadrimaculatus s.l.	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.3	-
Cx. nigripalpus[2,3]	5.7±2.0a	8.7±4.4a	4.7±1.0a	6.0±1.6a	3.0±1.3a	7.0±2.0a	0.19
Cx. (Melanoconion) spp.	1.5±0.0a	3.3±1.8a	2.8±1.1a	4.3±1.2a	2.3±1.1a	3.2±1.3a	0.65
Cx. quinquifaciatus	0.0±0.0	0.2±0.2	0.3±0.3	0.3±0.3	0.0±0.0	0.5±0.3	-
Ps. ciliata[2]	1.2±0.7a	1.7±0.8a	2.8±1.5a	1.2±0.7a	2.7±1.1a	2.3±1.2a	0.54
Ps. columbiae	0.7±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.2	0.0±0.0	-
Ps. ferox[2,3]	26.0±4.9a	26.0±5.8a	18.8±6.5a	17.3±3.6a	28.0±12.0a	27.5±4.9a	0.47
Ps. howardii	0.3±0.3a	0.2±0.2a	0.3±0.2a	0.2±0.2a	0.0±0.0a	0.2±0.2a	0.88
Cq. perturbans	1.2±1.0a	1.3±1.1a	1.5±0.5a	1.0±0.5a	4.3±2.8a	1.2±0.7a	0.51
Cs. melanura	9.2±3.8ab	18.7±5.7ab	16.7±5.6ab	24.3±5.2a	4.7±2.2b	20.8±6.3ab	0.03
Ur. sapphirina	1.2±0.6ab	3.0±2.6ab	1.0±0.4b	1.5±0.8ab	0.0±0.0b	8.7±3.9a	0.04
Tx. rutilus	0.0±0.0	0.5±0.3	0.2±0.2	0.2±0.2	0.2±0.2	0.0±0.0	-
Total No. Mosquitoes[2]	940.2±104.0a	952.0±163.1a	725.0±113.6a	793.7±119.1a	1157.3±190.8a	759.7±122.1a	0.08

[1]Adults could not be distinguished from *Ae. tormenter.*

[2]Significant day effect ($p < 0.05$).

[3]Significant position effect ($p < 0.05$).

Table 4-2. CDC Trap counts using colored LEDs or incandescent light only (Means ± SEM). Means within each row having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). n = 6 nights.

Species	Red	Orange	Yellow	Green	No Light	Incandescent	P-Value
Ae. dupreei [2,3]	34.5±10.8a	33.3±13.7a	26.5±7.2a	48.2±24.2a	33.2±8.6a	32.7±11.6a	0.51
Ae. fulvus pallens	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	-
Ae. atlanticus [1,2,3]	8.3±1.3abc	15.3±6.9ab	7.2±2.2bc	9.8±3.6abc	4.8±1.2c	17.5±5.1a	0.009
Ae. canadensis	0.2±0.2	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	-
Ae. infirmatus [2]	1.7±0.7a	4.8±2.9a	2.7±1.0a	2.7±0.8a	0.3±0.2a	4.3±1.3a	0.1
An. crucians s.l. [2]	0.8±0.5b	0.7±0.3b	0.7±0.4b	1.3±1.1b	0.0±0.0b	4.5±1.4a	0.004
An. quadrimaculatus s.l.	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.2	-
Cs. melanura	0.8±0.5a	2.0±1.3a	1.3±0.9a	2.5±1.0a	0.0±0.0a	3.5±0.8a	0.13
Cx. nigripalpus	0.3±0.2a	0.7±0.7a	0.2±0.2a	0.3±0.2a	0.2±0.2a	0.8±0.4a	0.68
Cx. (Melanoconion) spp.	0.2±0.2a	0.2±0.2a	0.7±0.3a	0.2±0.2a	0.0±0.0a	0.7±0.3a	0.25
Ps. ciliata	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.2	0.0±0.0	0.0±0.0	-
Ps. ferox [3]	0.2±0.2a	0.2±0.2a	0.0±0.0a	0.2±0.2a	0.2±0.2a	0.0±0.0a	0.72
Ur. sapphirina	0.7±0.3b	0.8±0.3b	0.7±0.3b	0.8±0.3b	0.0±0.0b	4.5±1.0a	0.001
Total No. Mosquitoes[2,3]	47.8±11.8a	58.2±23.4a	39.8±9.2a	66.2±30.5a	38.7±9.0a	68.7±16.1a	0.24

[1]Adults could not be distinguished from *Ae. tormenter*.

[2]Significant day effect ($p < 0.05$).

[3]Significant position effect ($p < 0.05$).

Table 4-3. CDC Trap counts using colored LEDs or incandescent light with 200 ml/min CO2 (Means ± SEM). Means within each row having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). n = 8 nights.

Species	IR	Red	Orange	Yellow	Green	Blue
Diachlorus ferrugatus[2,3]	19.6±4.5a	16.9±3.2a	18.1±4.1a	15.5±2.7a	21.5±5.0a	25.8±4.4a
Corethrella spp.[3]	2.6±0.8c	21.1±4.2c	45.0±9.9c	43.5±7.3c	54.1±12.3c	119.3±28.9b
Ae. dupreei[2,3]	1.0±0.4a	0.6±0.3a	0.6±0.5a	0.6±0.5a	0.6±0.3a	0.3±0.2a
Ae. fulvus pallens	0.4±0.2a	0.4±0.2a	0.6±0.3a	0.3±0.2a	0.3±0.2a	0.5±0.4a
Ae. atlanticus[1,2,3]	13.1±3.1a	14.1±3.4a	15.3±3.9a	12.6±4.1a	17.5±5.5a	11.6±3.4a
Ae. canadensis	1.1±0.4a	0.8±0.3a	0.8±0.4a	0.0±0.0a	0.4±0.2a	0.4±0.4a
Ae. infirmatus[2]	2.9±0.4a	5.3±0.7a	5.0±1.8a	3.9±1.1a	5.5±1.7a	3.5±0.7a
An. crucians s.l.[2,3]	2.0±0.4d	6.1±1.7cd	7.8±1.9bc	8.1±1.7bc	5.9±0.9cd	12.1±3.3b
An. quadrimaculatus s.l.	0.1±0.1	0.0±0.0	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.0
Cq. perturbans	1.5±0.6a	1.5±0.4a	0.9±0.3a	1.3±0.3a	1.8±0.5a	2.3±0.8a
Cs. melanura	2.4±0.8d	9.4±2.3cd	25.1±4.2ab	20.6±2.7cb	25.9±2.7ab	18.5±2.9bc
Cx. nigripalpus	2.8±0.25b	4.8±1.1ab	6.5±1.1ab	6.0±1.1ab	8.0±1.2a	7.8±1.7ab
Cx. (Melanoconion) spp.[2]	8.5±1.3a	11.5±2.0a	10.8±3.0a	11.0±2.8a	11.4±2.6a	17.4±2.9a
Cx. salinarius	0.3±0.2	0.3±0.2	0.4±0.3	0.4±0.2	0.5±0.3	0.5±0.3
Ma. dyari	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.1	0.0±0.0
Ps. ciliata	0.0±0.0	0.0±0.0	0.1±0.1	0.0±0.0	0.0±0.0	0.1±0.1
Ps. columbiae[2]	0.4±0.3ab	0.0±0.0b	1.0±0.8ab	0.5±0.4ab	0.9±0.5ab	1.9±1.1a
Ps. ferox[2]	4.9±1.3a	4.6±1.3a	4.3±1.6a	4.6±1.8a	5.6±2.1a	4.0±0.8a
Ur. sapphirina[2,3]	2.8±1.3b	13.4±3.3b	25.5±6.1b	22.3±4.1b	25.9±6.2b	53.9±13.4a
Total No. Mosquitoes[2,3]	44.0±5.1e	72.6±9.5cde	104.8±11.7bc	92.3±11.4bcd	110.1±10.9bc	134.6±20.3b

[1]Adults could not be distinguished from *Ae. tormenter.*

[2]Significant day effect ($p < 0.05$).

[3]Significant position effect ($p < 0.05$).

Table 4-3 --continued.

Species	No Light	Incandescent	P-Value
Diachlorus ferrugatus [2,3]	20.9±5.3a	15.5±5.2a	0.26
Corethrella spp. [3]	6.9±2.0c	211.9±30.1a	0.002
Ae. dupreei [2,3]	0.3±0.2a	0.4±0.2a	0.52
Ae. fulvus pallens	0.6±0.3a	0.4±0.2	0.89
Ae. atlanticus [1,2,3]	15.8±5.7a	14.9±5.0a	0.43
Ae. canadensis	0.6±0.4a	1.0±0.4a	0.38
Ae. infirmatus [2]	3.4±1.1a	7.0±3.5a	0.42
An. crucians s.l. [2,3]	2.0±0.5d	20.1±2.9a	0.0001
An. quadrimaculatus s.l.	0.0±0.0	0.3±0.2	-
Cq. perturbans	2.4±0.7a	1.6±0.7a	0.58
Cs. melanura	4.3±1.4d	33.6±4.7a	0.0001
Cx. nigripalpus	3.6±0.7ab	7.1±1.6ab	0.02
Cx. (Melanoconion) spp. [2]	11.4±4.0a	20.0±7.6a	0.07
Cx. salinarius	0.1±0.1	0.5±0.2	-
Ma. dyari	0.0±0.0	0.0±0.0	-
Ps. ciliata	0.0±0.0	0.0±0.0	-
Ps. columbiae [2]	0.5±0.4ab	0.4±0.4ab	0.08
Ps. ferox [2]	3.5±1.4a	6.9±3.1a	0.47
Ur. sapphirina [2,3]	2.4±1.1b	71.5±16.7a	0.0001
Total No. Mosquitoes[2,3]	50.8±10.4ed	185.6±26.8a	0.001

[1]Adults could not be distinguished from *Ae. tormenter.*

[2]Significant day effect ($p < 0.05$).

[3]Significant position effect ($p < 0.05$).

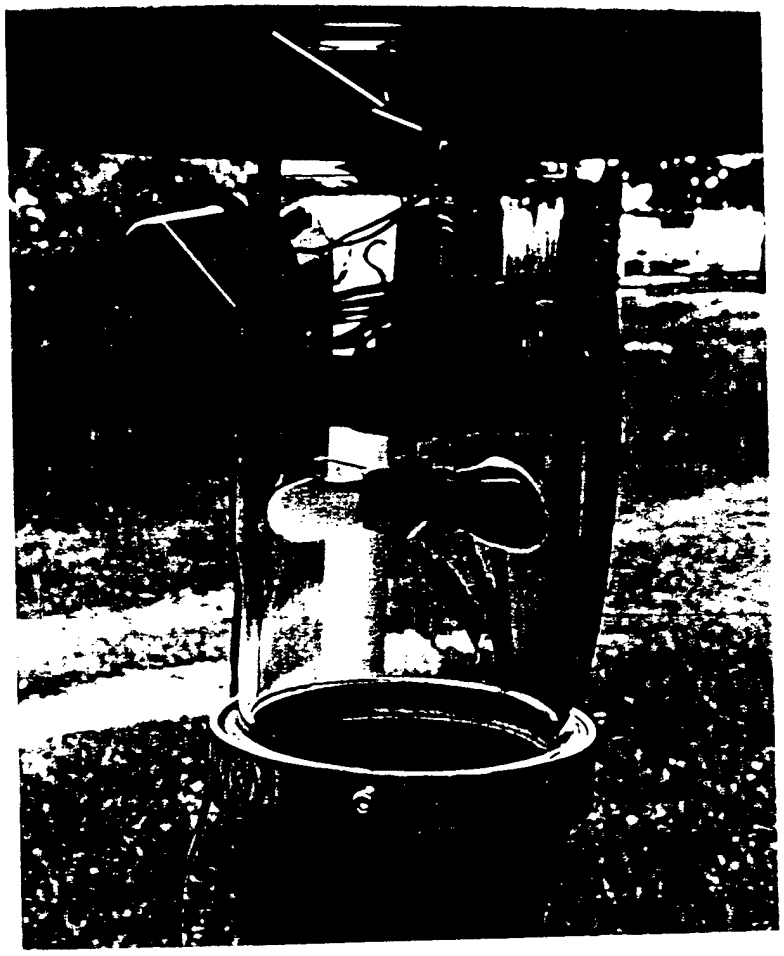


Figure 4-1. Modified CDC-type trap equipped with LED (shown with arrow).

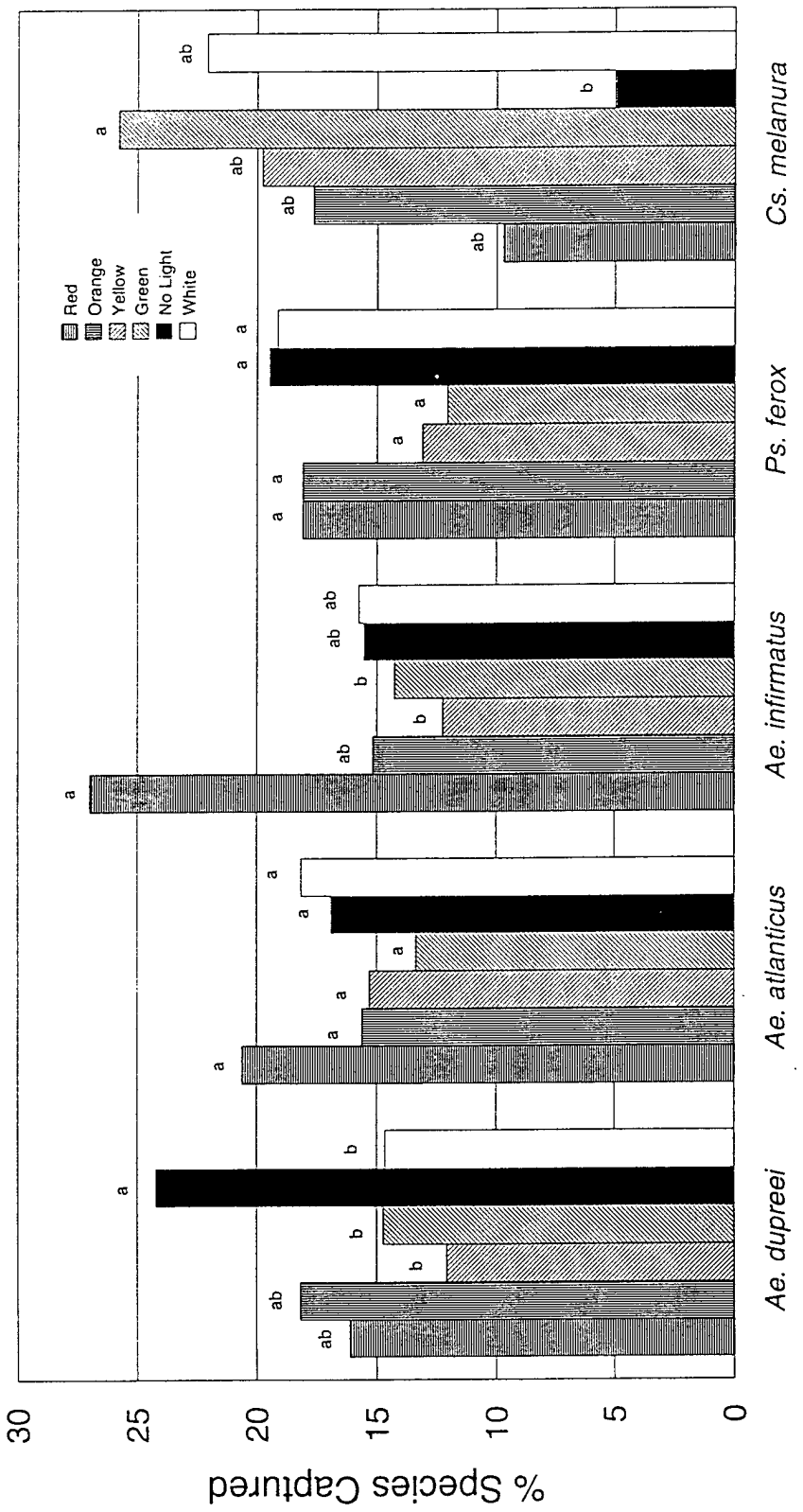


Figure 4-2. Relative percent composition of mosquito species captured in CO2 baited CDC-type traps using colored LEDs or incandescent light only. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 6 nights.

