# INFLUENCE OF FLIGHT ACTIVITY AND OCTOPAMINE ON HEMOLYMPH TREHALOSE TITERS IN *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae)

by

Deborah Ann Davidson

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Entomology

APPROVED:

J.L. Eaton, Chairman

R.D. Fell

D.E. Mullins

E.C. Turner, Jr.

D.M. Orcutt

April, 1990
Blacksburg, Virginia

# INFLUENCE OF FLIGHT ACTIVITY AND OCTOPAMINE ON HEMOLYMPH TREHALOSE TITERS IN *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae)

by

Deborah Ann Davidson

Committee Chairman: John L. Eaton

Entomology

(ABSTRACT)

Hemolymph trehalose concentrations of male and female *Heliothis zea* were quantitated by high performance thin-layer chromatography (HPTLC) at various ages and times of the day and related to flight activity. Effects of octopamine injection or stress on trehalose levels were also quantified.

Flight activity was measured with a 32-channel computerized actograph that simulated sunrise at 0300 EST (Eastern Standard Time) and sunset at 1700. Males exhibited greater flight activity than females at all ages examined. Flight began near sunset, continuing through the night and ending around sunrise. Females flew continuously throughout the night, whereas males exhibited two peaks in activity: the first between 1700 and 1900, followed by a second peak between 2000 and 2400. Flight activity peaked on days 3-4 in males and days 4-5 in females.

Trehalose was the predominant hemolymph sugar, comprising 82-100% of total hemolymph sugars. Glucose was the second most frequently observed sugar. Trehalose concentrations were variable, ranging from < 1 ug/ul to 37

ug/ul. Lab-reared moths had higher and more variable trehalose concentrations than field collected (wild) moths. Trehalose levels were relatively constant several days after emergence in both sexes but decreased significantly by day 6 in males. When examined over a 24 hour period, trehalose concentrations gradually increased throughout the day in day 4 males and females, peaking one hour before sunset at approximately 18 ug/ul. This peak is hypothesized to be related to flight preparation. Females showed a second peak in trehalose levels at 1800, but males' trehalose levels continued to decline until 1900. Males and females exhibited more similar patterns in trehalose concentrations when sampled every 15 minutes over the sunset period (1500-1900) than when sampled at hour intervals.

Injections of octopamine, reputed to induce hyperglycemia in other insects, failed to elicit significant increases in trehalose levels in either fed or starved moths. Starved moths had lower trehalose concentrations than fed moths. Method of analysis (HPLC, HPTLC and anthrone) produced no differences in measured levels of trehalose or glucose. Various forms of stress (handling, shaking), also reported to induce hyperglycemia in other insects, similarly did not significantly increase trehalose titers in moths.

ACKNOWLEDGEMENTS

I am indebted to my advisor, Dr. John L. Eaton, for his support and good humor throughout the pursuit of this degree. My entire committee, Drs. John Eaton, Richard Fell, Donald Mullins, Craig Turner and David Orcutt, was invaluable to me in providing their patience, advice (at committee meetings or otherwise) and assistance (whether in the lab or in the cold room!) and I am truly grateful. Dr. James Palmer provided his HPLC and assistance for which I thank him. Thanks also to Holly Ferguson for maintenance of the moth colony and assistance with the recalcitrant actograph, and Keith Tignor for advice and assistance ranging from the mechanical to the cerebral. Of the many VPI & SU graduate students who provided friendship and encouragement, I will always be especially grateful to Karen M. Vail and Colleen A. Cannon.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Section 1

# INTRODUCTION

The flight of insects consists of two types of movement: migratory and non-migratory. Non-migratory movements tend to be short flights (Provost 1952; Kennedy 1961; Southwood 1962) and involve within habitat movements associated with activities such as feeding, mating and oviposition. Migratory movements, on the other hand, entail movement away from the habitat enabling colonization of new habitats, recolonization of old habitats, hibernation or aestivation (Johnson 1969; Southwood 1962; Dingle 1972). From the preceding observations, it is apparent that flight activity occupies a prominent place in the lives of many insects.

The energy for flight may be derived from carbohydrates, lipids, amino acids or some combination thereof. Carbohydrates may be used during either short or long flights. During prolonged flights, carbohydrates have been shown to be used initially, with a subsequent switch to lipid metabolism for the rest of flight (Beenakkers et al. 1985). Insects that use carbohydrates as a source of energy for flight use them in the form of glucose. For many insects this occurs by the breakdown of glycogen in the fat body with the subsequent synthesis of the disaccharide trehalose. Trehalose is released from the fat body and transported to the flight muscles via the hemolymph (Friedman 1985). The neurohormone octopamine has been implicated in causing increased fat body metabolism (Downer 1979b). Other evidence suggests that octopamine may be

released from the nervous system at flight initiation increasing in the hemolymph to concentrations known to affect flight muscle metabolism *in vitro* (Goosey & Candy 1980).

In addition to regulation of metabolic functions, octopamine has been suggested to influence arousal in insects (Orchard 1982). Arousal is an increased state of awareness or excitability to stimuli that occurs before the onset of activity. In nocturnal insects, the onset of activity is triggered by decreasing light intensities occurring during twilight (Dreisig 1980). These changes are probably perceived by the compound eyes or the ocelli. Dreisig (1980) has hypothesized that a preparatory physiological process precedes the onset of activity. Octopamine has been suggested to regulate the arousal process in insects and other invertebrates (Claassen & Kammer 1986; Arnesen & Olivo 1988; Kravitz et al. 1984). If this is true, an increase in octopamine may thus result in a simultaneous increase in both arousal and metabolism, indicating multiple roles for octopamine in the control of insect locomotor activity.

*Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) is a nocturnal moth known to utilize lipids for flight (Van Handel 1974; Judge 1988); however, the role of hemolymph sugars in flight activity is unknown. There is a lack of adequate information linking the physiological relationships of photoreceptors, neurohormonal systems and locomotor related metabolism. Further, a lack of knowledge of the fundamental biology of this major agricultural pest limits the development of new control strategies.

The hypothesis of this investigation was that a relationship exists between photoreceptor input and the mobilization of carbohydrates in adult *Heliothis zea*. Specific objectives directed at elucidating this hypothesis were to:

1) determine the basal levels and the individual variability in titers of hemolymph trehalose;

2) measure daily variations in hemolymph trehalose titers and examine their association with flight activity;

3) characterize on a fine time scale specific daily variations identified in objective 2;

4) examine the effects of octopamine and related agents on sugar titers in the hemolymph.

Section 2

# LITERATURE REVIEW

## 2.1 Life History & Pest Status of *Heliothis zea*

*Heliothis zea* (Boddie) (Lepidoptera: Noctuidae), variously known as the corn earworm, cotton bollworm and tomato fruitworm, ranges between 45°N and 45°S in both the Old and New Worlds. At 25°C, development from egg to adult requires about 30 days. Eggs hatch after two to three days, with caterpillars passing through five instars over a two week period. Larvae then burrow into the soil where pupation occurs. Adults emerge in approximately two weeks, with females emerging two or three days before males. Mating and oviposition begins two to three days after eclosion. Moths are largely nocturnal, although some crepuscular activity has been observed (Callahan 1958; Agee 1969; Adler 1987). Short flights are typical on the first night after eclosion. Moths may feed before settling, usually within the same habitat (Lingren et al. 1988). At later ages, moths engaged in local movement typically takeoff at dusk for one to two hours during which time they feed, move and oviposit. This is followed by a quiet time until about midnight when mating activity begins and continues until 3:00 or 4:00 AM. Females release pheromone from the tops of plants, while males make high-speed directed flights in search of pheromone plumes (Raina et al. 1986; Callahan 1958; Fitt 1989).

*H. zea* is one of three *Heliothis* species to achieve major pest status. The economic importance of *H. zea* is indisputable. Over one billion dollars in damages to crops may result from *H. zea* and *H. virescens* in the U.S. annually (King & Coleman 1989). Four characteristics contribute to the pest status of *Heliothis* species: 1) polyphagy, 2) high mobility, 3) high fecundity and 4) a facultative diapause. These characteristics enable the moths' survival in unstable habitats and allow the colonization and exploitation of agricultural systems (Fitt 1989). Each of these characteristics is discussed below for *Heliothis* species in general, with specific examples given for *H. zea* when possible.

Polyphagy, the ability to feed on a wide variety of foods, is the first attribute contributing to the success *Heliothis* species as pests. *H. zea* feeds on a variety of crops including corn, cotton, sorghum, tomatoes and lettuce. Corn and sorghum are preferred over other crops (Fitt 1989). Selection of a host depends on the temporal and spatial availability of hosts at the preferred stage of development. Eggs are laid singly on or near growing points or buds with individual females ovipositing on many plants (Farrer & Bradley 1985; Fitt 1987). Preference is shown for the flowering stage of the host (Johnson et al. 1975; Quaintance & Brues 1905; Schneider et al. 1986).