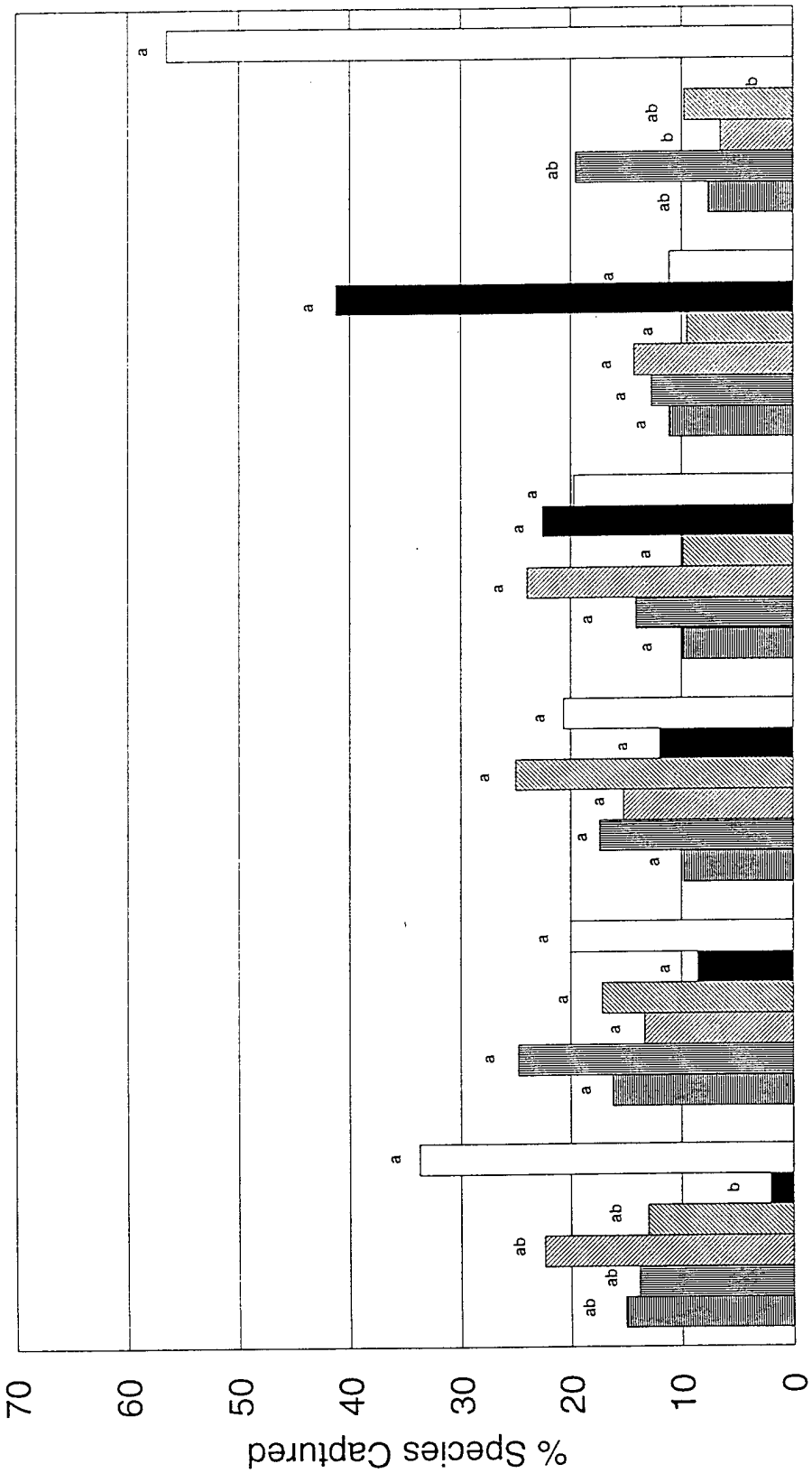


Figure 4-3. Relative percent composition of mosquito species captured in CO2 baited CDC-type traps using colored LEDs or incandescent light only. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 6 nights.

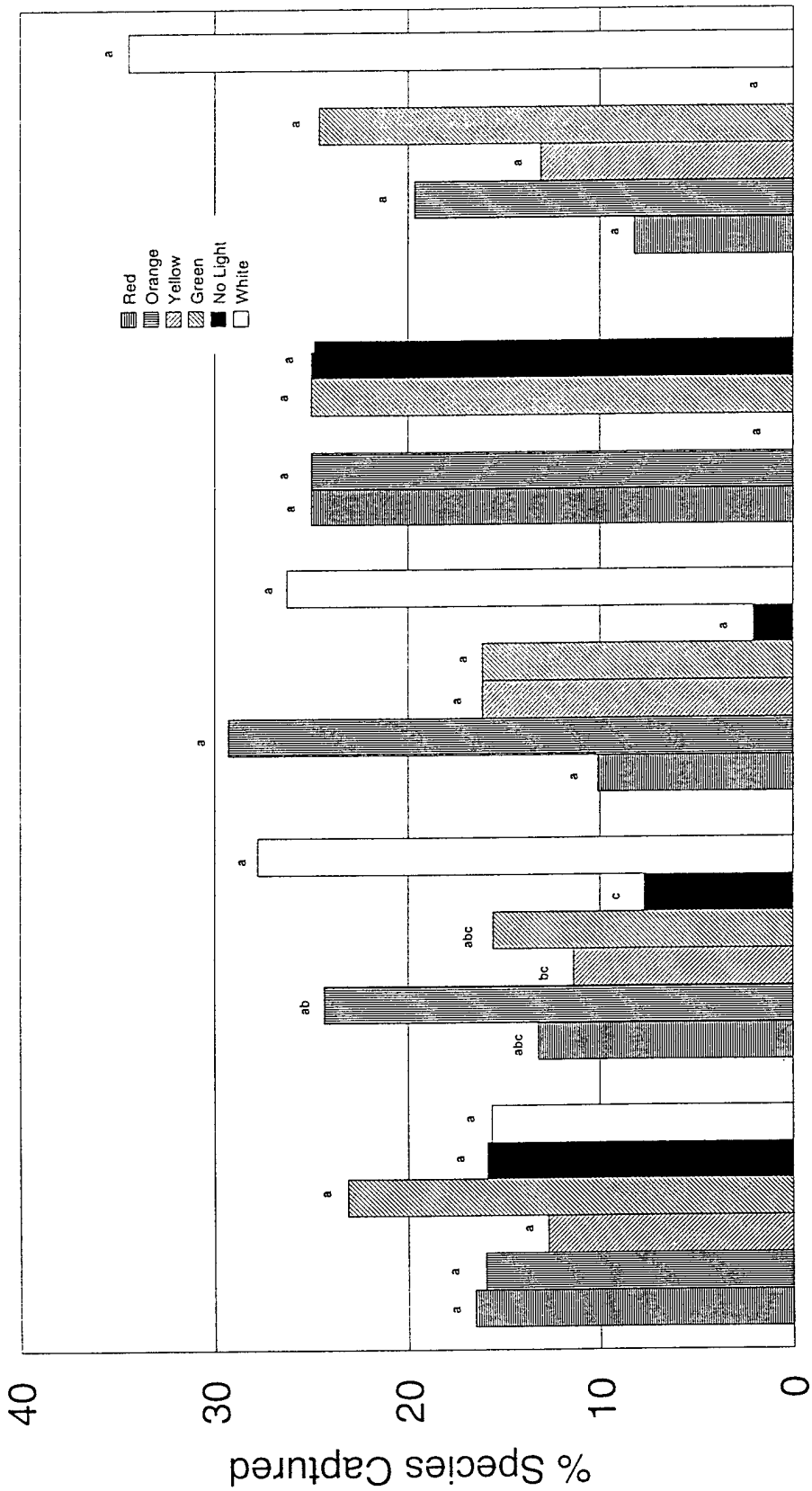


Figure 4-4. Relative percent composition of mosquitoes captured in CDC-type traps using colored LEDs or incandescent light only. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 6 nights.

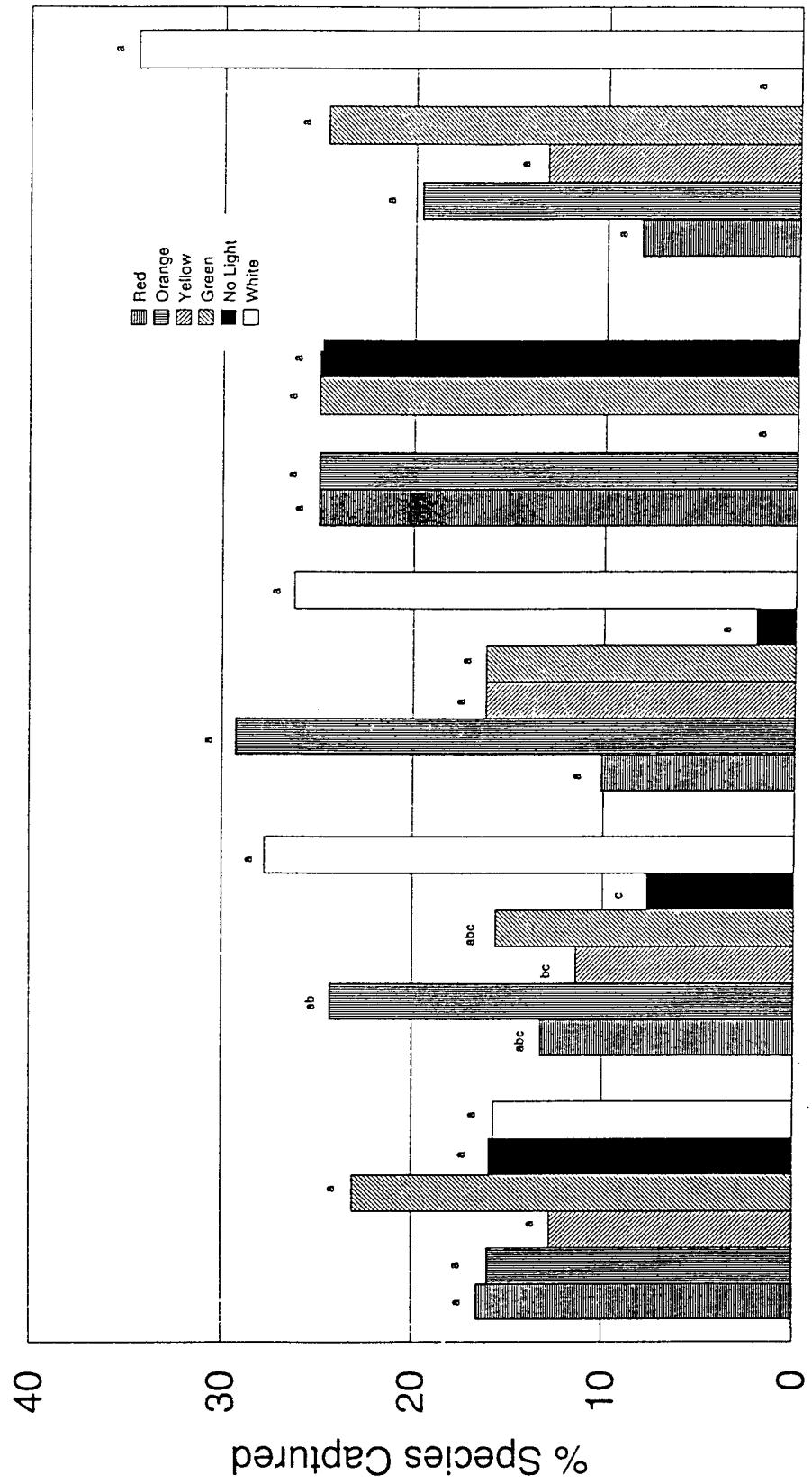


Figure 4-4. Relative percent composition of mosquitoes captured in CDC-type traps using colored LEDs or incandescent light only. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 6 nights.

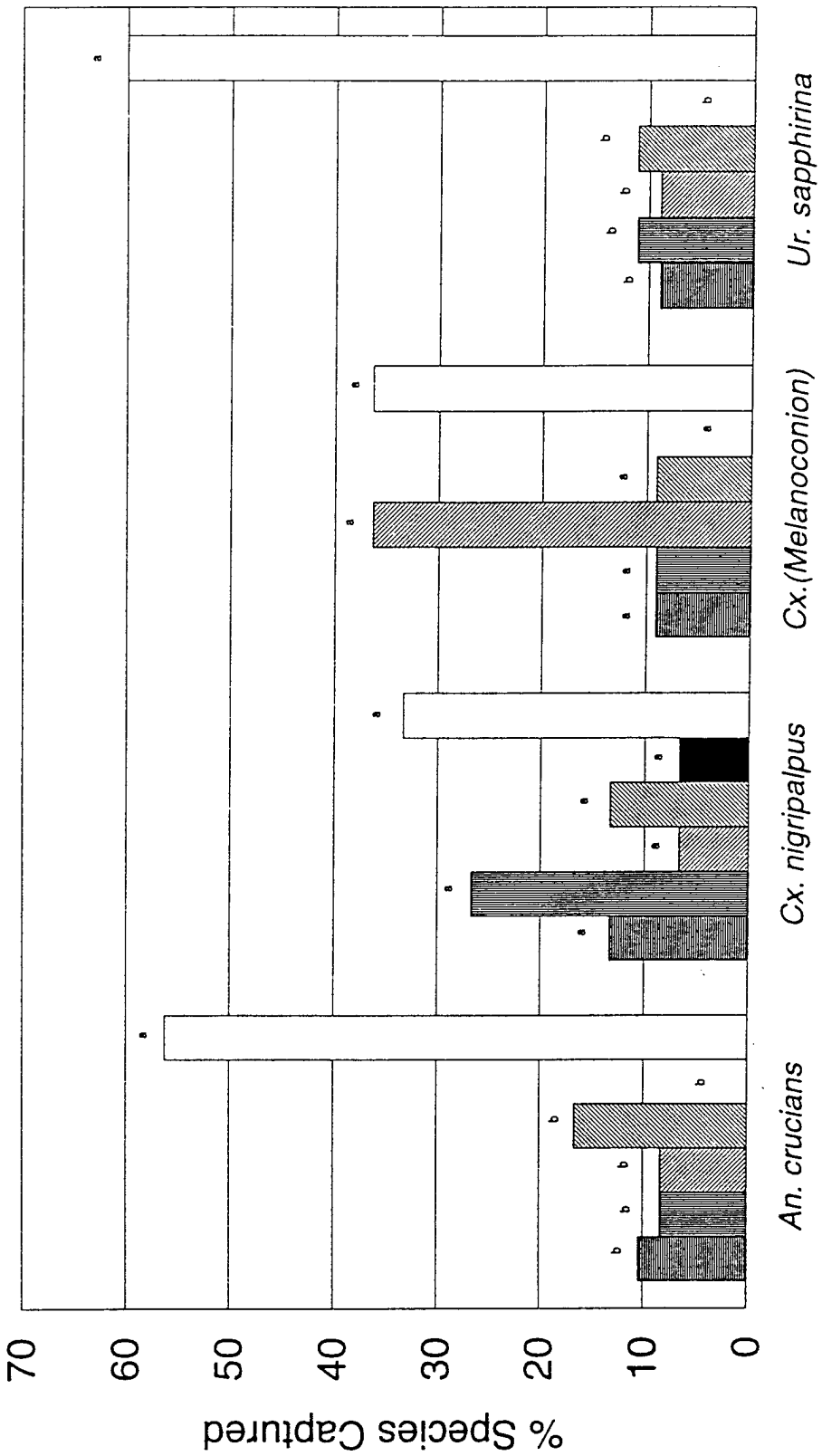


Figure 4-5. Relative percent composition of mosquitoes captured in CDC-type traps using colored LEDs or incandescent light only. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 6 nights.