Polyphagy increases the potential for population persistence and increase. Populations may develop simultaneously on several hosts or continuously on a succession of hosts. In apparently unsuitable areas, low density populations are possible because females are likely to locate a suitable host (Fitt 1989).

The second key element in the success of *Heliothis* species is their capability of undertaking within and between habitat flights. Three types of movement are recognized in *Heliothis* species: 1) short range, 2) long range and 3) migratory. Short range movement involves feeding, mating, oviposition and sheltering and ranges from 100 to 1000 m. Long range movement ranges from 1 to 10 km, involving movements between crops, between feeding and oviposition sites and between emergence and local oviposition sites. Long range movement overlaps migratory movement, which takes place above the boundary layer, continuing for several hours. Weather systems are typically used and moths may be displaced hundreds of kilometers. Unlike some noctuids, *Heliothis* species are facultative migrants. *H. zea* appears to migrate in response to a shortage of breeding sites rather than poor larval nutrition (Fitt 1989).

Until recently, most evidence for *Heliothis* migration has been circumstantial. With the use of various types of traps, *H. zea* and other species have now been collected flying above the boundary layer (Farrow & Daly 1987). Hendrix et al. (1987) have identified pollen from geographically restricted plants not located in the trapping region on trapped *H. zea* males. Additional evidence includes asynchrony between emergence from diapause and peak captures of males in the Southern U.S., hence suggesting immigration (Hartstack et al. 1982; Lopez et al. 1984; Rummel et al. 1986; Stadelbacher & Pfummer 1972).

The third characteristic, a facultative diapause as pupae, allows moths to pass winters in subtropical and temperate parts of their range and contributes to their pest status by maintaining local populations at times when hosts are not available or conditions are not suited to reproduction and population survival

(Fitt 1989; Eger et al. 1982). Diapause is induced by decreasing temperatures and daylength during late larval and prepupal development (Cullen & Browning 1978; Roach & Adkisson 1970). Overwintering pupae reach a uniform physiological state before morphogenesis resumes so that the time of emergence is not related to the time of pupation (Lopez et al. 1984). Emergence from diapause occurs over a three to six week period, with females emerging earlier than males (Fitt 1989).

The last major factor contributing to *Heliothis*' pest status is a high fecundity and a short generation time, resulting in a high capacity for population increase. The pre-reproductive period varies from two to five days, depending on temperature (Ellington & El-Sokkari 1986). For *H. zea*, a reproductive lifetime of eight to ten days has been reported, with individual females laying 1000-1500 eggs. In some females up to 3000 eggs have been recorded (Fye & McAda 1972). Fecundity has been found to be influenced by temperature, humidity, larval and adult nutrition and has been tenuously linked to body size (Fye & McAda 1972; Isley 1935; Willers et al. 1987). Unfortunately, there are no estimates of realized fecundity in the field for any species.

## 2.2 Flight

Insects are unique among invertebrates in their flight ability, currently sharing the air with only birds and bats. Unlike the wings of the latter, however, insect wings are not modified legs but rather result as outgrowths of integument on the dorsal thorax. Through evolution, many structural and physiological adaptations have occurred resulting in an efficient and effective mode of

locomotion. Wings are moved by muscle contraction in all insects, although the wings and musculature differ among insect orders. The complex of processes which provides the requisite energy for this locomotion is considered below.

## 2.3 Fuels for Flight

The flight of insects is an extremely energy demanding process involving 50-100 fold increases in metabolic rate beyond resting levels (Beenakkers et al. 1984, 1985). The energy to sustain flight derives from chemical reactions occurring within the flight muscles. Insect thoracic muscles contain little available ATP. Only 7 umoles/g wet weight of ATP (Sacktor & Hurlbut 1966) are present in the flight muscles of the blowfly, *Phormia regina*, sustaining flight a mere tenth of a second (Crabtree & Newsholme 1975). The initial fuel for flight is provided by intramuscular carbohydrates, i.e. glycogen and sugars (Kammer & Heinrich 1978), which are usually exhausted after several minutes; therefore, maintenance of a high metabolic rate is dependent on mobilization of fuels from the fat body, gut or both, and their transportation via the hemolymph to the flight muscles (Gilbert 1967; Wyatt 1967; Bailey 1975). High concentrations of fuels in the hemolymph insure unimpeded metabolism (Crabtree & Newsholme 1975), as is clearly demonstrated in the blowfly, where, as sugar concentrations drop, wingbeat frequency is reduced (Clegg & Evans 1961a, b).

Substrates fueling flight in insects typically include carbohydrates, lipids and amino acids (Beenakkers et al. 1984, 1985; Ziegler 1985). Recent reviews on the metabolism of these substrates are given by Friedman (1985), Downer (1985) and Chen (1985). General flight metabolism has been covered by

Beenakkers et al. (1984, 1985) and Kammer & Heinrich (1978). Specific aspects have also been reviewed, including hormonal control and substrate transport (Steele 1976; Candy 1981; Chino 1981; Wheeler 1989), substrate utilization in flight muscles (Steele 1981; Candy 1989), flight and lipid metabolism (Beenakkers et al. 1981a), proline and insect flight (Bursell 1981), and oxygen consumption during flight (Casey 1989).

## 2.4 **Carbohydrates**

Insect carbohydrates fall into three general types: storage (glycogen and sugars), structural (chitin) and mixed function (glycoproteins) forms (Friedman 1985). It is the storage forms that are of interest here. Glycogen is the storage form of glucose ($C_6H_{12}O_6$) and is most often located in the fat body. It is from this site that glucose is provided to other tissues after transformation into trehalose ($C_{12}H_{22}O_{11}$), a sugar commonly found in insect hemolymph (Friedman 1985). Fat body concentrations of glycogen vary with the species, for example 117 umol/g wet weight in *Locusta migratoria* (Van Marrewijk et al. 1980) and 275 umol/g wet weight in *P. regina* (Clegg & Evans 1961b) have been reported. Glycogen is also stored in the flight muscles among insects using it as an immediate source of glucose for flight metabolism. Values of 91 umol/g wet weight have been recorded for *Periplaneta americana* (Downer & Parker 1979). By flying cockroaches to exhaustion, Downer & Parker (1979) determined that 91% of the total glycogen consumed during flight originated from the flight muscles. Other values found in flight muscle include 7 and 150 umol/g wet

weight from *Schistocerca gregaria* and *P. regina* respectively (Rowan & Newsholme 1979; Childress et al. 1970).

Sugars may be stored in both the fat body and, because of their solubility, in the hemolymph (Beenakkers et al. 1985). In contrast to the blood of vertebrates, insect hemolymph often contains high concentrations of sugars (0.5 to 8.1%) (Wyatt 1967). Trehalose, a non-reducing disaccharide composed of glucose, is the typical sugar found in the hemolymph; however, some insects lack it entirely or possess only small amounts (Bedford 1977). Reported trehalose concentrations range from 0 (non-feeding *Celerio euporibae* larvae) to 71 ug/ul (*Megoura viciae* adult). Glucose is usually present at lower concentrations than trehalose (0 to 20 ug/ul), also depending on the species and stage (Wyatt 1967). Among Lepidoptera, trehalose levels range from 0 (*C. euporibae* larvae) to 24 ug/ul (*Sphinx ligustri* pupae). Glucose ranges from 0 (*Hyalophora cecropia* larvae, *Samia cynthia* pupae) to a high of 20 ug/ul (*C. euphorbiae* pupae); however, most glucose levels are between 0.01 and 0.9 ug/ul (Wyatt 1967).

Numerous other sugars have been reported from insect hemolymph including arabinose, cellobiose, fructose, fucose, galactose, maltose, mannose, ribose and sucrose (Mullins 1985). These sugars are often a reflection of specialized diets (Wyatt 1967). It is not unusual for adult honey bee hemolymph to contain fructose in addition to trehalose and glucose. In fact, sugar content is known to vary with caste, season and diet. Other sugars detected in honey bee hemolymph include sucrose, maltose and fructomaltose (Maurizio 1965).

In addition to those mentioned above, factors affecting hemolymph sugar concentrations are time of day (some sugars show circadian rhythms - see 2.9),

level of activity, starvation, molting and parasitization. Trehalose levels decrease during both flight and starvation (Van der Horst et al. 1978; Saito 1963). Parasitized cabbage loopers exhibit higher concentrations of hemolymph trehalose as well as fat body glycogen (Thompson 1986).

Carbohydrates have long been associated with insect flight metabolism and are an important fuel for many insects. Among many Hymenoptera and Diptera they may be the only substrate for flight, while in many Lepidoptera and Orthoptera they may be used in conjunction with lipid (Beutler 1936; Beenakkers et al. 1985; Clegg & Evans 1961a, b; Nayar & Van Handel 1971b). Most insects investigated thus far use trehalose as their carbohydrate source (Friedman 1978); however, it is not the only sugar to be utilized. Nayar & Van Handel (1971b) demonstrated that, in mosquitoes, "Trehalose did not change during vigorous flight and made a negligible contribution to exhaustive flight." It appeared, rather, that glucose was of primary importance in mosquito flight.

The fact that trehalose appears to be the predominant sugar in insects with well developed flight abilities has led to the suggestion that trehalose may be an exclusive carbohydrate reserve for flight (Bedford 1977). Van der Horst et al. (1978) have demonstrated the utilization of trehalose during the flight of *L. migratoria*, where trehalose levels decline rapidly upon initiation of flight. After 30 minutes, a steady state is established, with trehalose replenished by breakdown of glycogen in the fat body at a rate equal to utilization. Conversely, trehalose exhibits a low turnover rate in the resting locust. Other insects in which a similar sequence of events appears to occur are *Manduca sexta* (Ziegler & Schulz 1986a, b; Jutsum & Goldsworthy 1976) and *Anticarsia gemmatalis*

(Teo et al. 1987). Specific aspects of mobilization and utilization of trehalose are detailed in 2.7.

## 2.5 **Other Fuels**

In 1938 Kozhantschikov reported that lipids were an energy source for flight in Lepidoptera. Subsequently, lipids have been identified as a fuel in many insects. Lipids are often used in combination with carbohydrates, particularly in insects involved in migratory flights. Lipid utilization is advantageous to migratory insects because lipids contain eight times the energy content of an equal weight of glycogen, as well as providing metabolic water (Beenakkers et al. 1985).

Lipids are stored in the fat body as triacylglycerides and released as 1,2-diacylglycerides (DG). Since lipids are relatively insoluble, they must be transported through the hemolymph by lipoprotein carriers. On arriving at the flight muscles, DG are hydrolyzed to fatty acids and glycerol (Beenakkers et al. 1967). The fatty acids are degraded to two carbon units, acetyl CoA, and enter the citric acid cycle, ultimately supplying the ATP necessary for flight (Beenakkers et al. 1981b). The glycerol appears to cycle back to the fat body where it is used as a source for DG formation (Van der Horst et al. 1983). Mobilization of lipids is regulated by adipokinetic hormone (AKH), produced and released from the corpus cardiacum. It stimulates the fat body to produce DG, thereby resulting in increased DG concentrations in the hemolymph (Goldsworthy & Mordue 1974; Beenakkers et al. 1978). The neurohormone

octopamine has also been suggested to cause an increase in DG in addition to stimulating the release of AKH.

Less common than the use of carbohydrate and/or lipid, is the use of amino acids as a substrate for flight, as exemplified by the tsetse fly, *Glossina morsitans* (Bursell 1963), the Colorado potato beetle (*Leptinotarsa decemlineata*) (Weeda et al. 1980) and the Japanese beetle (*Popillia japonica*) (Hansford & Johnson 1975). In these insects, proline and glutamate concentrations drop during flight. It appears that glutamate is used initially, and is replenished by the oxidation of proline. Proline may act as a primary energy source or may act as a "sparker" for the citric acid cycle. In the latter instance, intermediates of the citric acid cycle needed for the oxidation of acetyl-CoA are supplied by proline, even though the primary energy source may be carbohydrate or lipid (Beenakkers et al. 1985).

## 2.6 **Octopamine**

Octopamine ($C_8H_{11}NO_2$), a biogenic amine, is widespread in the insect nervous system, functioning in several sometimes overlapping roles: as a neurotransmitter, neurohormone and neuromodulator (Orchard 1982; Evans 1985). Structurally, it is similar to the catecholamine noradrenaline, which is commonly found in vertebrates. Invertebrates lack or possess little noradrenline. In insects, many of the roles of noradrenline and adrenline appear to be combined in the functions of octopamine.

A variety of responses have been attributed to octopamine in its role as a neurohormone. Many of these have been examined in relation to flight and

stress, which produce similar physiological effects. Metabolically, octopamine has been implicated in causing both hypertrehalosemia and hyperlipemia. These conditions may be brought about by the direct effect of octopamine on the fat body (Orchard et al. 1982; Downer 1979b) as well as by causing the release of hypertrehalosemic or hyperlipemic factors (Orchard & Loughton 1981; Downer et al. 1984). Downer (1979a) reported a 100% increase in hemolymph trehalose titers within 15 minutes of injection of octopamine into the hemocoel of *P. americana*. He also demonstrated that octopamine can increase glycogenolysis in isolated cockroach fat body via a c-AMP dependent pathway and suggested that this effect may be a source of increased hemolymph trehalose (Downer 1979b). Furthermore, injection of octopamine increased trehalase activity in hemolymph and muscle in *P. americana* (Jahagirdar et al. 1984).

Goosey & Candy (1980) demonstrated a rapid elevation of octopamine in the early flight stages of locusts while Orchard & Lange (1983) reported increased hemolymph lipid during the same time frame. Octopamine has also been shown to act directly on the fat body to release lipid (Orchard et al. 1982); hence, Orchard & Lange's (1983) suggestion that octopamine may be responsible for the above elevated lipid levels. Further, in a study on working perfused locust thoracic muscles, octopamine stimulated the oxidation of glucose, trehalose, butyrate and diacylglycerol, as well as increasing the size of muscle contractions (Candy 1978).

In adult male *P. americana*, incubation of corpora cardiaca (CC) with octopamine results in the release of a factor that causes increased hemolymph trehalose titers when injected into adult cockroaches (Downer et al. 1984). In a

similar fashion, locust CC incubated with octopamine results in the release of hyperlipemic hormone. This release can be blocked by phenoxybenzamine, an alpha-adrenergic blocker (Orchard & Loughton 1981).

American cockroaches that have been stressed in a variety of ways also exhibit increases in hemolymph sugar levels (Matthews & Downer 1973,1974; Matthews et al. 1975). Stress in cockroaches and locusts results in increased hemolymph octopamine levels, three-fold and ten-fold respectively (Davenport & Evans 1984). When looked at over a 24 hour period in cockroaches, the highest hemolymph octopamine concentration coincided with the peak in locomotor activity, while in the nervous tissue octopamine accumulated in the photophase and was reduced in the scotophase (Davenport & Evans 1984). More recently, Woodring et al. (1988) demonstrated a free running endogenous brain rhythm for octopamine in the house cricket, *Acheta domesticus*. Furthermore, the peak in hemolymph octopamine also occurred at night. Interestingly, both cockroaches and crickets are nocturnal insects. Observations such as these have led to the suggestion that octopamine may play a stimulatory role in preparing insects for activity, in a manner similar to adrenaline in vertebrates (see 2.9).

As mentioned previously, some effects of octopamine can be eliminated by the use of alpha-adrenergic antagonists such as phentolamine, gramine, cyproheptadine (Downer et al. 1984) and phenoxybenzamine (Orchard & Loughton 1981). Recently it has been shown that injection of American cockroaches with taurine ($C_2H_7NO_3S$), a naturally occurring hemolymph amino acid, results in a decrease in excitation-induced elevation of hemolymph octopamine titers (Hayakawa et al. 1987). Taurine's effects are long lasting

(15 hours) and it is suggested that taurine may be part of a dual control mechanism for excitation, with octopamine serving as a stimulant and "taurine serving as an inhibitory modulator of octopamine release" (Hayakawa et al. 1987).

## 2.7 Mobilization & Utilization of Trehalose

Mobilization of trehalose occurs in two ways: 1) by feedback of reduced trehalose titers on the enzymatic pathways for trehalose synthesis and 2) by hormonal factors. Maintenance of a high trehalose concentration in the hemolymph is a result of trehalose synthesis in the fat body. Trehalose is synthesized by the following two step reaction, the same pathway followed by yeast (Candy & Kilby 1961):

1) UDP-glucose + glucose-6-phosphate

Trehalose-6-phosphate + UDP and

2) Trehalose-6-phosphate + $H_2O$

Trehalose + $P_i$.

UDP-glucose and glucose-6-phosphate are also substrates for glycogen synthesis. In the locust high trehalose concentrations inhibit trehalose synthesis and favor glycogen synthesis. As trehalose concentrations decrease during flight, glycogen synthesis is inhibited and trehalose synthesis occurs at the expense of glycogen (Applebaum & Schlesinger 1973). These events occur because the activity of glycogen phosphorylase, the enzyme responsible for the breakdown of glycogen, is inhibited competitively by glucose-6-phosphate. Synthesis of trehalose reduces the amount of glucose-6-phosphate, thus

removing phosphorylase inhibition (Beenakkers et al. 1985). In addition to the effects of glucose-6-phosphate, phosphorylase activity is also affected by hormonal factors.