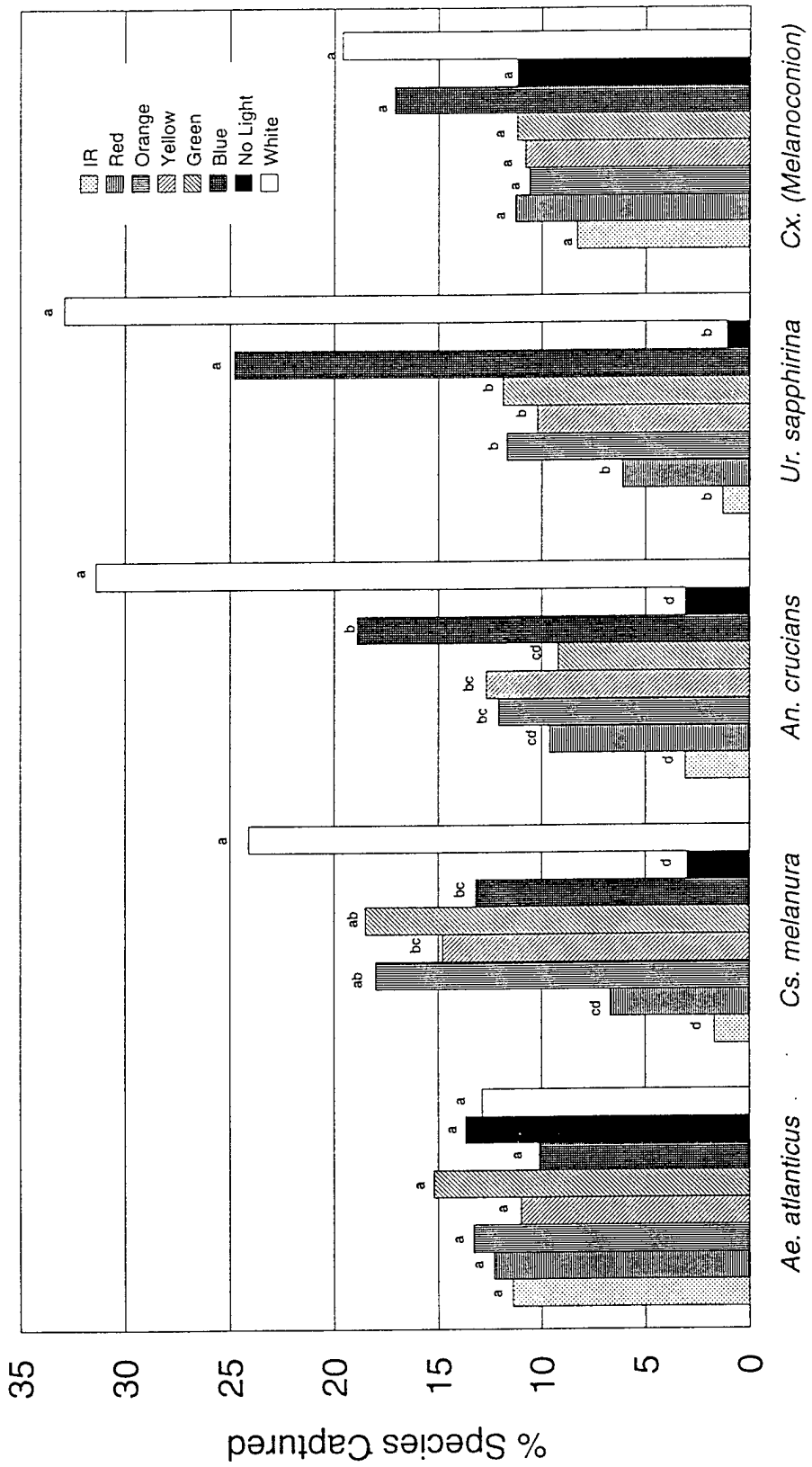


Figure 4-6. Relative percent composition of mosquitoes captured in CO2 baited CDC-type traps using colored LEDs or incandescent light only. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 8 nights.

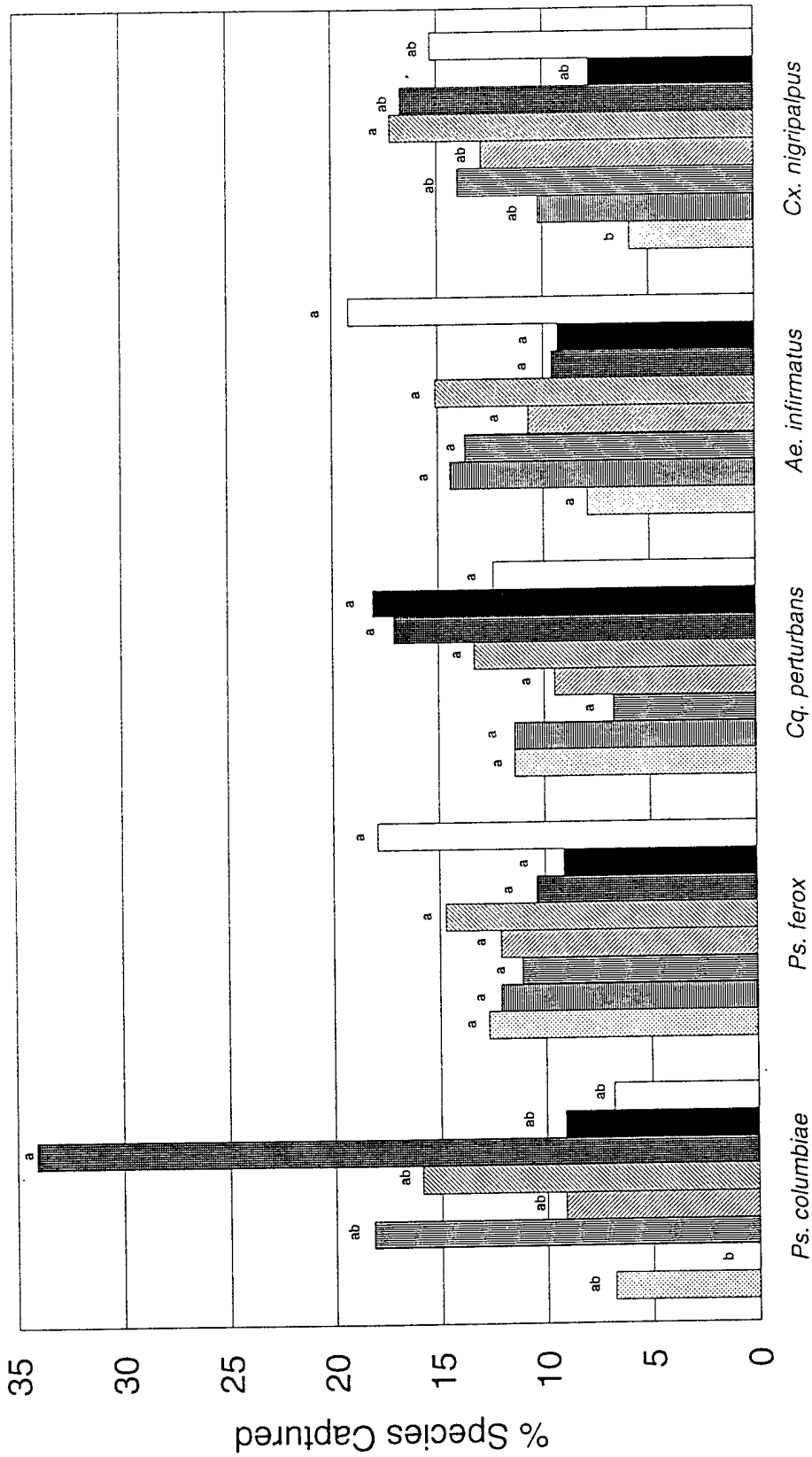


Figure 4-7. Relative percent composition of mosquitoes captured in CO2 baited CDC-type traps using colored LEDs or incandescent light only. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 8 nights.

CHAPTER 5
EVALUATION OF BLUE AND GREEN LIGHT EMITTING DIODES AS ATTRACTANTS FOR WOODLAND MOSQUITOES AND OTHER DIPTERA IN NORTH CENTRAL FLORIDA

Introduction

Field trials by Burkett et al. (1998a) evaluated capture numbers and species composition for light traps equipped with different colored light emitting diodes (LEDs) and compared them to those using standard incandescent bulb typically used in CDC-type traps. Results of these trials showed blue (450 ± 50 nm) and green (567 ± 50 nm) "super-bright" LEDs attracted nearly as many mosquitoes (both in numbers and species composition) as the standard bulb. The LEDs used in those trials were oriented "up" to reflect the LED "light" off the aluminum trap lid. The number of mosquitoes captured in light-baited traps is directly proportional to the intensity of the light (Barr et al. 1963). Our objective was to determine if trap efficiency could be improved by orienting the LED's away from the trap in a 360° pattern rather than reflecting the light off the trap lid. Orienting the LEDs away from the trap could increase the visible radius and thus allow the traps to be detected from a greater distance.

Aedes aegypti (Muir et al. 1992, Snow 1971), and other Diptera (Agee and Patterson 1983, Allan and Stoffolano 1986, Smith 1986) have bimodal spectral sensitivities with slight variations in λ max occurring in the green and blue areas of the

electromagnetic spectrum. Not all mosquitoes are attracted to light. Likewise, not all species are attracted to the same wavelengths. Much of the research on dipteran visual attractant research is based on the response of diurnal species to reflected light (Brett 1938, Bracken et al. 1962, O'Gower 1963, Granger 1970, Bradbury and Bennett 1974, Browne and Bennett 1980, 1981, Allan and Stoffolano 1986). Visual attractant research based on transmitted light is less common (Headlee 1937, Williams et al. 1955, Breyev 1963, Bargren and Nibley 1956, Gjullin et al. 1973, Wilton and Fay 1972, Browne and Bennett 1981). Few reports detail the response of individual species and often focus on species easily reared in the laboratory, but not commonly captured in light-baited traps. Several colors (100 nm width) of highly efficient, low cost, "super bright" LEDs have recently been developed. LEDs are an improvement over standard incandescent bulbs because they produce greater light intensity and require significantly lower amounts of energy (ca. 0.125 ma/hr vs. 150 ma/hr). In this study, we evaluated the relationship between orientation of the LED on a light trap and capture numbers and species composition for woodland mosquitoes and other dipteran species.

Methods and Materials

Field trials conducted 1800 to 0800 hrs from August 9-14, 1997 in a 6 X 6 Latin square design evaluated light-trap captures of common woodland haematophageous Diptera including mosquitoes, *Corethrella spp.* (Diptera: Chaoboridae), *Diachlorus ferrugatus* Osten Sacke n (Diptera: Tabanidae), and phlebotomine sand flies (Diptera: Psychodidae). Trials were conducted in a ca. 1 hectare cypress swamp surrounded by

pine flat woods located north of Gainesville, FL. Light, day, and position effects were evaluated using a three-way ANOVA (SAS Institute, 1995). Multiple comparisons were made using the Ryan-Einot-Gabriel-Welsh multiple range test (alpha = 0.05). The trap and motor assembly remained stationary, but the lights were changed nightly so each would occupy every position during the six-day period. After each trap night, trap collections were separated, weighed, identified and counted.

Standard CDC-type traps (John W. Hock Company, model 512, Gainesville FL) were modified by replacing the standard bulbs with the LEDs. LEDs oriented "out" in a 360° pattern were compared to those oriented "up" and reflecting off the aluminum trap lid. LEDs were soldered in series to insulated 22 gauge wire shaped to form a 40 cm diameter circle (Fig. 5) and were secured with rubber bands to the 1/4" mesh screen atop the trap lid assembly. The green or blue LEDs oriented up or out were compared to controls consisting of no light (fan only) and a standard incandescent bulb (John W. Hock Company, CM-47, 6.3 V, 520 millicandela [mcd], Gainesville, FL). The LEDs used were green (567 ± 50 nm, 2400 mcd, 8° [Toshiba Tosbright,® Martech Optoelectronics, Latham NY]) and blue (450 ± 50 nm, 800 mcd, 22° [Panasonic,® Digikey Corp, Thief River Falls, MN]). Power for the green and blue LEDs were supplied by 2 and 3 alkaline D cell batteries at (2.8 ± 0.2 volts, 18 ± 2 ma) and (4.3 ± 0.2, 20 ± 3 ma) respectively. A 10 ohm resistor was soldered in series to prevent over-driving the LED.

Trials used 9 kg compressed-gas (carbon dioxide) cylinders equipped with double-stage (Victor Equipment Company, model VTS453B-320, Denton TX) and microregulators (Nupro, Series M, Wiloughby OH) to maintain a constant gas flow of

200 ml per minute. Carbon dioxide was delivered to the trap through a 3 m piece of 8 mm transparent plastic tubing secured with a rubber band so the top of the tubing was even with the top of the trap opening. Gas flow was checked each morning and evening using an in-line flowmeter (Gilmont Instruments, no. 12, Great Neck NY). Mosquitoes attracted near the trap intake were drawn in by the trap fan, blown through a screen funnel and into a quart polypropylene jar containing a 6 X 6 cm piece of dichlorvos impregnated vinyl strip used as a killing agent. Six volt, 10 ampHrs rechargeable gel cell batteries (Powersonic Corp, San Diego CA) were used to run the fan motor and standard incandescent light. Traps were hung 165 cm above ground level and placed every 30 meters around the outer perimeter of the swamp. Due to the thick vegetation, none of the traps were visible to each other. The trapping period followed a 1.7, 2.3, 1.2, 2.4, and 1.9 cm rainfall on July 28, 29 and August 1, 2 and 7 respectively. The following species groups, *Cx. (Melanoconion) spp.* Theobald, *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann and *Aedes atlanticus/tormenter* Dyar and Knab were pooled, as these taxa could not be distinguished with confidence.

Results

During the six trap-nights, 102,917 mosquitoes, 94 tabanids (*Diachlorus ferrugatus* Osten Sacken), 2,841 chaoborids (*Corethrella spp.*), and 26 *Lutzomyia shannoni* (Dyar) were collected respectively. Proportions of trap collections captured at the different light color/orientation combinations for the most common species are shown in Figures 5-2 and 5-3. Means, standard errors, p-values, and significant differences for

the common species are shown in Table 5-1. As noted in the tables, there were significant trap-position and day effects for some species. No significant differences were observed for the total number of mosquitoes captured at the light color/orientation combinations ($p = 0.21$). However, there were significant differences in capture numbers for individual species including *An. crucians* s.l. ($p=0.0001$), *Culiseta melanura* (Coquillett) ($p=0.02$), *Cx. (Melanoconion) spp.* ($p=0.03$), and *Uranotaenia sapphirina* (Osten Sacken) ($p=0.001$). *Anopheles crucians* and *Ur. sapphirina* were captured in the largest numbers at the incandescent bulb and the blue LEDs oriented out. Conversely, *Cs. melanura* was captured in greatest numbers in the traps baited with the incandescent bulb, and blue and green LEDs oriented up. Blue LEDs "out" were most attractive for *Cx. (melanoconion) spp.* Although female *Lutzomyia shannoni* (Dyar), were collected, no significant color differences were observed given the small sample size. The tabanid, *Diachlorus ferrugatus* Osten Sacken, also showed no significant color preference. Chaoborids (*Corethrella spp.*), however showed significant color preferences ($p=0.0009$) for the incandescent, and both blue orientations. No colors were found to be repellent to mosquitoes when compared to the no-light controls. The mosquito species composition attracted to the incandescent light trap agree with those found by Mann (1993) and Burkett et al. (1998a).

The weights of non-target insects collected in the different CDC light trap combinations showed the incandescent bulb to have significantly more ($p=0.001$) than any of the LED color/orientations.

the traps, there were significant differences in trap collection for only 4 species. This may be due to the heavily vegetated and confined habitat of the cypress swamp. Greater differences in trap collections due to differences in intensity may result in more open habitats.

We recommend additional work aimed at species from different habitats and in different geographical locations. Furthermore, the attraction of phlebotomine sand flies deserves additional investigation. Light-emitting diodes require significantly lower amounts of energy (ca. 1 ma/8 hrs) than incandescent bulbs (See Burkett et al.1998a) saving both battery life and expense during use. For convenient use, LEDs could be soldered directly into the existing trap circuitry (in series) using a 100 and 200 ohm resistors for blue and green LEDs respectively. Future trap designs should incorporate a mixture of green and blue diodes oriented both "out and up." The use of "super-bright" LEDs warrants serious consideration as a replacement for standard incandescent bulbs used in light-baited traps. These results have potential for use in population dynamics studies or for enhancing the attractivity of certain species.

Table 5-1. CDC Trap counts using colored LEDs or incandescent light in different orientations with 200 ml/min CO2 (Means ± SEM). Means within each row having the same letter not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). n = 6 nights.