In 1961 Steele reported that, in the American cockroach, injection of CC extracts increased hemolymph trehalose levels. From this he deduced the presence of a hypertrehalosemic hormone (also known as hyperglycaemic hormone). Since that time a variety of other insects, *P. regina* (Friedman 1967), *Calliphora erythrocephala* (Normann & Duve 1969) and *L. migratoria* (Mordue & Goldsworthy 1969) to name a few, have been shown to possess hypertrehalosemic hormone. Presence of hypertrehalosemic hormone was determined by injecting CC extracts from the previously mentioned insects into *P. americana*. Interestingly, and perhaps perversely, injection of CC extract from the locust into the locust does not cause hypertrehalosemia (Beenakkers et al. 1978). This result may be explained by the fact that this species relies mainly on lipid for its energy. According to Van der Horst et al. (1978), there is a significantly higher trehalose turnover rate in the flying locust compared to the resting locust, thereby indicating enhanced release of trehalose from the fat body and thus providing a role for the hormone in flight. In the blowfly, the presence of hypertrehalosemic hormone appears crucial for the maintenance of a constant trehalose concentration during flight (Vejbjerg & Normann 1974).

Hypertrehalosemic hormone affects glycogen phosphorylase (Steele 1963; Weins & Gilbert 1967; Goldsworthy 1970; Ziegler 1979) in a cascade fashion through the second messenger c-AMP by converting phosphorylase from its inactive (b) to its active (a) form, resulting in the release of glucose-1-phosphate from glycogen. Recent studies have indicated a third form (ab) of

phosphorylase in the locust fat body (Van Marrewijk et al. 1988a, b). It is believed that phosphorylase ab provides an additional level in the regulation of glycogen breakdown.

Other hormones may cause increases in trehalose as well. Adipokinetic hormone (AKH) has been identified in CC extracts of *L. migratoria* and *S. gregaria*. In addition to causing an increase in hemolymph lipids (Beenakkers 1969; Mayer & Candy 1969), AKH has demonstrated hypertrehalosemic activity in American cockroaches (Jones et al. 1977). As indicated (2.6), octopamine appears to exert a similar effect.

Upon reaching the flight muscles, trehalose is hydrolyzed to glucose by the enzyme trehalase. The actual site of hydrolysis varies with the insect. Lepidoptera, Orthoptera and Dictyoptera have microsomal (membrane bound) trehalase. In these insects, trehalose is thought to be hydrolyzed at the cell surface, with the glucose then transported into the cell. Diptera and Hymenoptera trehalases appear to be associated with mitochondria, and thus trehalose may be hydrolyzed inside the cell (Candy 1989). Changes in intramuscular pH may be responsible for the activation of trehalase.

## 2.8 Fuels for Flight in Lepidoptera

For many years Lepidoptera were thought to utilize only lipids during flight (Kozhantschikov 1938; Domroese & Gilbert 1964), even if they fed on nectar (a carbohydrate source). It was believed that carbohydrate had to be converted to lipid for use. The idea that all Lepidoptera use only lipid in flight was eventually rejected after Stevenson (1968) demonstrated that homogenates of flight muscle

in *Prodenia eridania* could oxidize glucose, trehalose, glycogen and phosphorylated hexoses. Van Handel and Nayar (1972b) determined that the lipid metabolizing moth *Spodoptera frugiperda* could use dietary sugar directly. They rightly point out "As fatty acids and carbohydrates in flight muscle both provide acetyl-CoA, there is no biochemical reason why nectar-feeding Lepidoptera would use only the acetyl-CoA derived from fatty acids for flight energy". Lepidopterans which do not feed as adults (e.g. *Philosamia* and *Actias*) possess the enzymes for fatty acid oxidation while those that do feed (e.g. *Pieris* and *Agrotis*) have a muscular enzyme pattern similar to locusts (Beenakkers 1969). Migratory Lepidoptera use lipid as their main flight substrate (Brown & Chippendale 1974; Kammer & Heinrich 1978; Ziegler & Schulz 1986a; Van Handel 1974). As with the locust, *M. sexta* and *A. gemmatalis*, appear to use carbohydrates initially and then switch to lipid utilization (Jutsum & Goldsworthy 1976; Teo et al.1987).

In a study on separated lipid classes in the hemolymph of *H. zea*, Judge (1988) found that DG concentration was highest before flight, decreasing during flight in day 4 moths. Females' DG levels decreased from approximately 30 ug/ul before flight to between 23 and 15 ug/ul during flight. Males flew more than females and exhibited a similar DG pattern over a 24 hour period. The observed decrease of DG during flight is similar to results obtained from studies on *A. gemmatalis*, suggesting that lipid utilization is greater than lipid mobilization. These results differ from those of the migratory locust which appears to mobilize lipids at a higher rate than it utilizes them (Van Handel & !ayar 1972a). DG were the major lipid class detected, as they are in most

insects (Beenakkers et al. 1985). Triacylglycerides (TG) were present in relatively high concentrations, about 35% TG (w/w) versus 50% DG.

## 2.9 Locomotor Activity & Circadian Rhythms

The activity rhythms of insects, such as general locomotion, flight, feeding and oviposition, are usually restricted to specific times of the diel cycle. Most rhythms have been shown to be a combination of endogenous and exogenous components, with the rhythm of activity controlled by an endogenous oscillation which is directly modulated by the exogenous environmental cycles of temperature and light. Changes in light intensity are especially important in setting activity rhythms (Saunders 1982; Dreisig 1980). It is likely, indeed, most probable, that physiological rhythms preparatory to the onset of activity occur (Dreisig 1980) and that these are part of the phenomenon known as arousal.

Octopamine has been suggested to "elicit flight production by enhancing the efficacy of sensory transmission thereby increasing excitability or arousal" (Claassen & Kammer 1986), possibly in a manner similar to that of adrenalin in vertebrates, i.e. preparing for "fight or flight" (Orchard et al. 1982; Storey 1985). A similar role for octopamine in arousal has been implicated in the crayfish (Arnesen & Olivo 1988) and the lobster (Kravitz et al. 1984). As indicated in 2.6, octopamine exhibits several effects, including increased fat body metabolism and release of metabolically active neurohormones from the CC, leading Bailey et al. (1983) to suggest that rapid increases "in hemolymph octopamine may activate or potentiate a number of related physiological processes that facilitate

flight". These processes may include metabolic rhythms. Although many metabolic functions with demonstrated diurnal cycles ($O_2$ consumption, $CO_2$ release, trehalose and glycogen levels) often reflect activity cycles (Saunders 1982; Brady 1974), Nowosielski and Patton (1964) showed that trehalose titers in the house cricket appeared to be independent of feeding and locomotion.

Section 3

# DAILY & LIFETIME VARIATION IN HEMOLYMPH TREHALOSE IN *Heliothis zea* (Boddie) ADULTS MEASURED BY HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

Carbohydrate and lipid are important fuels for flight, particularly in many migratory insects (Beenakkers 1969; Nayar & Van Handel 1971a; Van Handel & Nayar 1972a, b; Brown & Chippendale 1974; Van der Horst et al. 1980). For example, *Locusta migratoria* utilizes carbohydrate heavily initially while prolonged flight results in a switch to lipid utilization. This usage pattern is reflected by a decrease in hemolymph trehalose concentrations to a steady state (Van der Horst et al. 1978, 1980). Migratory Lepidopterans may follow a similar pattern (Teo et al. 1987).

For flight initiation, the insect must be in an appropriate state of arousal. Dreisig (1980) has suggested that certain physiological processes must occur before insects are aroused. Among nocturnal insects, including *Heliothis zea*, these processes may be triggered by decreasing light intensities preceding the start of the activity period (Dreisig 1980). Elevation of trehalose titers prior to initiation of flight could be one such process. Trehalose levels are known to exhibit diurnal changes in several insects, possibly reflecting rhythms of locomotor activity (Saunders 1982).

The corn earworm is known to use lipids during flight (Van Handel 1974; Judge 1988), whereas the role of trehalose is unknown. The purpose of this investigation was to examine hemolymph trehalose titers at various times in the adult life to look for trehalose cycles related to locomotor activity. Basal daily and lifetime trehalose levels as well as variability between individuals were determined.

## 3.1 MATERIALS & METHODS

### 3.1.1 Experimental Animals

Moths used in these experiments were reared from eggs obtained from a laboratory colony established in summer 1986 at VPI & SU. The colony was periodically supplemented with moths from the USDA laboratory in Gainesville, FL. After surface sterilization (Henneberry & Kishaba 1966), eggs were placed in paraffin-coated cups (Sweetheart$^{R}$ 18 oz.) and allowed to hatch. Upon hatching, larvae were transferred to 30 ml plastic cups containing diet (Henneberry & Kishaba 1966) and left until pupation. Three days after pupation, pupae were weighed, sexed and placed in individual emergence cages. After eclosion, adults (except for day 1) were provided with 10% sucrose solution in a vial stoppered with a cellulose sponge. The sucrose was removed one day prior to sampling. Moths placed in the actograph were also provided with tap water. Larvae and adults were housed under a 14:10 (L:D) light regime in an environmental chamber maintained at 25$^{o}$C. Wild moths were collected as

larvae (instars 2-5) from corn, taken to the lab and placed on diet to complete development. Wild adults were handled similarly to lab adults.