Species	Blue Out	Blue Up	Green Out	Green Up	No Light	Incandescent	P-Value
Sample Wt (mg)[2]	878.5±200.2a	797.5±92.1a	767.2±113.1a	856.0±140.3a	856.3±278.5a	1268.0±398.5a	0.35
Trash Wt (mg)	48.8±7.5b	28.0±5.9bc	17.8±2.9c	13.0±1.0c	6.5±2.3c	97.2±13.6a	0.001
Ae. dupreei[2]	74.5±21.7a	85.2±10.4a	62.1±12.5a	116.8±38.1a	109.5±16.8a	170.8±85.1a	0.22
Ae. fulvus pallens	0.7±0.3	0.5±0.3	0.8±0.5	0.5±0.2	0.8±0.5	0.3±0.2	-
Ae. atlanticus[1,2]	626.0±127.7a	649.5±103.0a	651.5±104.3a	637.7±86.9a	786.7±211.5a	973.8±283.0a	0.39
Ae. triseriatus	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.3	0.7±0.4	0.0±0.0	-
Ae. canadensis	0.3±0.3	0.0±0.0	0.0±0.0	0.2±0.2	0.0±0.0	0.0±0.0	-
Ae. infirmatus[2]	117.8±41.8a	117.7±21.1a	100.0±18.3a	138.3±34.4a	136.0±41.4a	177.8±64.8a	0.57
Ae. mitchellae	0.2±0.2	0.0±0.0	0.2±0.2	0.0±0.0	0.7±0.5	0.0±0.0	-
An. crucians s.l.[2]	149.3±22.9ab	97.7±11.7bcd	107.7±23.2bc	88.7±3.3cd	43.3±6.6d	187.3±21.0a	0.0001
An. quadrimaculatus s.l.	7.7±1.9a	4.0±2.0a	5.3±2.8a	4.2±2.5a	1.0±0.4a	8.3±3.1a	0.26
An. puntipennis	0.0±0.0	0.2±0.2	0.0±0.0	0.3±0.3	0.0±0.0	0.0±0.0	-
Cx. nigripalpus[2,3]	78.5±11.8a	76.3±23.4a	66.7±16.2a	75.2±10.9a	49.3±11.2a	67.1±13.4a	0.35
Cx. (Melanoconion) spp.[3]	51.3±15.7a	42.5±6.6ab	40.2±6.1ab	36.8±2.9ab	19.5±3.3b	25.8±5.4ab	0.03
Cx. quinquifaciatus	7.3±4.1	4.3±2.2	1.5±0.4	4.0±1.8	1.5±1.0	1.7±0.9	-
Cx. territans	2.8±1.0	2.7±1.6	2.5±1.0	1.3±0.7	0.8±0.5	1.3±0.9	-
Ps. ciliata[2,3]	17.2±5.0a	16.2±6.5a	23.7±6.3a	22.7±7.4a	29.0±8.1a	13.5±5.3a	0.11
Ps. columbiae	1.3±1.3	0.8±0.8	0.0±0.0	0.5±0.3	0.0±0.0	2.3±2.3	-
Ps. ferox[2]	140.0±39.0a	138.7±36.5a	140.3±36.6a	181.2±41.7a	181.0±61.5a	266.2±105.9a	0.43
Ps. howardii	2.5±2.0	2.5±1.3	1.0±0.4	2.3±0.6	1.2±0.7	1.8±0.5	-
Cq. perturbans[2]	16±4.6a	10.5±2.7a	14.0±5.2a	5.3±2.5a	4.7±2.1a	9.0±3.6a	0.1
Cs. melanura[3]	54.5±17.0ab	74.5±28.0a	49.5±12.2ab	83.0±23.8a	9.8±4.3b	77.7±14.9a	0.02
Ma. dyari	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.2	0.3±0.2	0.2±0.2	-
Ur. sapphirina[2]	100 0±23.0ab	72.5±8.6bc	26.2±4.6dc	25.0±6.8dc	2.5±1.5d	141.8±21.4a	0.001
Tx. rutilus	0.3±0.3	0.0±0.0	0.5±0.3	0.5±0.5	0.0±0.0	0.2±020	-
Total No. Mosquitoes[2]	1448.3±285.8a	1396.2±188.1a	1293.7±194.7a	1425.0±153.4a	1378.3±331.2a	2127.7±568.1a	0.21
Diachlorus ferrugatus[2]	4.0±1.6a	2.3±1.5a	2.3±0.7a	1.7±0.6a	3.3±1.1a	4.2±1.7a	0.21
Corethrella spp.	103.8±16.3abc	123.3±27.1ab	19.5±4.2bc	33.8±7.7bc	3.3±1.1c	189.7±62.2a	0.0009
Lutzomyia shannoni	1.0±0.5a	0.7±0.3a	0.3±0.2a	1.3±0.3a	0.3±0.2a	0.7±0.5a	0.32

[1]Adults could not be distinguished from *Ae. tormenter.*

[2]Significant day effect ($p < 0.05$).

[3]Significant position effect ($p < 0.05$).

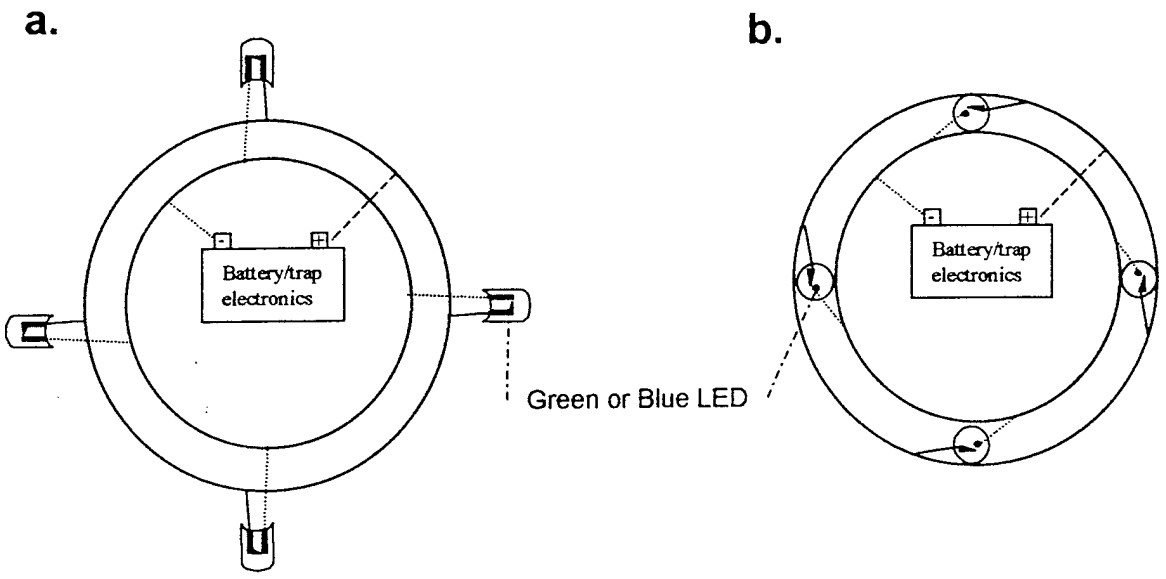


Fig. 5-1. Wiring assemble representation for LED orientations: a) Top view of LED assembly oriented 360 out and b) Top view of LED assemblely oriented "up." For green LEDs: 3 V with separate battery pack or if wired to trap electronics use 200 ohm resistor. For blue LEDs: 4.5 V with separate battery pack or if wired to trap electronics use 100 ohm resistor.

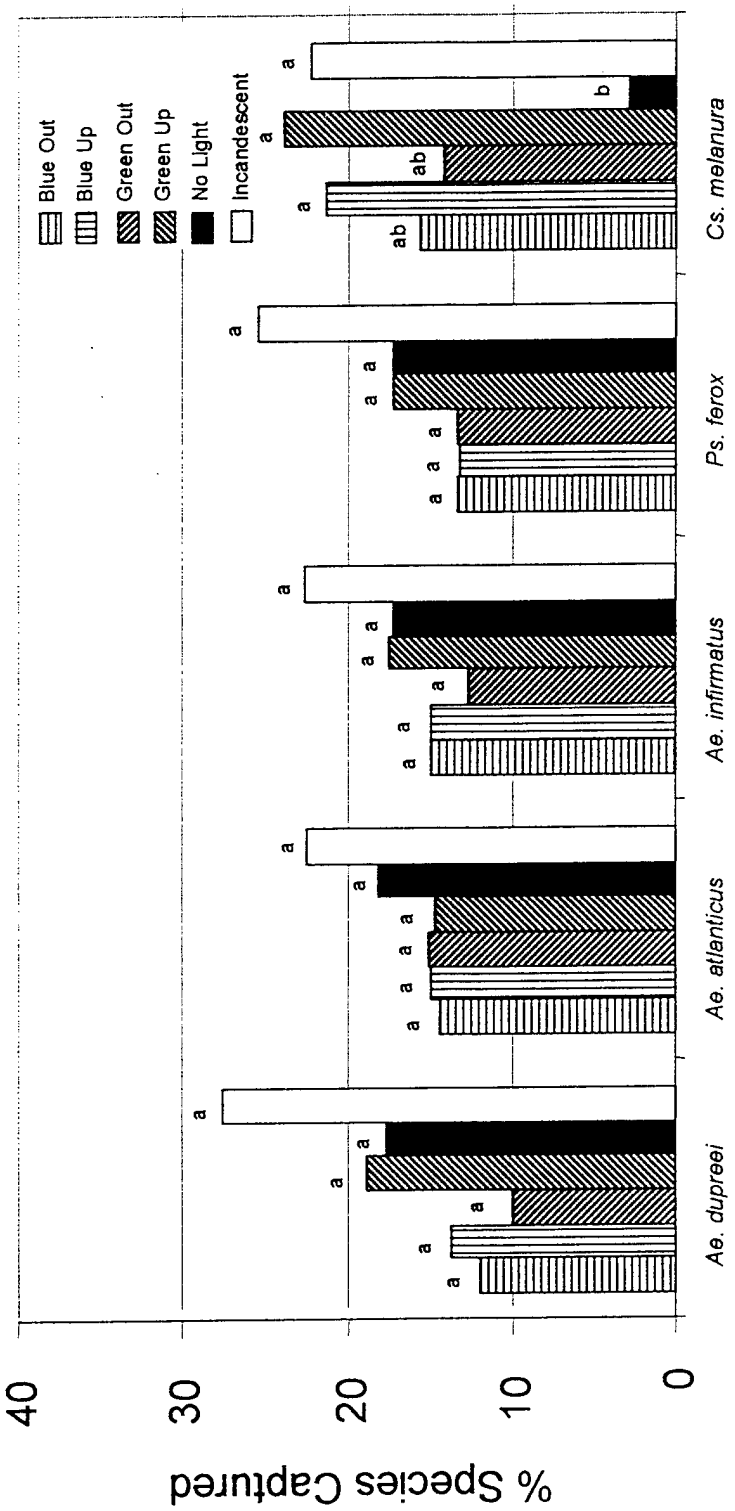


Figure 5-2. Relative percent composition of common mosquito species captured in CO2 baited CDC-type traps using different orientations of green or blue LEDs or incandescent light. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 6 nights.

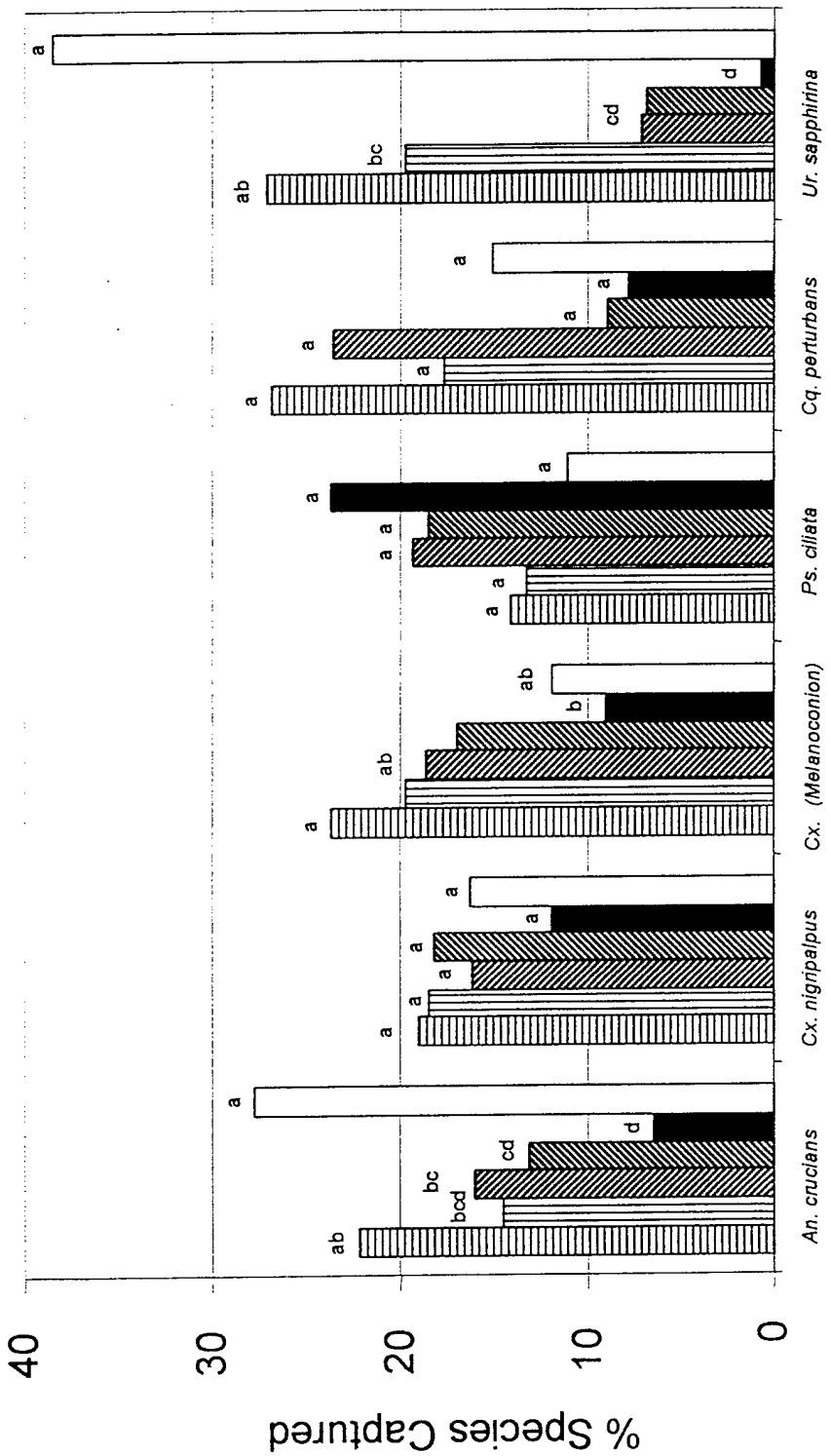


Figure 5-3. Relative percent composition of common mosquito species captured in CO2 baited CDC-type traps using different orientations of green or blue LEDs or incandescent light. Means within each species group having the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). alpha = 0.05, n = 6 nights.

CHAPTER 6
LABORATORY EVALUATION OF COLORED LIGHT AS AN ATTRACTANT FOR FEMALE *AEDES AEGYPTI, AEDES ALBOPICTUS, ANOPHELES QUADRIMACULATUS* AND *CULEX NIGRIPALPUS*

Introduction

That some species of mosquitoes and other medically important Diptera are attracted to artificial light or other visual stimuli has long been known and exploited in a variety of trap designs. Not all mosquito species respond equally to visual stimuli or to different wavelengths of light. Indeed, many mosquitoes do not respond to light traps at all (Service, 1995). Mating, dispersal, appetitive flight, and location of sugars, hosts, resting, oviposition and overwintering sites are all governed to some degree by vision. Many authors have examined the important visual components of host/resource finding and have divided them into shape, color (reflected and transmitted), size, contrast, light intensity, texture and movement (Allan et al. 1987). These factors alone or in combination appear to play an important role in a blood feeder's ability to successfully find a suitable host or other resource.

Most research on responses of haematophagous Diptera to various colors has evaluated the response of diurnal species to reflected light colors (Brett 1938, Brown 1954, Bracken et al. 1962, Barr et al. 1963, Granger 1970, Bradbury and Bennett 1974, Browne and Bennett 1980, 1981, Allan and Stoffolano 1986). Studies using colored

transmitted light are few, and even fewer provide information on individual species or emit light of known wavelengths and/or intensity (Headlee 1937, Breyev 1963, Bargren and Nibley 1956, Gjullin et al. 1973, Wilton and Fay 1972, Vavra et al. 1974a, Browne and Bennett 1981). None of these studies incorporate both reflected and transmitted light. Lack of information about the attractancy of different light wavelengths for different species of mosquito is a serious void in a science where mosquito control/research operations are often largely based on the numbers and types of mosquito captured in light-baited traps.

A laboratory method for the evaluation of the relationship between various light colors (wavelengths) of transmitted/reflected light and feeding preference (based on duration of feeding time in seconds) is presented herein for *Aedes albopictus* Skuse, *Ae. aegypti* (L.), *Anopheles quadrimaculatus*, Say (Type A) and *Culex nigripalpus* Theobald. Color preferences also are evaluated based on fecal speck counts beneath the illuminated artificial hosts. Fecal specks on white cards have long been used to evaluate resting site preferences and provide population indices for a variety of filth flies species (Axtell 1970). Fecal speck cards have not been used to evaluate resting site preferences for mosquitoes, but presumably, could provide an equivalent estimation of feeding or resting site preference in a confined area. Information obtained about mosquito responses to different wavelengths of light can be used to further exploit insects' attraction to artificial light and enhance our ability to conduct studies on population dynamics, species specific surveys and/or improve reduction strategies.