### 3.1.2 **Measurement of Flight Activity**

Flight activity was measured with a 32 channel computerized actograph. Moths were housed individually in cylindrical paper cartons (13 cm D x 16.5 cm H) topped with screen (mesh 10). Each cage possessed two opposing windows 2.5 cm from the top through which an infrared (IR) light beam emitted from a photodiode on one side was detected by a phototransistor on the other. Each time the moth flew through the beam a flight count was recorded. Each of the 32 channels was monitored independently and data from each channel was written to a hard disk at determined intervals. Daily start and stop times, recording interval and experiment duration were designated by the experimenter on the computerized actograph (DEC MINC 11). Data from the computer hard-disk was subsequently transferred to the IBM 370 mainframe for analysis by the Statistical Analysis System (Eaton 1985; SAS 1985a, b). In contrast to the environmental chamber, the actograph simulated day, sunset, night and sunrise, with durations of 12, 2, 8 and 2 hours respectively. Simulated sunrise began at 0200 EST (Eastern Standard Time): sunset began at 1600 EST.

Experiments were conducted to determine the flight activity patterns of male and female moths by placing either newly emerged single male or female moths into individual actograph containers. Moths were of the same sex for each single experiment. The actograph was programmed to monitor activity from 1530 to 0700 at 0.25 hour intervals for six days.

### 3.1.3 **Sampling Procedure**

Moths were cold immobilized by refrigerating them at 5°C for at least 15 minutes before sampling. Handling of insects has been reported to cause hyperglycemia (Downer 1979a; Matthews & Downer 1973). It was thought that cooling the moths before sampling would minimize any hyperglycemic response that might occur. An incandescent lamp in the refrigerator prevented dark adaptation of the compound eyes during light phase sampling. Hemolymph was collected in a 1 ul microcap (Drummond$^R$) from a puncture made on the posterior mesothoracic scutellum. When less than 1 ul was obtained, the volume was estimated by measuring the length of the hemolymph column in the microcap and setting up a ratio (a microcap = 32 mm). The hemolymph was then added to a known volume of 70% ethanol (10-15 ul) and centrifuged (Micro-centrifuge, Fisher Scientific Model 59A) at 9700 RPM. Samples were dried under $N_2$ and stored at 0°C until assayed.

### 3.1.4 **Analytical Methods**

#### 3.1.4.1 **High Performance Thin-Layer Chromatography**

Sugars were separated and analyzed by quantitative High Performance Thin-Layer Chromatography (HPTLC) (Fell in press). Chemicals were obtained as indicated. Sigma Chemical Company: citrate buffer, glucose, trehalose dihydrate; Fisher Scientific: ethanol, acetonitrile (HPLC grade); Ricca Chemical Corporation: ceric sulfate. Plates (Merck$^R$ HPTLC silica gel 60) were washed in

methanol in a linear developing chamber (Camag$^R$ HPTLC) and dried, then sprayed with 0.1 M $NaHSO_3$, dried, then sprayed again with citrate buffer:dd$H_2O$ (1:10 dilution), dried and activated in an oven at 100$^o$C for one hour prior to use. Plates not immediately used were stored in a desiccator. Plates stored more than two weeks were reactivated before use.

Hemolymph samples were resuspended in an appropriate volume of 70% ETOH (between 10 and 20 ul). One ul of each of the diluted hemolymph samples and of mixed glucose-trehalose standards (0.25, 0.5, 1.0, 2.0, 2.5 ug/ul) was applied to the pre-activated HPTLC plates using a Camag$^R$ Nanomat 1 applicator and Drummond$^R$ microcaps. Plates were spotted on two opposing sides, with each side having its own series of standards. Samples were run in duplicate.

After the samples were applied and dried, the plate was developed with acetonitrile:$H_2O$ (85:15 v/v) three times (dried with a hair drier one minute on each side after each run). Plates were developed to the center each time in a linear developing chamber and dried thoroughly after the final run. The spots were detected by dipping the plate into a 0.1 N ceric sulfate solution (in approximately 2 N sulfuric acid) diluted 1:10 with 15% sulfuric acid, and then charring the plates in an oven at 110$^o$C for 12-15 minutes. Plates were scanned in the absorbancy mode at 440 nM with a slit length of 4 mm and slit width of 0.3 mm in a Camag TLC Scanner II$^R$ connected to a Spectra-Physics SP4270$^R$ integrator. Sensitivity and span ranged from 160-170 and 10-15 respectively. Integrator parameters were as follows: PW = 1, PT = 1000, TB = 1, attenuation = 64, chartspeed = 4. Standard curves were constructed from which sample trehalose concentrations (ug trehalose/ul hemolymph) were calculated using a

SuperCalc$^{3\ R}$ spreadsheet, which adjusted for the amount of hemolymph collected and the dilution of the sample. These spreadsheet data were uploaded to an IBM 370 mainframe for statistical analysis with SAS.

### 3.1.4.2 **Validation & Accuracy of the HPTLC Technique**

The validity of the HPTLC technique has been evaluated by Fell (in press) but further tests were performed to insure technique reliability with *Heliothis* samples. A pooled hemolymph sample from *H. zea* adults was evaluated by High Pressure Liquid Chromatography (HPLC) and the anthrone technique (Palmer 1979; Trevelyan & Harrison 1952). Approximately 60 ul of hemolymph were diluted into 120 ul ETOH (70%). After centrifugation, the supernatant was transferred to a microcentrifuge tube. The supernatant was used directly for HPLC injection. For the anthrone procedure two dilutions were made: 12 ul of supernatant were added to 3588 ul dd$H_2O$ (double distilled water) and 18 ul of supernatant were added to 2082 ul dd$H_2O$. One ml aliquots were then analyzed by the anthrone technique (described in 4.1.3.1). For HPTLC, 5 ul of supernatant were diluted with ETOH (70%) before spotting. The HPLC analysis was performed using a Waters HPLC with a refractive index detector using a propylamine column and an 80:20 acetonitrile:water carrier solvent. The column was run at 2500 psi with a flow rate of 1.2 ml/min (Palmer 1979).

The trehalose recovery efficiency of the HPTLC procedure was examined by spikeover analysis on *H. zea* hemolymph samples. Two ul of hemolymph were diluted into 25 ul 70% ETOH. After centrifugation, the sample was divided into two equal aliquots in microcentrifuge tubes and dried under $N_2$. One

sample was diluted in 10 ul 70% ETOH, the other in 10 ul 70% ETOH with 5 ug of trehalose. Both samples were analyzed as described except that samples were spotted on one side only of each plate (3.1.4.1).

### 3.1.4.3 **Limits of Detectability**

The limits of detectability were determined by spotting a series of mixed trehalose-sucrose-glucose-fructose standards (0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 1.0, 2.0, 2.5 ug/ul) on one side of the plate, and then analyzing in the usual fashion. After detection, the spots were scanned with the densitometer settings routinely used.

### 3.1.5 **Identification of Hemolymph Sugars**

Hemolymph was examined to determine the major sugar components. Hemolymph samples (varying from 16 to 34 ul) from day 4 male or female moths were pooled and extracted (3.1.3), dried under $N_2$, then resuspended in an amount of ETOH (70%) equal to that of the collected hemolymph. The samples were diluted 2:1 sequentially, with the final dilution 1/16 the concentration of the original. The samples were separated, detected and quantified as described (3.1.4.1) except that the standards included sucrose and fructose and again plates were spotted on one side only. When spots which did not match any of the standards were noted, plates were respotted and visualized with two other reagents, 1) N-(1-naphthyl)-ethylenediamine dihydrochloride - heated 10 minutes (Bounias 1980) and 2) aniline diphenylamine - heated 30 minutes

(Hansen 1975). Percentages of spots thought to be sugars were calculated. Since equal amounts of the various sugar standards char to approximately the same degree, amounts for spots which did not match any standards were estimated from glucose and trehalose standard curves and averaged.

### 3.1.6 **Experimental Protocols**

Basal hemolymph trehalose titers were determined by sampling at least 20 moths (10 males, 10 females) from the day of emergence (day 1) to 8 days after emergence (day 9). Previous experiments indicated extreme variability in trehalose titers among moths of various sex and age classes. To minimize effects due to time of sampling (Nowosielski & Patton 1964), two specific sampling intervals were chosen (between 0930-1030 and between 2130-2230). The sampling intervals were 12 hours apart and occurred during the middle of the day and night phases respectively.