Methods and Materials

Visualometer and Data Collection

A pie-shaped olfactometer (Butler and Katz 1987, Marin et al. 1991, Wilson et al. 1991, Butler and Okine 1995, Okine 1994) electronically quantifies insect feeding activity on 10 compounds simultaneously for a set time period. The apparatus (hereafter called a "visualometer") was modified to incorporate 10 different light wavelengths which illuminated from below identical attractive food choices (termed "artificial hosts"). Simplified views of the top and side of the visualometer are shown in Figs.6-1a and 6-1b, respectively. Each artificial host was illuminated with unique wavelengths (ca 10 nm width) produced using filtered broad spectrum white light. The mosquito feeding response (time) on the illuminated artificial hosts was measured by two methods. First, when the mosquito fed and closed a circuit, mosquito feeding time (total seconds) was computer recorded, logged, and analyzed using touch and bite contact seconds (Fig. 6-1, K and J). Second, feeding preferences were evaluated by counting the number of mosquito fecal specks present within a 5 cm circle around the artificial host/colored light combination. Ten holes drilled into the bottom of the aluminum pie-shaped arena contained the tips of fiber optic cables that emitted light upwards and illuminated the artificial hosts from below (Fig. 6-1b, N). The fiber optic tips were covered with recessed interference filters (described below). As an additional attractant, CO_2 (0.5 l/min) was released through Tygon® tubing (Norton Performance Plastics Corp., Akron OH), directly below each artificial host (Fig. 6-1, G) for measured time intervals of 4 seconds "on" and

6 seconds "off". The visualometer was located in a temperature-controlled, light-proof, Faraday-cage room (Lindgren Enclosures, Model No. 18-3/5-1).

Artificial Host

The attractant food source used in the visualometer, termed "artificial host," consisted of fresh, citrated bovine blood mixed with agar and various feeding stimulants/attractants. The following mixture was used as the feeding stimulant/attractant: (J.F.B.,unpublished data): For 133 ml: 1.66 gms agar (U.S. Biochemical Corp, Cleveland OH), 33 ml fresh citrated bovine blood, 100 ml deionize water, 7.14 mg sodium chloride, 0.38 mg potassium chloride, 0.154 mg calcium chloride dihydrate, 0.2 mg magnesium chloride hexadydrate. 0.42 mg dibasic sodium phosphate, 2.1 mg sodium bicarbonate, 0.92 mg dextrose, 0.184 mg glutathione disulfide (oxidized glutathione), adjusted to a final pH of 7.4. The blood/agar/feeding attractant mixture was placed into the "cup" on the underside of a 35 mm plastic film canister lid where it was covered with a reinforced silicone membrane (Butler et al. 1984) held in place using a 4 mm retaining ring cut from the top of the film canister. The complete artificial host was then inserted into one of the ten holes cut into the transparent plexiglass visualometer lid. Between trials, the visualometer was disassemble and washed. Artificial hosts were replaced for each replicate and new mosquitoes were used for each trial.

Light Source and Filters

The light source used a wide spectrum tungsten-halogen bulb (Sylvania, no. DNF, Danvers, MA) transmitted through fiber optic cables (RTS Industries, Gainesville FL) (Fig.6-1b, N). Seven VIS-NIR broadband (± 5 nm) interference filters (350, 400, 450, 500, 550, 600, 650 and 700 nm) (Fig. 6-1b, L) with appropriate neutral density filters (NDF)(Fig.6-1b, M) to equalize intensities were used for each wavelength tested (Oriel Instruments, Stratford CT). The "white" light from the fiber optic cable (with NDF) and no light were used as controls.

Mosquito Species

All trials used 150, five to eight day old nulliparous, non blood fed females aspirated from cages containing both male and females. *Aedes albopictus*, *Ae. aegypti*, *An. quadrimaculatus*, and *Cx. nigripalpus* were the species evaluated. All species were tested separately. Laboratory colonies maintained at USDA ARS in Gainesville, FL provided recently colonized (1995) *Ae. albopictus*, and specimens from a long-established colony of *An. quadrimaculatus*. *Aedes aegypti* were reared as outlined in Gerberg (1970) and obtained from an established University of Florida departmental colony. Wild *Cx. nigripalpus* were reared from larva and pupae obtained from a sewage lagoon at the University of Florida Swine Research Unit. All mosquitoes were reared and maintained at 25° C, 95% RH and a 14:10 (L:D) photoperiod. All trials were run from 1600 to 0800 hrs.

Statistical Analysis

All touch/bite contact seconds and fecal specks were recorded for 8 and 16 hrs respectively. All species trials were analyzed using the first 4 hours of feeding activity, with the exception of *Cx. nigripalpus*, which analyzed the last 4 hrs of feeding times. A 10 X 10 Latin square design (3-way ANOVA) was used for *An. quadrimaculatus*. For other species, a randomized complete block (2-way ANOVAs) design with 8 to 10 replications was used. Duncan's multiple range test was used to delineate significant differences between the colored light treatment means. Differences between treatment means were considered significant at alpha = 0.10. Pearson's correlation coefficients (SAS Inst. 1995) were performed to test for relationships between feeding times and fecal specks on each of the artificial host/colored light combinations.

Results

With the exception of *Cx nigripalpus*, all species showed a period of "orientation/acclimation" lasting ca.10-15 minutes after which mosquitoes would begin aggressively probing and feeding on the artificial hosts. Of these, *Ae. albopictus* was the least aggressive and consequently had the lowest over all feeding times on the different host/color combinations. The wild *Cx. nigripalpus* presumably still under circadian control did not begin actively feeding until about 4 hours into the trial.

Aedes aegypti

Feeding durations (Fig. 6-2) and fecal speck (Fig. 6-6) results for this species were not significant different for feeding times (n=10, p= 0.17) or fecal specks (p=0.16) respectively, for any of the colors tested. No correlation (r=0.02, p=0.84) was found between the number of fecal specks and feeding durations. Significant differences for total seconds of feeding (p=0.04) and fecal speck counts (p=0.003) were observed for different replications (day effect).

Aedes albopictus

This species showed significant preferences (n=10, p=0.03) for certain wavelengths of light (Fig. 6-3). *Aedes albopictus* fed significantly longer on yellow-orange (600 nm), blue-green (500 nm), white, blue (450 nm), violet (400 nm), and black compared to other colors tested. Significant differences (p=0.001) also were found for the number of fecal specks (Fig. 6-7), with 550 nm (yellow-green) being significantly greater than the other wavelengths. The number of mosquito fecal droplets, however, did not correlate (r=0.06, p=0.55) with the feeding times at respective colors. *Aedes albopictus* had an overall mean (± SEM) feeding time of 244 ± 44.2 seconds which was significantly lower than the feeding times (p=0.002) and fecal speck (p=0.0005) numbers of the other mosquito species. As with all other trials, significant differences for total feeding durations (p=0.01) and fecal speck counts (p=0.004) were observed for different replications (day effect).

Anopheles quadrimaculatus

Results of the visualometer trials (Fig. 6-4) found weakly significant differences (n=10, p=0.094) in feeding times for several of the wavelengths tested. Mosquitoes fed for a significantly longer duration on the white and no light controls over the other wavelengths. Likewise, feeding durations on all other wavelengths were significantly greater than those on 350 nm (UV). Significant feeding duration differences were found on consecutive days (p=0.07), but not for different positions (p=0.73). There were significant differences in fecal speck numbers for *An. quadrimaculatus* (p=0.003, Fig. 6-8) with the greatest number of specks occurring on 500 nm, 550 nm, 600 nm and white. The number of fecal specks differed significantly between days (p=0.0001), but not for position (p=0.35). The number of mosquito fecal droplets correlated significantly (r=0.45, p=0.0001) with the feeding times at each of the illuminated artificial hosts.

Culex nigripalpus

Due to lack of activity during the first 4 hours of the feeding trials, the last 4 hours (2000 - 2400) were analyzed. Significant color preferences (n= 8, p=0.06) were observed for this species. *Culex nigripalpus* (Fig. 6-5) showed significantly higher feeding times on blue green (500 nm), orange (600 nm), blue (450 nm), white, red (650), and yellow-green (550 nm) over the other colors tested. Fecal specks (Fig. 6-9) showed significant preferences (p=0.09) for 500 and 600 nm over 700 nm and white. As with *An.*

quadrimaculatus, the number of fecal specks was significantly correlated ($r=0.47$, $p=0.0001$) with feeding times at each of the illuminated artificial hosts.

Discussion

Considering the variation in attractiveness of different mosquito species to light-baited traps (Huffaker and Back 1943, Bidlingmayer 1967), it is not unreasonable to expect that individual species will vary in wavelength preference. Such wavelength preferences (exhibited by behavioral responses) may or may not correspond to spectral sensitivities. For attraction to light-baited traps, intensity is considered more important than color (Barr et al. 1963). As such, many studies of color light preferences in Diptera are criticized because they fail to compensate for intensity (and/or hue) and make interpretation of the results difficult (Allan et al. 1987). These visualometer tests compensated for variations in light intensity by incorporating neutral density filters at each wavelength so that each treatment only varied by color and an accurate assessment of "color" preference could be obtained. Even so, different wavelengths may be physiologically more stimulating and result in greater behavioral responses.

For mosquitoes, electroretinograph studies for determining spectral sensitivities have only been published for *Ae. aegypti* (Muir et al. 1992, Snow 1971). These electroretinograph studies both provide evidence of bimodal sensitivities with a small peak at 350 nm and a large peak 550 nm. This bimodal pattern is similar to those found for tabanids (Allan et al. 1991, Smith 1986) and other insects (White 1985) and is assumed, but never tested, to be similar to the spectral sensitivities of other mosquito

species. Interestingly, spectral sensitivity research has focused mainly on diurnal species that are not generally attracted to standard light-baited traps. In our visualometer trials *Cx. nigripalpus* is the only species commonly captured in field trials using light baited traps. Results of our trials showed none of the mosquito species tested highly attracted to the assumed peaks in spectral sensitivities (550 and 350 nm) over the other wavelengths. Peak spectral sensitivities of ca. 550 and 350 nm may serve to allow discrimination in a environment dominated by greens and blues (Lythgoe 1979), but do not necessarily correspond with attractive wavelengths.

Aedes aegypti and *Ae. albopictus* are not frequently captured in mosquito traps baited primarily with light (Service 1995). As these species are diurnal, reflected light appears to be more important in resource location than transmitted light. In general, most successful *Ae. aegypti/albopictus* adult traps do not use light, but rather rely on strategic placement and low reflective colors (Fay 1968, Freier and Francy 1991). The relatively small numbers of *Ae. aegypti/albopictus* captured in light traps suggest that transmitted light is relatively unimportant in host/resource choice. Indeed, the duration of feeding times and fecal speck numbers for *Ae. aegypti* did not differ significantly between any of the wavelengths tested. Duration of feeding times and fecal speck numbers for *Ae. albopictus* however, were significantly greater for 600 nm , 500 nm, 450 nm, 400 nm and broad spectrum white light. Field trials with light emitting diodes or other sources of monochromatic light might result in similar attractive colors under field conditions.

Although nocturnally active, *An. quadrimaculatus* is another species poorly collected by light-baited traps (Bradley 1943). In our visualometer trials, *An.*

quadrimaculatus were most attracted to the strongly contrasting, no light and broad spectrum white controls followed by 550 nm light. Although ultraviolet lamps have long been known to increase the numbers of host or resource seeking mosquitoes captured at light traps (Headlee 1937, Weiss 1943, Williams et al. 1955, and Breyev 1963), 350 nm was the least attractive wavelength for *An. quadrimaculatus* and most of the other species tested in our study.

If the duration of feeding is a measure of attractiveness, then the feeding time results for *An. quadrimaculatus* differed slightly with those found in two field experiments using colored light emitting diodes which found no significant trap count differences for *An. quadrimaculatus* (Burkett et al.1998a), See Chapter 5). In either case, the color of light does not appear to be important in the host/resource seeking behavior of *An. quadrimaculatus* based on these studies. The significant differences in color feeding durations/specks found for *An. quadrimaculatus* in these trials may be an artifact of the close confines of the visualometer, but are unimportant for trapping wild individuals. Correlations between the number of specks around the artificial hosts and the feeding durations shows significant, though not necessarily strong correlations for both *An. quadrimaculatus* and *Cx. nigripalpus*. This negates the possibility of using one as a predictor of the other, but does serve as a means of checking the reliability of the visualometer's touch and bite contact second (feeding time) electronics.

With the latter being more effective, field trials with CDC-type light traps baited with light and those with light and CO_2 are effective at collecting *Cx. nigripalpus* (Nayar 1982). Field research using narrow wavelength LED's (Burkett et al. 1998a) also found

this mosquito attracted to light traps, and in one field trial, *Cx. nigripalpus* was significantly attracted to green (567 nm) followed by blue (450 nm) and white over the other colors tested. This agrees with what was found in the visualometer trials for this species. Other follow-up studies (See Chapter 5) which compared the blues, greens and whites found no significant color preferences for *Cx. nigripalpus*. Given the weakly significant results, and general lack of supporting field data, light color is largely unimportant in host/resource acquisition for both *Cx nigripalpus* and *An. quadrimaculatus*.

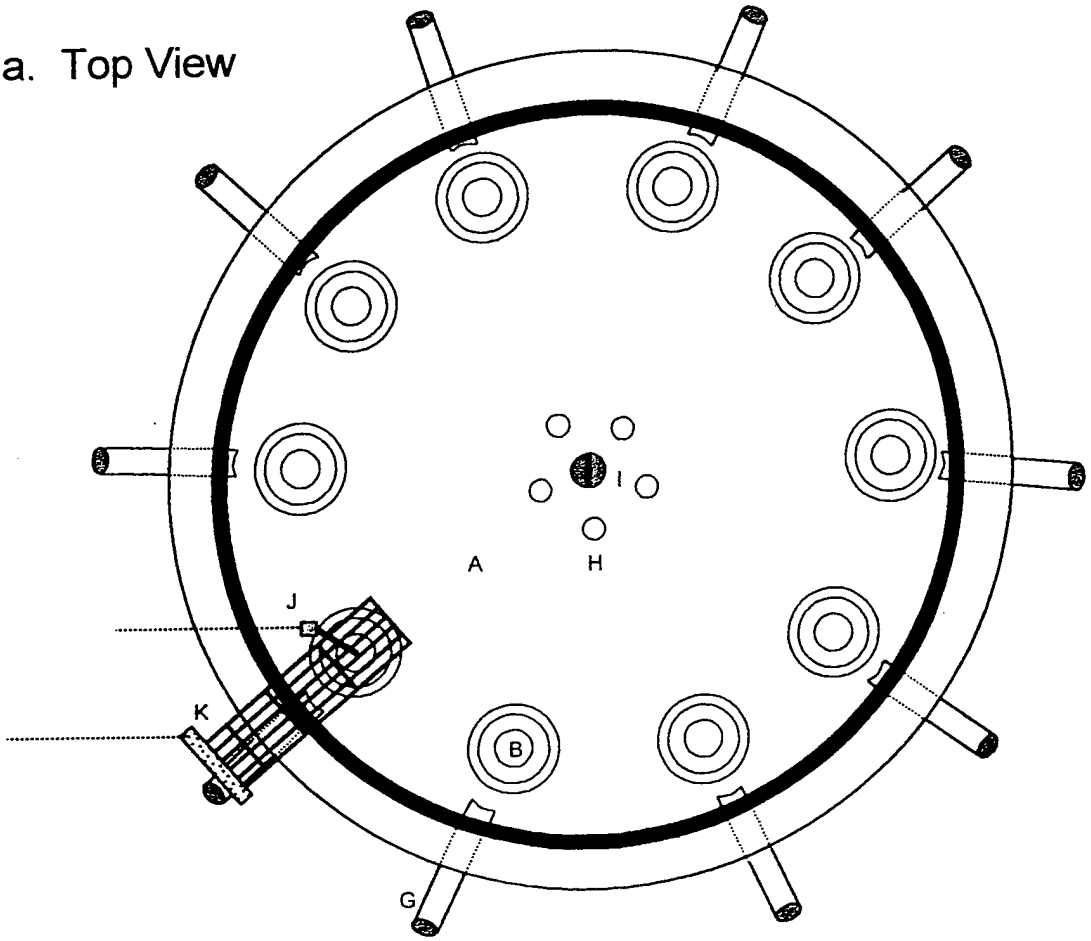
According to Clements (1992) mosquito excretion and defecation both produce fly specks, the former being a clear to yellowish spot produced soon after feeding; and the later being a dark speck produced several hours after feeding (rate is temperature dependent). Unfortunately, mosquitoes were not observed while in the visualometer and fly speck counts were not differentiated by color. *Ae. albopictus* showed no correlation between feeding durations and corresponding fecal specks, yet showed significant color preferences for both feeding time and fecal specks numbers on the different colors. Better correlation between feeding durations and fly speck numbers may have been possible if speck counts were differentiated by color within four hours of initiation of the assay. Likewise, the clear specks may have provided feeding color preferences, and the darker specks may have given resting site information.