Studies of the diurnal trehalose cycle were conducted by collecting hemolymph samples from at least 20 day 4 moths (10 males, 10 females) at specified times (0300, 0700, 1100, 1500, 1600, 1700, 1800, 1900 and 2300) over a 24 hour period with concentrated sample collection around the sunset period (1700).

The sunset period (1500-1900) was examined more closely by collecting hemolymph samples from at least 20 day 4 moths (10 males, 10 females) at 15 minute intervals beginning two hours before sunset and continuing until two hours after sunset.

### 3.1.7 **Data Analysis**

Data were analyzed by ANOVA and Students t test using the Statistical Analysis System (SAS 1985a,b). Tests were considered significant at $a < 0.10$.

## 3.2 RESULTS & DISCUSSION

### 3.2.1 **Flight Activity**

Flight activity was recorded from the day of eclosion (day 1) through day 6 (Figures 1-3). Flight activity is presented as total flight counts of all moths (either male or female) per interval.

Moths commenced flight between 1600 (one hour before sunset) and 1730 (Figures 1-3). Males demonstrated a peak in flight activity between 1700 and 1900; activity increased again approximately between 2000 and 2400. Females tended to be more continuously active throughout the night. Males usually ceased flying between 0200 and 0300 (sunrise), flying only rarely after sunrise; whereas females were more likely to continue activity. Flight activity immediately after sunset is most likely related to feeding behavior, which has been observed to be crepuscular in nature, while other behaviors occur later at night (Adler 1987; Callahan 1958). The bimodal nature of the male flight data agrees with the findings of Callahan (1958) for *H. zea* but is at odds with that of Hsiao (1978) who reported activity as more continuous throughout the night, lacking the pronounced drop two to three hours after dark, and Leppla et al. (1979) who reported only one peak in activity for males. The continuous activity

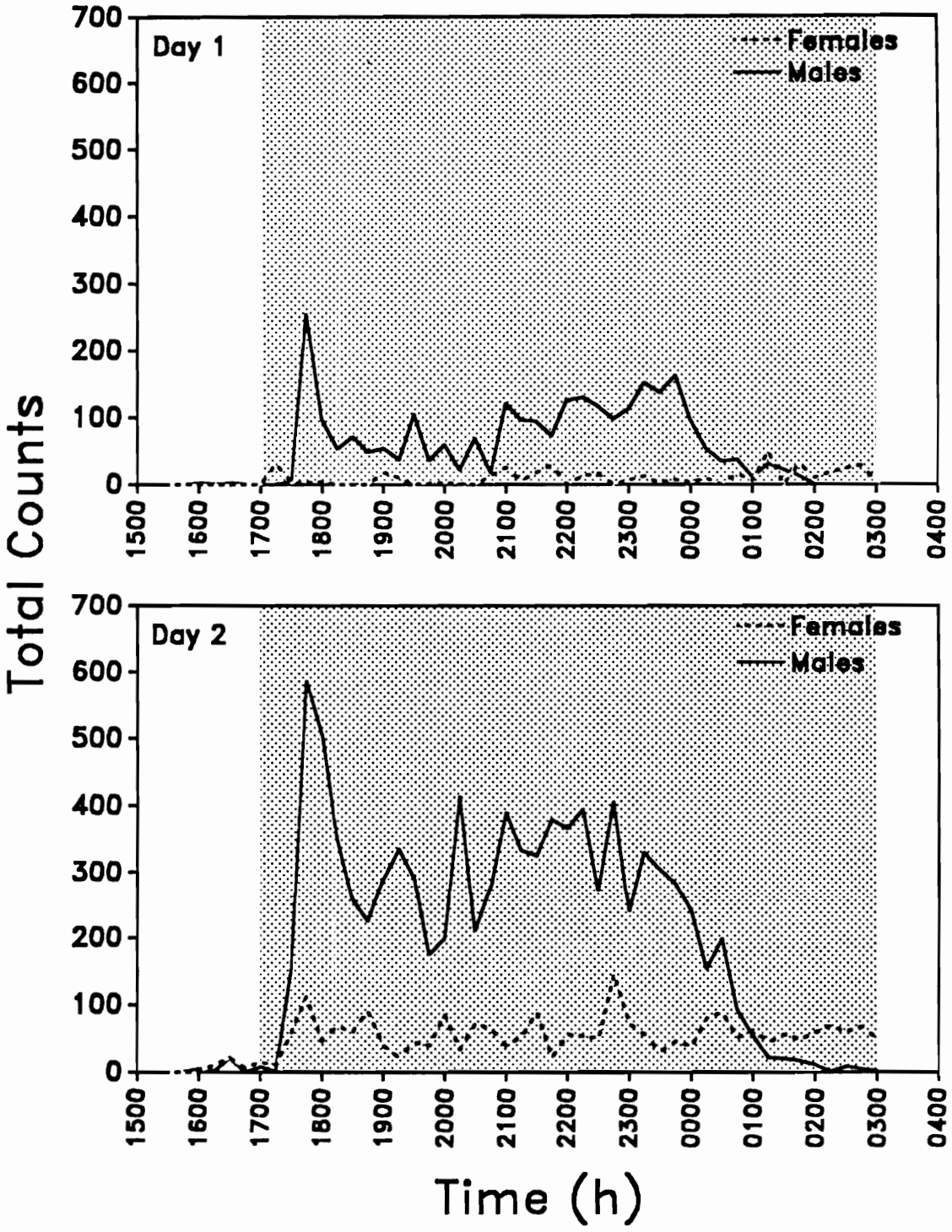


Figure 1. Flight Activity for Day 1 and 2 Female and Male *Heliothis zea*.
(Sunset = 1700, sunrise = 0300; n = 25 except for day 1 females; n = 17 for day 1 females, counts adjusted to equivalent of n = 25.)

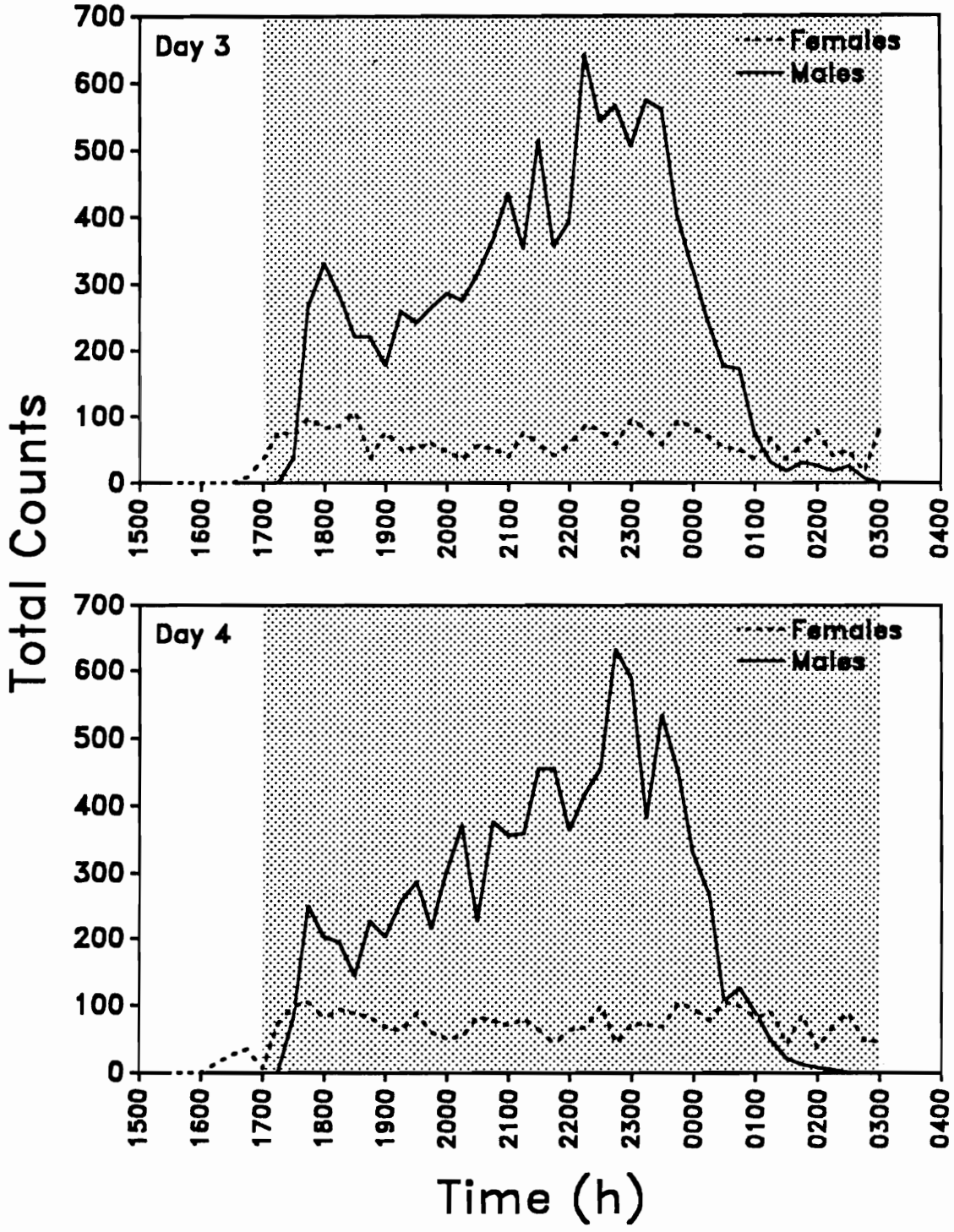


Figure 2. Flight Activity for Day 3 and 4 Female and Male *Heliothis zea*.
(Sunset = 1700, sunrise = 0300; n = 25.)