Future trials using the visualometer need to concentrate on species known to be attracted to artificial light. Information obtained about medically important mosquitoes can be used to further improve current light-based trapping methods and, ultimately,

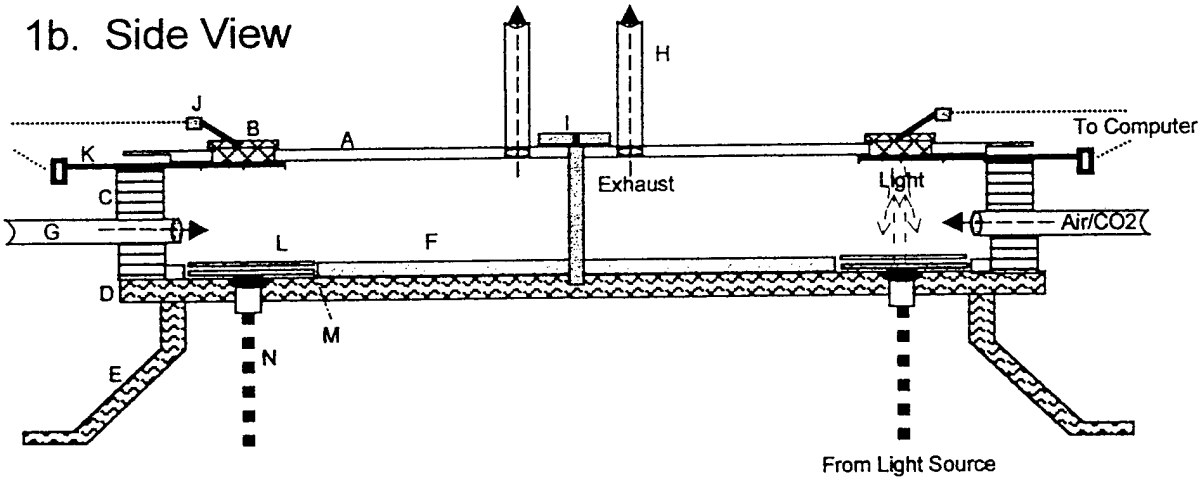
enhance studies on population dynamics, species specific surveys and improve reduction strategies.

Figure 6-1. **Visualometer** (a) Top view (b) Side view. (A) 430 x 5 cm dia. transparent plexiglass lid (B) Artificial host, (35 mm film canister lid) (C) 30 cm high plastic side piece with holes for plastic tubing (D) Aluminum base with holes for fiber optic cables (E) Support leg (F) Plexiglass filter support (G) 10 cm diameter Tygon® tubing for incoming air/CO2 (H) Tubing for exhaust (I) Assembly screw (J and K) Probe inserted into top of artificial host and bottom sensor fitted under artificial host (feeding mosquitoes complete circuit logged by computer), one sensor per artificial host (L) Interference (bandbass) filter (M) Neutral density filter (N) Fiber optic cable (attached to light source). Drawing not to scale.

1a. Top View



1b. Side View



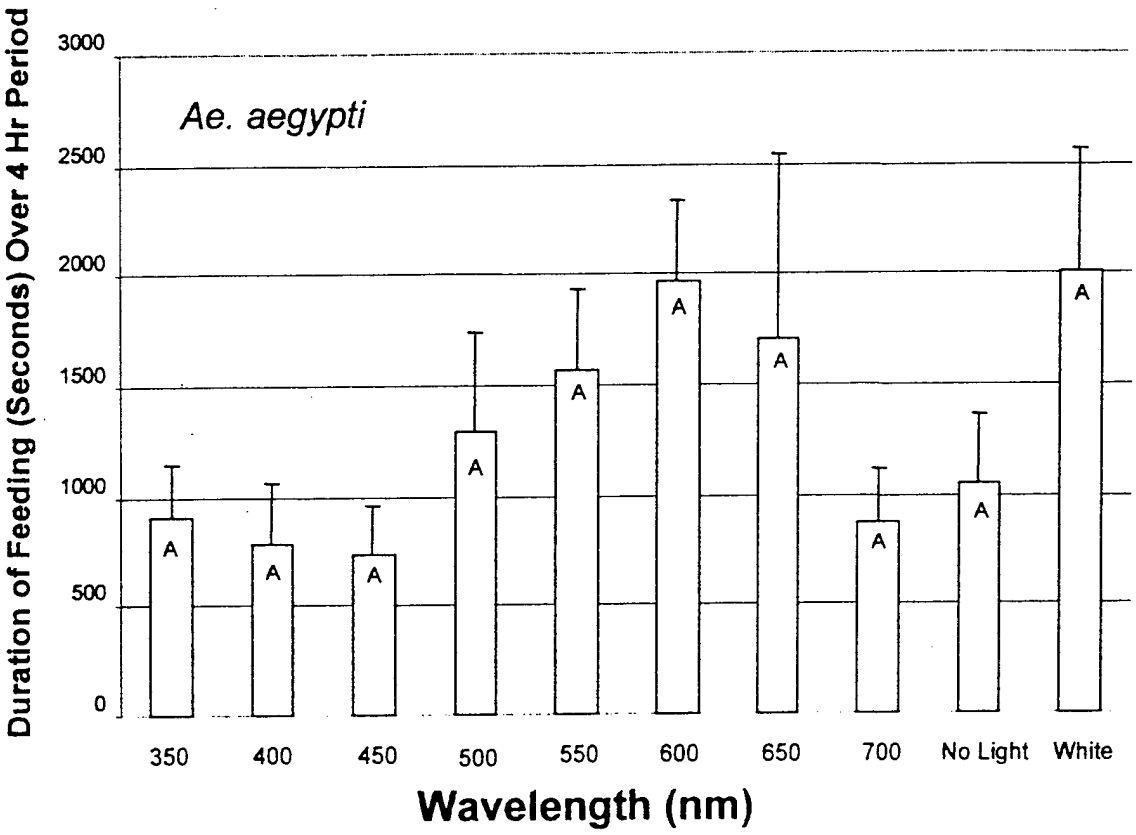


Figure 6-2. Duration of feeding (seconds) during a 4 hour exposure (means ± SEM) for *Aedes aegypti* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

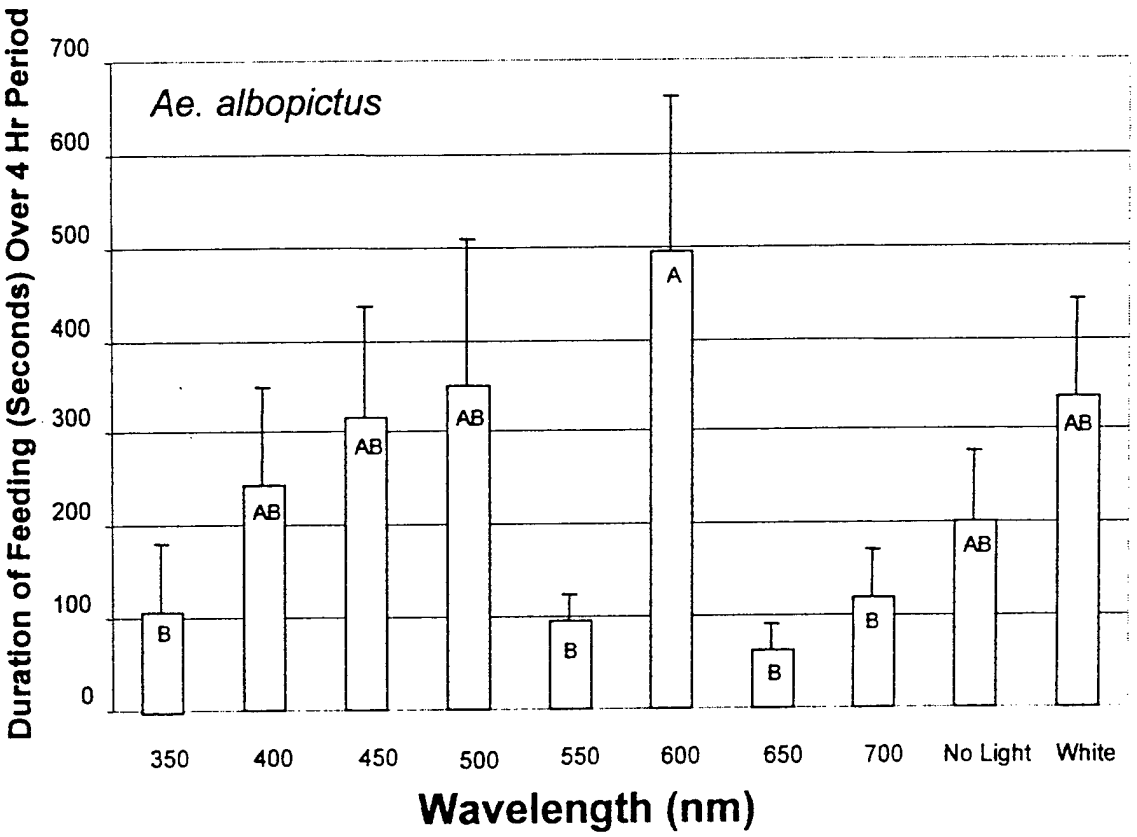


Figure 6-3. Duration of feeding (seconds) during a 4 hour exposure (means ± SEM) for *Aedes albopictus* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

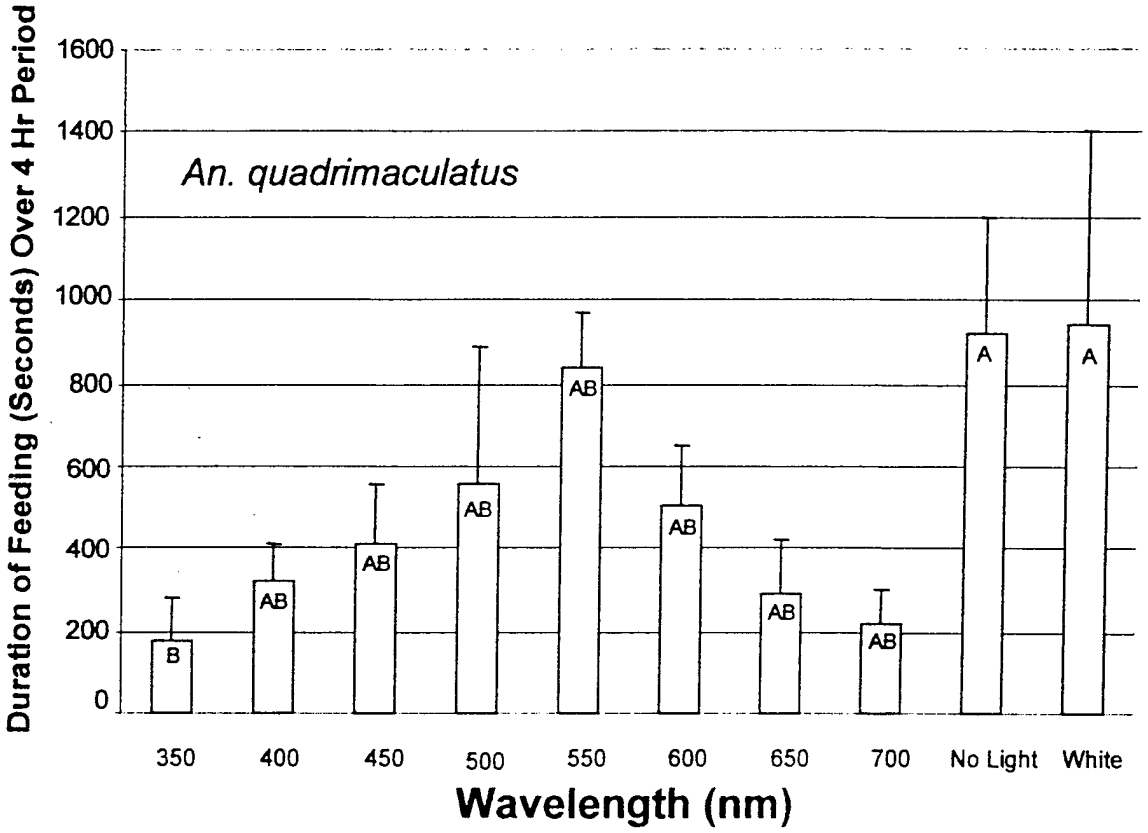


Figure 6-4. Duration of feeding (seconds) during a 4 hour exposure (means ± SEM) for *Anopheles quadrimaculatus* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

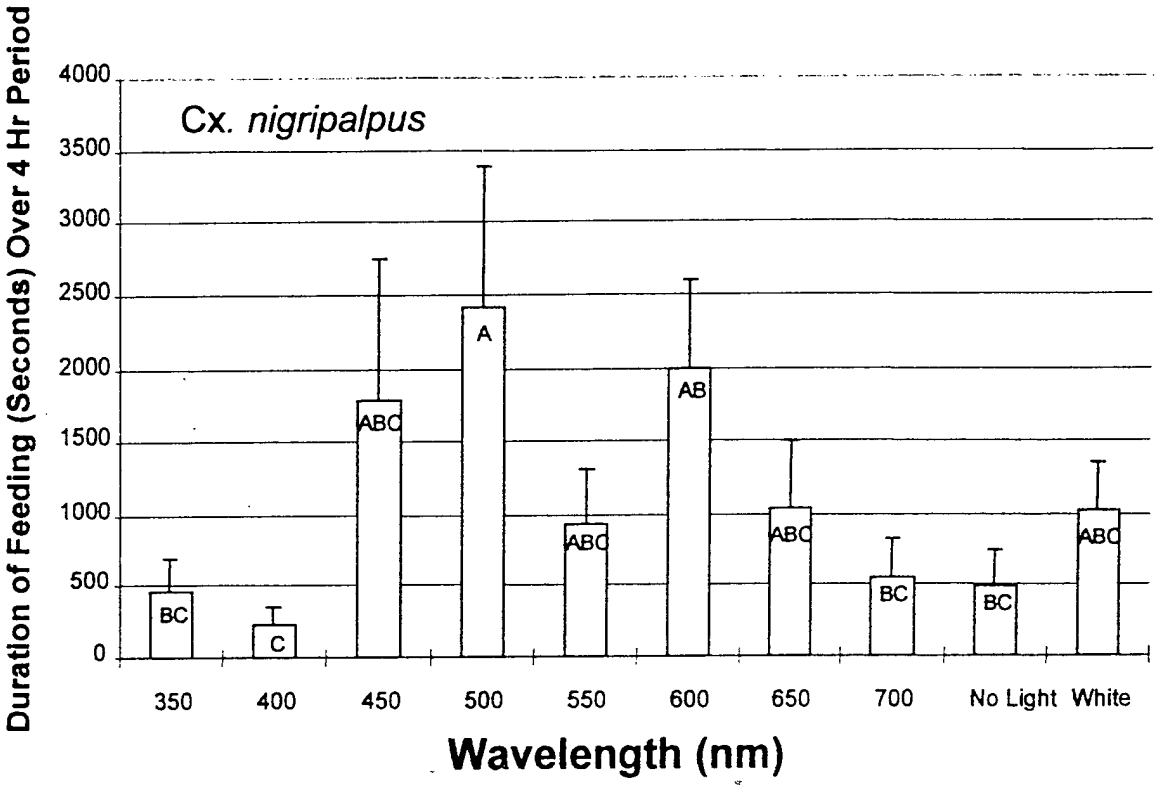


Figure 6-5. Duration of feeding (seconds) during a 4 hour exposure (means ± SEM) for *Culex nigripalpus* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

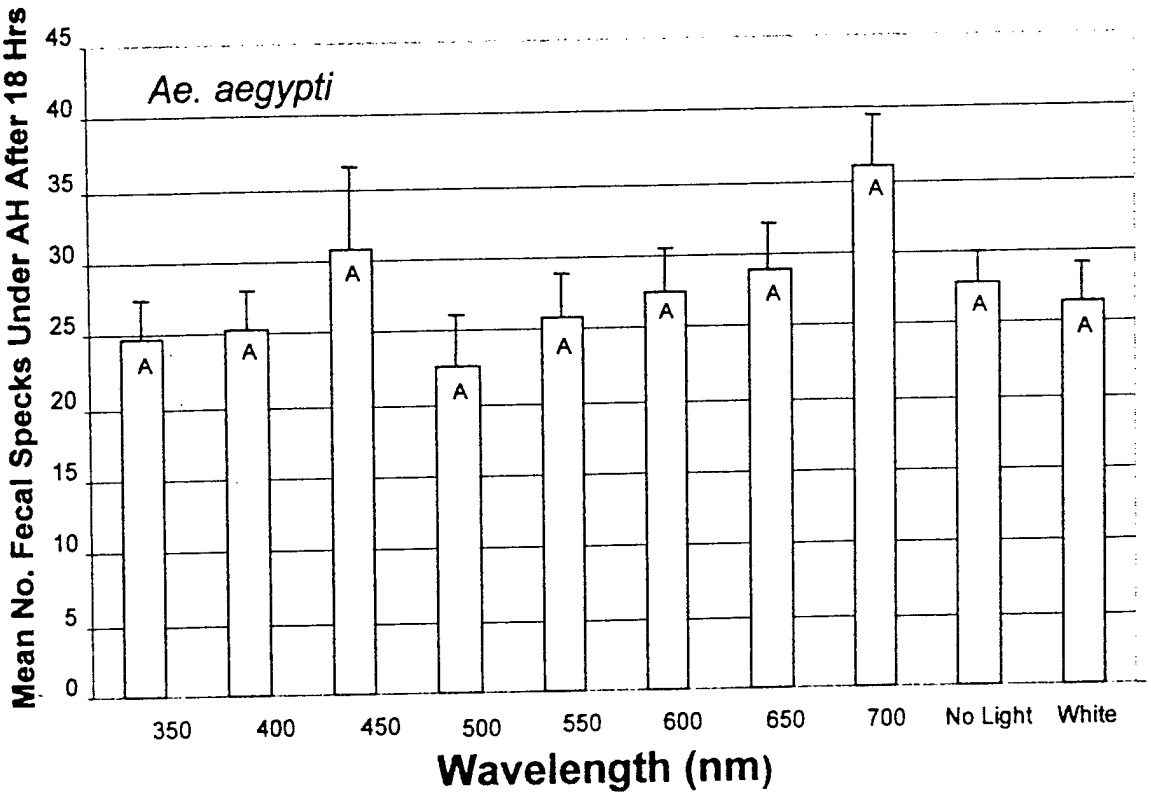


Figure 6-6. Numbers of fecal specks (means ± SEM) after an 16 hour exposure in a 5 cm circle around each artificial host/wavelength combination for *Aedes aegypti* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

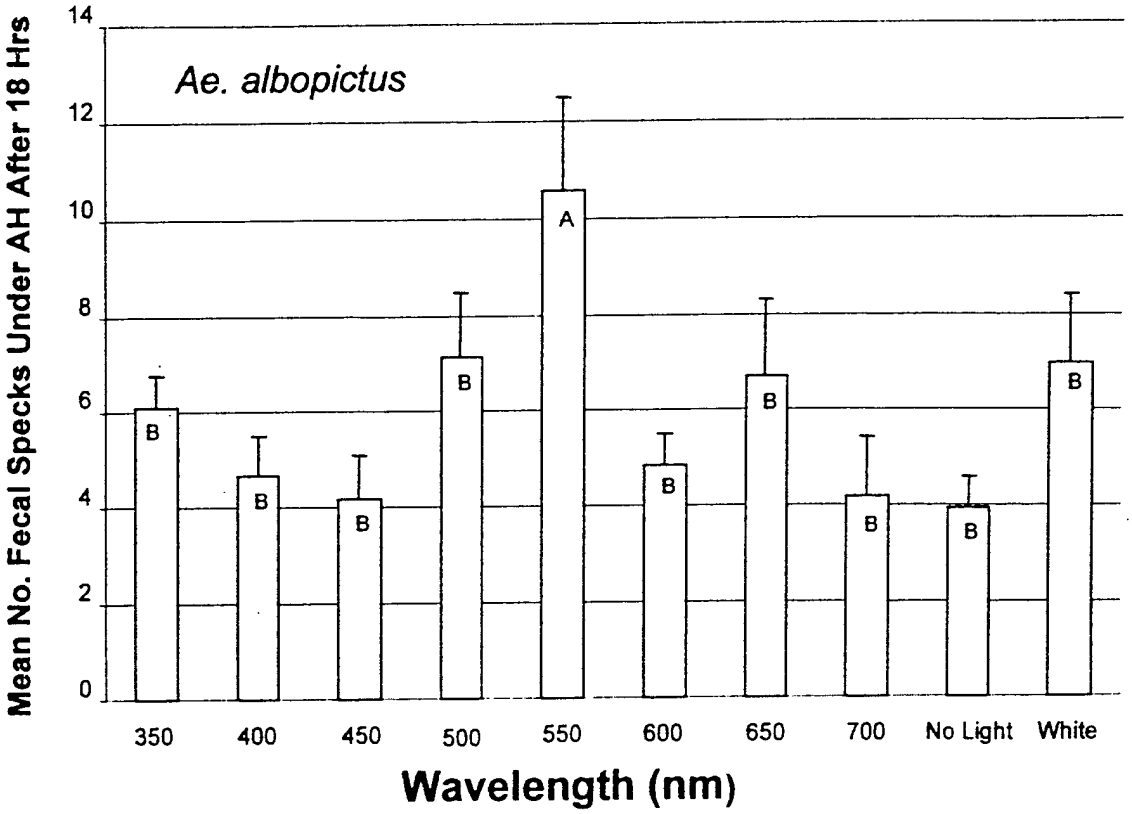


Figure 6-7. Numbers of fecal specks (means ± SEM) after an 16 hour exposure in a 5 cm circle around each artificial host/wavelength combination for *Aedes albopictus* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

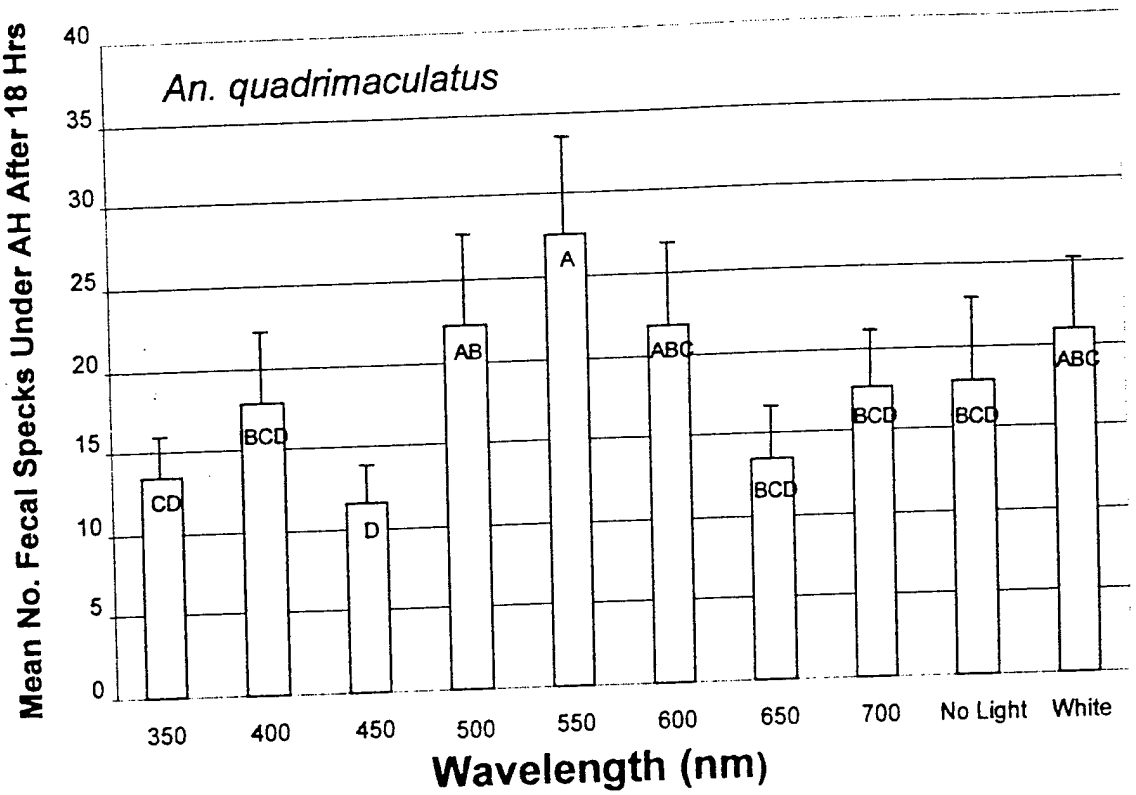


Figure 6-8. Numbers of fecal specks (means ± SEM) after an 16 hour exposure in a 5 cm circle around each artificial host/wavelength combination for *Anopheles quadrimaculatus* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

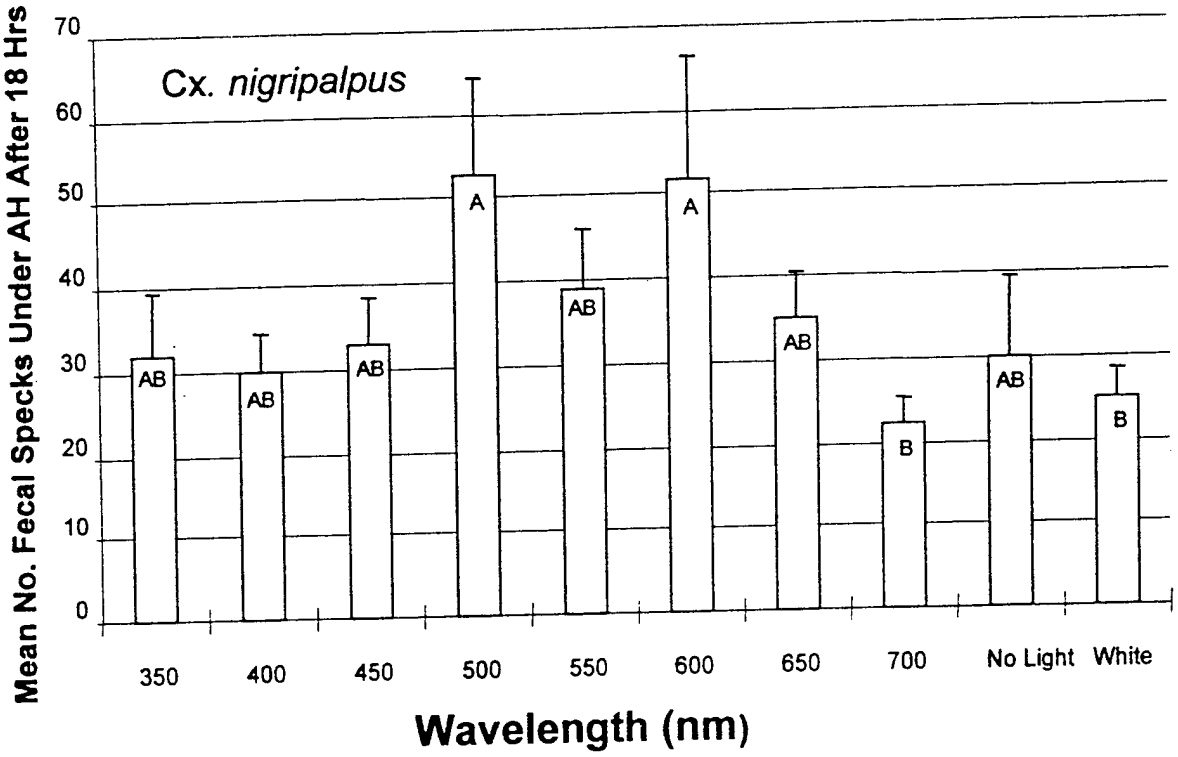


Figure 6-9. Numbers of fecal specks (means ± SEM) after an 16 hour exposure in a 5 cm circle around each artificial host/wavelength combination for *Culex nigripalpus* on artificial hosts illuminated with different wavelengths of light. Means within each species group with the same letter are not significantly different (alpha=0.10, Duncan's Multiple Range Test).

CHAPTER 7
MOSQUITO HOST/RESOURCE FINDING: THE IMPORTANCE OF SUGAR FEEDING AND ATTRACTION TO ARTIFICIAL LIGHT

Introduction

Waning public health budgets, drug resistant pathogens, pesticide resistant arthropods, exponential human population growth, deforestation and rapid global transportation are all contributors to the resurgence and maintenance of the world's most insidious arthropod-borne diseases. Employing an effective surveillance program is the primary means used by vector control specialists to anticipate, prevent, or control disease in human or domesticated animals. These surveillance programs however, are biased and limited in their ability to collect representative age classes. Nor do these programs provide an accurate species profile. A recent account of many of the commonly used mosquito sampling methods and traps is detailed by Service (1995). For most mosquitoes and many other medically importance insects, light traps, in one form or another, are the primary means of tracking vector populations. Many mosquito species do not respond well to light traps (e.g., *Aedes aegypti/albopictus, Culex pipiens* complex, and many anophelines). Furthermore, use of humans as bait for landing/biting collections is becoming increasingly unethical, and potentially dangerous due to the emergence of drug resistant malaria and other arthropod-borne pathogens.

Light traps collect only flying populations, and catches are influenced by light source, wavelength, intensity, trap placement, and many other intangibles (Moore and Gage 1996). Because of the selectiveness of sampling inherent to light trap collections, surveillance programs are continually in need of improved trap designs. The ideal trap would collect representative age distributions, species composition, and if desired, larger numbers. To enhance collection efficiency, light traps are usually supplemented with additional attractants such as carbon dioxide, lactic acid, octenol, acetone, heat, water vapor and others. In some instances, light is not used, only chemical attractants. Any improvement to mosquito trapping/attractant technology represents a positive contribution to vector ecology. Depending on the trapping/surveillance objective, finding the correct attractant combination can result in improved or even selective trapping. Potential trap improvements and guidance for additional attractants research are provided within this dissertation. These are (1) the use of super bright LED's as replacements for the standard incandescent bulb used in light traps and (2) determining the attractive chemical components contained within natural sugar sources. Utilizing both of these host/resource seeking parameters into future trap designs may allow sampling of additional age classes and/or species not otherwise normally captured in light traps.

A major requisite for survival and reproduction for mosquitoes and other haematophageous insects is their ability to find and exploit suitable hosts or other resources. Much has been written and research continues to progress on how mosquitoes are able to find these scattered resources. Sutcliffe (1987) for example, classifies host/resource finding into three elements: (1) Appetitive Flight: an internally driven flight

initiated by an unmet physiological need; (2) Activation and Orientation: acquisition of external olfactory or visual stimuli from the environment (e.g. host), and (3) Attraction: goal-oriented attraction flight to the objective. This three-step paradigm, redefined by Klowden (1996) as "ranging behavior," describes how host/resource seeking behavior can be applied toward light attraction and location of sugar sources. Bidlingmayer (1994) provides additional information on resource seeking females and concludes that long range stimuli used by resource seeking females on appetitive flights are directed toward selected visual targets. This flight continues until terminated by either the start of a long-range attractant flight or a short-range response to avoid the target. A long-range attraction flight is initiated and guided by various goal associated external cues, which may be visual, olfactory, or other, that indicate the existence and direction of the goal. The attraction flight is terminated either by making contact with the goal or by a short-range response rejecting the goal and a return to appetitive flight.

The purpose of this dissertation is to evaluate aspects of visual and sugar feeding ecology and to provide a basis for the incorporation of their attributes into future trapping systems. This is accomplished by evaluating sugar feeding and artificial light attraction as two important behavioral elements in mosquito host/resource seeking. A better understanding of resource seeking behavior will be a positive contribution to vector ecology and allow the development of improved surveillance/control equipment. This dissertation contains five chapters of original research that provide reasonable evidence that mosquitoes (at least for the species studied) are opportunistic, and that dietary sugars used for flight fuel and immediate metabolic needs are obtained from a wide diversity of

sources including homopteran honeydew. Many of these sugar sources have no detectable odor, but likely have similar volatile components warranting additional investigation. Furthermore, these dissertation results show a species-specific response (for common north central Florida woodland mosquitoes) to traps baited with different wavelengths of artificial light. In combination, these dissertation chapters are an important contribution to the science of mosquito/vector ecology. The results from both the sugar feeding and light attractancy chapters form the basis for future trap improvements used by vector control specialists and field ecologists. The wide diversity of sugar sources used by mosquitoes leads this author to conclude that these sources have common, but undiscovered, attractants.

Sugar Feeding

Blood feeding arthropods including mosquitoes consume blood meals which satisfy protein deficits needed to promote ovarian development (Clements 1992). With few exceptions, blood contains insufficient sugar to sustain immediate metabolic needs and must be obtained from other sources. An extensive review of mosquito sugar feeding is found in chapter 1; however, a few additional points not made elsewhere in this dissertation are included below. Laboratory survivorship data for sugar fed females for several of Florida's common mosquito species indicates species specific variations (Nayar and Sauerman 1975a). All species lived for about 24 hours post emergence without a sugar meal, but survival of mosquitoes fed once to repletion on 50% sugar solutions ranged from 144 (*An. quadrimaculatus*) to 336 hours (*Ae. taeniorhynchus*). In general,

smaller mosquito species were more efficient than larger species at utilizing their sugar reserves. In a research project not included elsewhere in this dissertation, Burkett (1998, unpublished) found that survivorship length did not differ significantly for *Cx. nigripalpus* fed once to repletion on 10% solutions of glucose, sucrose, or melezitose. Assuming wild mosquitoes approach anything close to the 6-14 days of survival following a full sugar meal, it seems reasonable that wild mosquitoes can go at least 3-4 days without sugar feeding. Sugar feeding frequency in wild populations has likely evolved to meet the needs of specific species. Some species are known to feed at all stages of their gonotrophic cycle (Nasci and Edman 1984, Magnarelli 1978), but sugar feeding frequency data for wild populations species remains unavailable. Sugar feeding likely varies by species, and depends on the mosquito's physiological and environmental conditions.