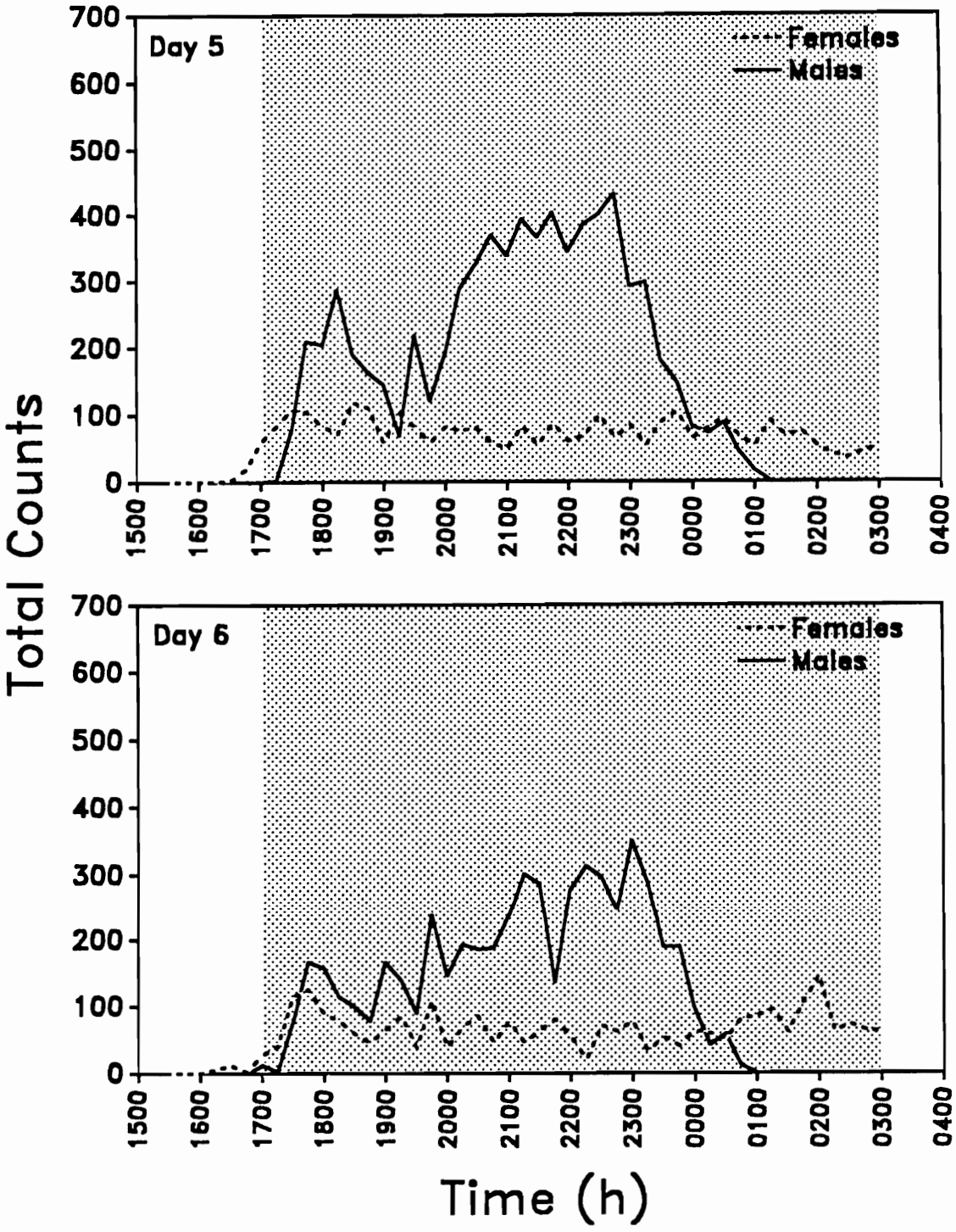


Figure 3. Flight Activity for Day 5 and 6 Female and Male *Heliothis zea*.
(Sunset = 1700, sunrise = 0300; n = 25 except for day 6 females; n = 23 for day 6 females, counts adjusted to equivalent of n = 25.)

of females concurs with the findings of Leppla et al. (1979) although they reported this only for days 4-6.

Moths flew least on the day of eclosion (Figure 1): short flights are typical for this time period (Lingren et al. 1988). Males flew most on day 3, closely followed by day 4 (Figure 2), unlike females which flew most on day 5, then day 4 (Figures 2-3). Previous studies on both *H. zea* (Judge 1988) and *Trichoplusia ni* (Eaton 1985) in this lab have indicated maximum flight on day 4. The observed increase in flight activity over the first three days in both sexes corroborates similar findings of Leppla et al. (1979). Male flight activity was higher than female flight activity for every day. One reason that females fly less than males may be due to the time they spend calling, i.e. emitting sex pheromone to attract males, a sedentary activity. Calling begins within an hour of sunset and peaks three to seven hours later (Raina et al. 1986). Most females mate on days 2 and 3, when pheromone titers are highest, which may explain the observed lag behind males in peak flight activity. Pheromone titers decline significantly after day 3 regardless of whether mating has occurred. Increased flight activity at this time may be directed toward location of habitats occupied by potential mates. Sprint and Eaton (1987) also found lower flight activity for female *T. ni* but Leppla et al. (1979) stated that male and female *H. zea* produced approximately the same amount of activity. Although the females were unmated, they often deposited eggs in their cages. Presumably, this behavior would also reduce total flight activity. Male moths were also unmated: it is unknown if this state affected their flight behavior. The results of this study differ from those of Judge's study (1988). He found that female moths flew most on the first day of eclosion and that they also flew more than males at this time.

Additionally, he observed a peak in flight activity for females shortly after sunset, which is not very pronounced in this study. Population differences may account for some of the differences observed as the flight studies were conducted over several years.

### 3.2.2 **Validation & Accuracy of HPTLC Technique**

Both HPLC and the anthrone procedure were employed to confirm the results of the HPTLC technique. The mean concentrations (ug trehalose/ul sample $\pm$ SE) from each procedure are as follows: HPTLC, 4.68 $\pm$ 0.34; HPLC, 4.65 $\pm$ 0.14; anthrone, 5.24 $\pm$ 0.05. Perhaps not surprisingly, the anthrone value is higher than the other two. The anthrone reagent is nonspecific, reacting with any reducing substances present in the sample. Additionally, the anthrone work utilized the low end of the standard curve, which was observed to produce somewhat higher sample concentrations than the middle of the curve did. Both factors could have contributed to the higher anthrone values.