Natural sugar availability may limit successful host; mate, oviposition, resting sites, or other crucial ecological factors needed to maintain even a minimum level of fitness for mosquitoes. As the ancestral stock of mosquitoes must have needed sugar for these same reasons, it is logical to conclude that mosquitoes evolved and finely tuned their ability to locate sugar resources long before they became blood feeders. As many mosquito species are autogenous, their elongate stylets may have initially evolved in response to sugar feeding. The widely scattered, often rare availability of hosts and other resources in the mosquito's environment necessitates periodic sugar feeding to provide flight energy and other immediate metabolic needs. Whether the host/resource seeking insect employs the "opportunistic/passive" or the "actively search" mode of host/resource

finding (O'Meara 1987), an external source of sugar for energy is required. Unlike other species, mosquitoes do not use fat reserves (triglycerides) for flight and immediate energy requirements (Nayar and Van Handel 1971, Van Handel 1984), but instead use dietary sugars and glycogen. Fat stores are used for long term survival. Adequate dietary sugar then, becomes a critical component of successful host finding.

Sugar and blood feeding are mutually exclusive events, yet often share similar activity periods. As with blood-feeding, sugar-feeding for anautogenous mosquitoes is subject to circadian events and associated with metabolic needs. According to Foster (1995), the behavioral context of feeding decisions is not fully understood but can be divided into two possible paradigms. In a fixed-search paradigm, a mosquito in a particular physiological state would be locked into a dominant behavioral mode at some point before the insect encounters food stimuli. Hence, its activity would be part of an appetitive sequence with a specific goal. Alternatively, in a conditional-response paradigm, the mosquito's decision to choose among foods (simultaneous or sequential encounters) would be governed by instantaneous internal state and stimulus strength. When confronted with a choice of sugar or blood, Hancock and Foster (1993) found female *Cx. nigripalpus* preferred sugar when energy reserves were low and blood when reserves were high. Likewise, Yee et al. (1992) found all sugar feeding ceased in the presence of a host, but resumed once the host-stimuli was removed. Blood-host attraction should dominate earlier in the activity window but then gradually give way to sugar attraction as persistence diminishes and reserves are consumed in continued flight or unsuccessful attempts to obtain blood. This later statement is supported by Xue and

Barnard 1997, who found sugar-starved *Ae. aegypti* were not as aggressive/persistent (lower avidity) then those with adequate sugar reserves. Although having lower avidity, Foster and Eischen (1987) found sugar-starved *Ae. aegypti* (but not for *An. quadrimaculatus*) resulted in a greater frequency of blood feeding over mosquitoes supplied with sufficient sugar.

Several papers have linked visual and sugar feeding ecology, as visual cues may be important in finding sugars for some mosquito species. Many authors have noted a propensity for crepuscular/nocturnal mosquitoes to feed from floral nectars of background contrasting pale-colored or white flowers (Sandholm and Price 1962, Grimstad and Defoliart 1974, Magnarelli 1977, 1978, 1979, 1983, and Gadawski and Smith 1992). At least for north central Florida, the generalization of feeding on pale-colored flowers does not appear to be true. At least from this author's field observations, pale-colored flowers were not an important source of sugar for mosquitoes endemic to the Gainesville area. Indeed, during observations made from sunset through midnight during July and August 1997, not a single mosquito (and few other insects) was present foraging on pale-colored composites (e.g., *Bidens pilosa*, *Erigeron quercifolius*, *Eupatorium capillifolium*, *Rudbeckia hirta,* or *Cnidoscolus stimulosus* (Euphorbiaceae)) which were abundant in one of the study areas. Mosquitoes were only observed feeding on the extrafloral nectaries of partridge pea (*Cassia occidentalis*) and on the sugary exudates (apparently produced only at night) from the developing fruits of bahaigrass (*Paspalum distichum*). Neither of these sugar sources had detectable odors; yet photographs taken by the author show these plants were visited by a wide range of insects including several

moths (especially noctuids and geometrids), flies, green lacewings, earwigs, ants and even German or Asian cockroaches. For *Culex nigripalpus*, decaying fruits from elderberry (*Sambucus canadensis*) were also found to be an important sugar source for males and females. Samples obtained near a primary mosquito breeding site in a grove of elderberry contained numerous males and females with distended bright red abdomens which contained elderberry juice as verified using GC. Photographs were also taken of *Aedes albopictus* feeding on honeydew from aphid infested corn and sorghum at the University of Florida's Santa Fe Beef Unit. These same mosquitoes were observed puncturing the corn leaves with their stylets and apparently imbibing liquid. Based on these field observations and the wide diversity/ratios of sugar species (see chapters 2 and 3) found in the crops of wild mosquitoes, one can safely conclude that mosquitoes are opportunistic feeders, and obtain sugar from a wide variety of sources. Since many of these natural sugar sources (e.g., extrafloral nectars, exudates, decaying fruit, etc.) are not dominated by strong "flowery" scents characteristic of some nectar sources, mosquitoes and the other visiting insects must cue in on olfactory stimuli which have yet to be determined. In any case, isolating the attractive sugar source components and incorporating them into traps would allow for sampling a different age structure and perhaps species composition then would otherwise be possible in a standard attractant baited or unbaited light trap.

In this dissertation, gas chromatography (GC) analysis was used to evaluate the composition of sugars found in the crops of five field collected mosquito species from a variety of habitats over the course of two seasons. The percentage of mosquitoes

containing crop sugars ranged from 10-11% in *An. quadrimaculatus* and *Ps. ferox* to 47.7% in *Cq. perturbans*. Crop sugar occurrence was significantly greater in females than males in *An. quadrimaculatus* (p=0.0006) and *Cs. melanura* (p=0.00004). These findings agree with those of other Florida mosquitoes. Bidlingmayer and Hem (1973) for example, found similar sugar positivity results using the cold anthrone test for *An. quadrimaculatus* (♀=15%); *Cs. melanura* (♀=36%, ♂=29%); *Ps. ferox* (♀=20%) and *Cx. nigripalpus* (♀=17%, ♂=17%). Moreover, Magnarellli (1978) obtained similar results for *Cq. perturbans* (♀=57%). The results in this dissertation differ only in that more female *An. quadrimaculatus* contained sugars than males. The diverse sugar composition, proportion, and occurrence found in the crops of all species tested strongly suggests that these mosquitoes are opportunistic and that sugar is not a limiting resource.

All species tested contained melezitose and/or erlose (*An. quadrimaculatus* (55%), *Cs. melanura* (33%), *Cx. nigripalpus* (15%), *Cq. perturbans* (10%), and *Ps. ferox* (7%)). The data shows honeydew to be an extremely important resource for both *Cs. melanura* and *An. quadrimaculatus* comprising 1/3 and 1/2 of the sugar meals respectively. These observations make sense, because unlike *Cq. perturbans* (Grimstad and DeFoliart 1974, 1975), *Ps. ferox* (Magnarelli 1980) and *Cx. nigripalpus* (see Nayar 1982), which have been observed feeding on a variety of natural sugar sources, *An. quadrimaculatus* and *Cs. melanura* have not been reported to feed on floral/extrafloral nectars.

Percival (1961) and Van Handel et al. (1972) characterized nectar sugars and ratios for many plant species. We initially hoped that GC analysis could be used for

determining which plant(s) or even family of plants that mosquitoes use for sugar meals based on direct comparisons with published reports. Unfortunately, unlike melezitose and other trisaccharides, sucrose is rapidly hydrolyzed into fructose and glucose in insect crops (Schaefer and Miura 1972, Burkett et al. 1998) which negates any possibility of determining the source plant of the sugar meal. One interesting observation, however, is that some crop samples from *An. quadrimaculatus*, *Cs. melanura*, and *Cx. nigripalpus* contained relatively large amounts of sucrose in their crops showing that either some sugar sources produce enzyme inhibiting compounds, or salivary sucrase is not always produced and shunted to the crop with sugar meals.

When compared to the traditional use of the cold anthrone test, GC has proved to be an excellent and powerful tool for qualifying and quantifying the important ecological dietary parameters such as sugar source, preference, occurrence and composition of sugar feeding flies.

Attraction to Artificial Light

Many authors have shown that certain mosquitoes are attracted to transmitted light (Headlee 1937, Weiss 1943, Williams et al.1955, Breyev 1963, Bargren and Nibley 1956, Gjullin et al. 1973, Wilton and Fay 1972, and Browne and Bennett 1981). In conjunction with olfaction, mosquito vision plays a critical role for mosquitoes in locating mates, hosts, sugar sources, and oviposition or resting sites. Chapter 1 reviews light attractancy and the importance of vision in the ecology of mosquitoes and other medically important flies. Bidlingmayer (1994) reviews the visual responses of female

mosquitoes during appetitive and attractive flights. His treatise doesn't address attraction to lights per se, but his ideas can be applied to attractive flights toward artificial lights. Long range orientation behavior toward artificial light is likely stimulated by the extreme contrast of an artificial light against the dark background of the mosquito's environment. Upon approaching the light source, short-range orientation behavior takes over and the mosquito is either sucked into the trap when it gets too close, or else repelled.

Mosquitoes' attraction to artificial light has long been known and exploited in a variety of trap designs (Service 1995); however, the relationship between light attraction and host/resource seeking is somewhat ambiguous. As the name implies, "light traps" use light as the primary attractant for baiting insects toward a sampling device. Even though light traps have been used since the 1950's, surprisingly little work has been done to evaluate the importance the individual wavelengths comprising broad spectrum "white" bulbs typically used in light baited traps. Light, often supplemented (or not used at all) with other attractants, is an extremely important surveillance tool used by worldwide vector control agencies. Considering the importance of light traps, it remains somewhat perplexing that more work hasn't been published to establish the behavioral effects toward different wavelengths. Amazingly few studies have evaluated mosquitos' response to different wavelengths of transmitted monochromatic light, and even fewer have detailed the response of individual species.

Many mosquito control programs throughout the world, and especially in the US, use light trap capture numbers to dictate control programs. The number of mosquitoes per trap used as a "spray threshold" varies depending on geographical location, species of

interest, trap type/ set-up, and the experience of the control personnel. At a recent short course composed of mosquito personnel from around the state of Florida, I posed the question about which light traps were being used and the numbers they used as a "spray threshold." Few of the programs around the state used the same traps, procedures or threshold numbers. Similar experiences were had while reviewing various Air Force pest management programs. Often, little thought is given to the quality or consistency of "light" used in light traps and comparison with other programs is apparently unimportant. For some programs, any bulb found around the shop sufficed.

According to Allan et al. (1987), crepuscular and nocturnal biting flies are unlikely to have well-developed color vision, but their abilities to detect differences in intensity contrast are likely to be well developed. Spectral sensitivity (i.e., relative sensitivity of the retina to light of different wavelengths) studies consistently showed most flies possess a bimodal spectral response with peaks around 340 and 525 nm (White 1985). Considering the variation in mosquito species attractiveness to light traps (Huffaker and Back 1943, Bidlingmayer 1967), it is reasonable to expect differences in color preference based on variations in their spectral sensitivities. Alternatively, attractiveness may not be due to color per se. Different wavelengths may be physiologically perceived as more intense and subsequently more attractive. Barr et al. (1963) concluded that more intense light (up to a point) is more attractive than less intense light.

For those species attracted to light, diodes emitting blue (450 ± 50 nm) and green (567 ± 50 nm) were most effective in collecting wild mosquitoes. *Uranotaenia*

sapphirina, *Anopheles crucians*, and *Psorophora columbiae*, were significantly attracted to the blue over the other colors (excluding the incandescent bulb). Likewise, *Culiseta melanura*, and *Culex nigripalpus*, were captured in the greatest numbers at green and orange, and green respectively. Spectral sensitivity studies using the laboratory rat of medical entomology, *Ae. aegypti* (Muir et al. 1992, Snow 1971), and other Diptera (Allan et al.1991, Smith 1986, and White 1985) show bimodal spectral sensitivities peaking around blue (350 nm) and green (550 nm) regions of the electromagnetic spectrum. Unfortunately, all mosquito spectral sensitivity studies have focused on *Ae. aegypti*, and none have been conducted on nocturnal or other species actually commonly attracted to artificial light. It remains unclear as to whether mosquitoes attracted to the traps are responding to "color," or if those colors possess a greater perceived intensity and hence attractive from a greater distance. Unlike standard incandescent bulbs which radiate light in a 360° pattern, LEDs emit narrow beams (8-32°). In chapter 5, trap numbers based on LED orientation were compared using LEDs oriented up and reflecting off the shiny aluminum pan covering the CDC trap with those oriented out in a 360° pattern. Because of the narrow, but bright beams of light produced by the LED's, those oriented "out" would be visible from a farther distance than those oriented "up." As such, one would expect trap capture numbers to be significantly greater in the traps having the LED "out" orientation. Surprisingly, of the 11 common species collected, only *An. crucians* and *Ur. sapphirina* were significantly attracted to blue LED's oriented "out." over the other colors and orientations. Future studies and trap designs should focus on combinations of blues and greens oriented in different directions. Hopefully, future technology will

produce "super-bright" LEDs or other high efficiency lights capable of producing wavelengths between 450 and 550 nm, as these may produce excellent results.

Summary

Incorporating the practical elements of visual and sugar feeding ecology into future trapping systems is the primary goal of this dissertation. This is accomplished by examining the behavioral response of mosquitoes to different wavelengths of light and by evaluating sugar meal composition of wild mosquito populations and laboratory colonies. Both light attraction and sugar feeding are important components of host/resource seeking and deserve to be included in one dissertation. Ecologically, different wavelengths of light (mostly blues and greens) were found to be most attractive to those species attracted to light. Attraction to these wavelengths corresponds to spectral sensitivity peaks found in most dipteran electroretinograph (ERG) studies. Mosquitoes and other insects attracted to light probably evolved photo receptors sensitive to these colors (ca. 550 and 350 nm) because they live in an environment dominated by greens and blues (Lythgoe 1979). Insects must be able to discriminate among similarly colored objects if they are to find the hosts and sugars they require for survival. In terms of practical use, LED's are a viable option to use as an attractive light source. Their use reduces many of the logistical problems associated with typical surveillance programs. Blue and/or green LED's collect nearly as many mosquitoes (both in numbers and species composition), are more attractive to some species, collect less non target insects, are highly efficient providing up to two times the battery use length when compared to a

standard C-47 incandescent bulb. As for the ecological nature of sugar feeding, mosquitoes were found to contain a diversity of sugars and feed on a wide variety of sources and appeared to be opportunistic. All species were found to feed on honeydew and over 50% of *An. quadrimaculatus* containing crop sugars fed on honeydew. From a practical stand point (at least for the species studies), sugars did not seem to be a limiting resource from which control practices could be developed. Yuval and Warburg (1989) for example controlled phlebotomine sand flies by spraying host plants and animal burrows with *Bacillus thuringiensis* var. *israelensis* mixed with a sugar solution. Mosquitoes must find sugar sources in the wild even though they are diverse and widely dispersed. Of the primary sugar sources discovered during the course of these investigations, none had an odor detectable by humans. The olfactory stimuli used by mosquitoes and other insects to locate sugar sources are unknown but likely have common chemical components yet to be identified. Future research should focus on isolating these compounds and incorporating them into traps. This would allow vector control personnel and vector ecologists to sample additional species (perhaps even autogenous ones) and age classes not previous available.

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BIOGRAPHICAL SKETCH

Douglas A. Burkett was born on January 25, 1964 in Libertyville, Illinois. He was the third child of Robert and Betty Burkett. Summer and part-time jobs cultivated his interest in entomology where he held positions working for a mosquito abatement district, the Chicago Botanical Gardens and served as an assistant cooperative extension agent for Cook County Illinois. Two years were spent at College of Lake County after which he transferred and completed a duel Bachelor of Science degree in Entomology and Pest Management from Iowa State University in 1987. In June 1988, he married his long-time girl friend, Laura L. Koeppen, also of Libertyville, IL. He then finished his Masters of Science in Medical and Veterinary Entomology at Kansas State University and was commissioned a First Lieutenant in the U.S. Air Force's Biomedical Sciences Corps in 1990. In his first assignment, he served as active duty advisor/medical entomologist for the Air Force's C-130 aerial spray squadron. He also supervised the DoD Aerial Application Certification Course and managed the research and development of new application techniques including biological insecticides, oil dispersants, and decontamination agents. He has served as a pest management consultant for the Air Force Reserve and taught contingency entomology to the Air National Guard. In July 1996 he was selected by the Air Force to return to school and complete his Ph.D. Upon

completion at University of Florida, he will transfer to Okinawa, Japan and serve as a pest management professional and medical entomologist for the Department of Defense in South East Asia. He and his wife Laura have two daughters, Alexis and Sara.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Assistant Professor of Entomology and Nematology

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Assistant Scientist of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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