HPTLC $R_f$ values of standards and samples are given in Table 1. As indicated, good agreement occurred between the two. These values are somewhat higher than those obtained by Fell (pers. comm.) for this technique. Such differences may have occurred due to variation in procedure, i.e. developing the plates in a linear chamber rather than a twin trough tank, as well as other factors (development distance, humidity, ambient temperature (Fried & Sherma 1986)). Figure 4 shows a comparison of scan results for a mixture of standards (trehalose, sucrose, glucose and fructose) and for a typical hemolymph sample. The peak of the hemolymph sample occurs at about the

Table 1. $R_f$ Values Obtained for Standards and Hemolymph Samples on HPTLC Silica Gel 60 Plates Developed in a Linear Chamber

| Standard | $R_f$ |
|---|---|
| Fructose | 0.53-0.60 |
| Glucose | 0.49-0.56 |
| Sucrose | 0.36-0.44 |
| Trehalose | 0.25-0.33 |
| **Hemolymph Sample** | |
| Spot 1 - Glucose | 0.49-0.54 |
| Spot 2 - Sucrose | 0.41 |
| Spot 3 - Trehalose | 0.25-0.32 |
| Spot 4 - Unknown | 0.45-0.47 |
| Spot 5 - Unknown | 0.13 |

Plates pretreated with 0.1 M $NaHSO_3$ and citrate buffer, developed 3x with acetonitrile-water (85:15 v/v)

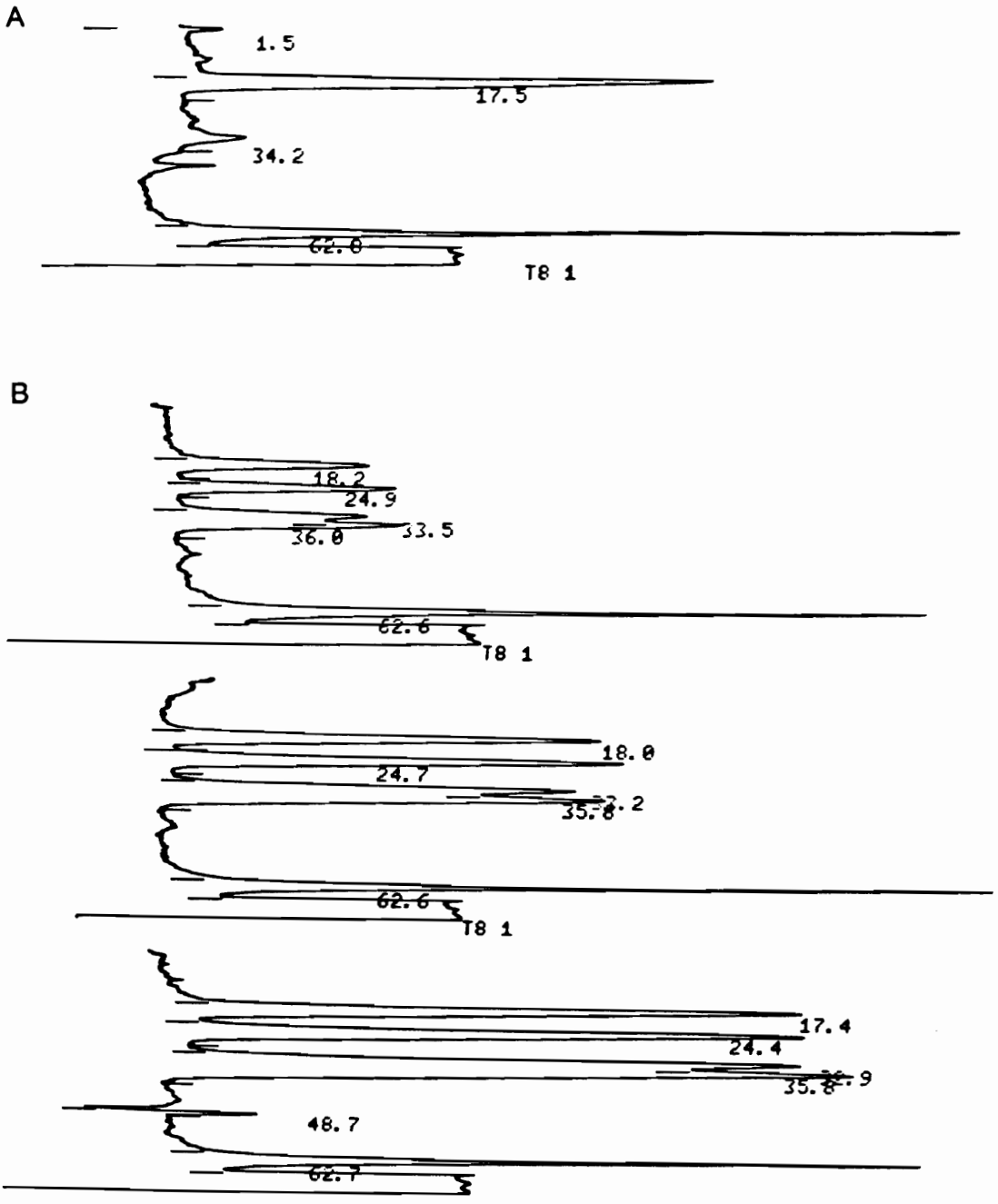


Figure 4. Comparison of Camag Densitometer Recordings of Sugar Chromatograms of HPTLC Plates After Visualization.

A) Hemolymph sample. B) Three concentrations (0.5, 1.0, 2.0 ug/ul) of a mixed standard. (RT = retention time. Trehalose: RT = 17.4-18.2, Sucrose: RT = 24.4-24.9, Glucose: RT = 32.9-33.5, Fructose: RT = 35.8-36.0, solvent front: RT = 62.0-62.7).

same place as that of the trehalose.

Spikeover-analysis indicated good recovery of trehalose although there was some variation among individual samples. The mean value (n = 6) of trehalose in an unspiked sampled was 1.47 $\pm$ 0.60 (ug trehalose/ul spotted $\pm$ SE), while that in a spiked sampled was 1.93 $\pm$ 0.79, yielding a difference of 0.46 $\pm$ 0.08. The expected difference was 0.50 ug/ul. This represents an 8% underestimation of trehalose.

### 3.2.3 **Limits of Detectability**

Since hemolymph samples sometimes lacked any discernible sugars, it was of interest to determine the lower limit of detection using the parameters routinely employed in scanning plates. The lowest amount readable was 0.15 ug/ul; however, trehalose was detectable down to 0.1 ug/ul. Since 0.05 ug/ul was not detectable, the actual limit of detection is somewhere between these values (0.05-0.1 ug/ul). Therefore, hemolymph samples considered undetectable contain less than 0.1 ug sugar/ul spotted sample or in most cases less than 1 ug sugar/ul hemolymph.

### 3.2.4 **Hemolymph Studies**

#### 3.2.4.1 **Identification of Hemolymph Sugars**

Trehalose was the predominant hemolymph sugar identified in these experiments. Among males, one sample consisted entirely of trehalose while

the other contained trehalose (82.2%), glucose (6.6%) and sucrose (11.2%). The presence of sucrose is probably a reflection of the diet of the moths: in many insects, sugars other than glucose and trehalose result from specialized diets (Wyatt 1967). Trehalose was also the major sugar of females, ranging from 91.4-93.8%; however, there was some evidence of other sugars as well. Glucose ranged from 1.0-2.3%. Other spots, possibly sugars, were observed on the plates. To obtain evidence that these were indeed sugars, the plates were respotted and sprayed with two other reagents which react with sugars upon heating: 1) N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) and 2) aniline diphenylamine. All three methods indicated a spot below trehalose ($R_f$ = 0.13). The fact that its $R_f$ is lower even than that of melezitose, a trisaccharide, may indicate that this is also a trisaccharide. This spot has frequently been noted in newly emerged moths but disappears rapidly with age. Fell (pers. comm.) has obtained positive results using the NEDD reagent on hemolymph samples from day 1 moths. In preliminary experiments, *T. ni* exhibited what appeared to be the same "sugar" at higher concentrations over a longer time frame than *H. zea*. Perhaps this is a carbohydrate from the pupal stage that lingers into the adult stage. Interestingly, day 4 females possessed this "sugar", while males did not. An estimate of the concentration of this "sugar" based on trehalose and glucose standards showed that it ranged from 4.6 to 5.2% of the total hemolymph sugar. Another faint spot below glucose appeared in two of the pooled samples but it could not be visualized conclusively with the other two detecting agents. If this is a sugar, it is present in about the same concentration as glucose. Overall, these results agree with those of Jaffe et al.

(1988), who, using HPLC, found trehalose to account for 90% of the hemolymph sugars of *H. zea* adults.

### 3.2.4.2 **Variability of Hemolymph Sugar Titers**

Some moths had no measurable trehalose or other sugars in their hemolymph, a phenomenon that began at day 3, continuing thereafter (Figure 5). Mortality was also observed to begin around this time but unfortunately was not quantified. Anelli and Friedman (1986) reported that moribund blowflies have reduced hemolymph and fat body trehalose levels. This may be the case for corn earworms as well. Another possibility is the existence of moths within a population with low or undetectable sugar levels. Such moths may display the same types of sugar cycles as the other moths but at very low levels. Since it was questionable whether these moths were the same as the others, they were eliminated from subsequent analyses.

Few studies of hemolymph sugar titers have examined samples from individual insects: rather, hemolymph samples from several insects are usually pooled for analysis (Nowosielski & Patton 1964; Van der Horst et al. 1978; Mullins 1985). One consequence of examining single samples is the extreme variability in concentrations detected (Brown & Mazzone 1977; Nowosielski & Patton 1964), a problem that recurred throughout this study. Trehalose concentrations ranged from undetectable to a high of 37.4 ug/ul. Frequently, the variability of the within group data is so great that differences between groups are often indiscernible, even with sample sizes of 10 moths/group. Nowosielski and Patton (1964) attempted to examine individual house crickets

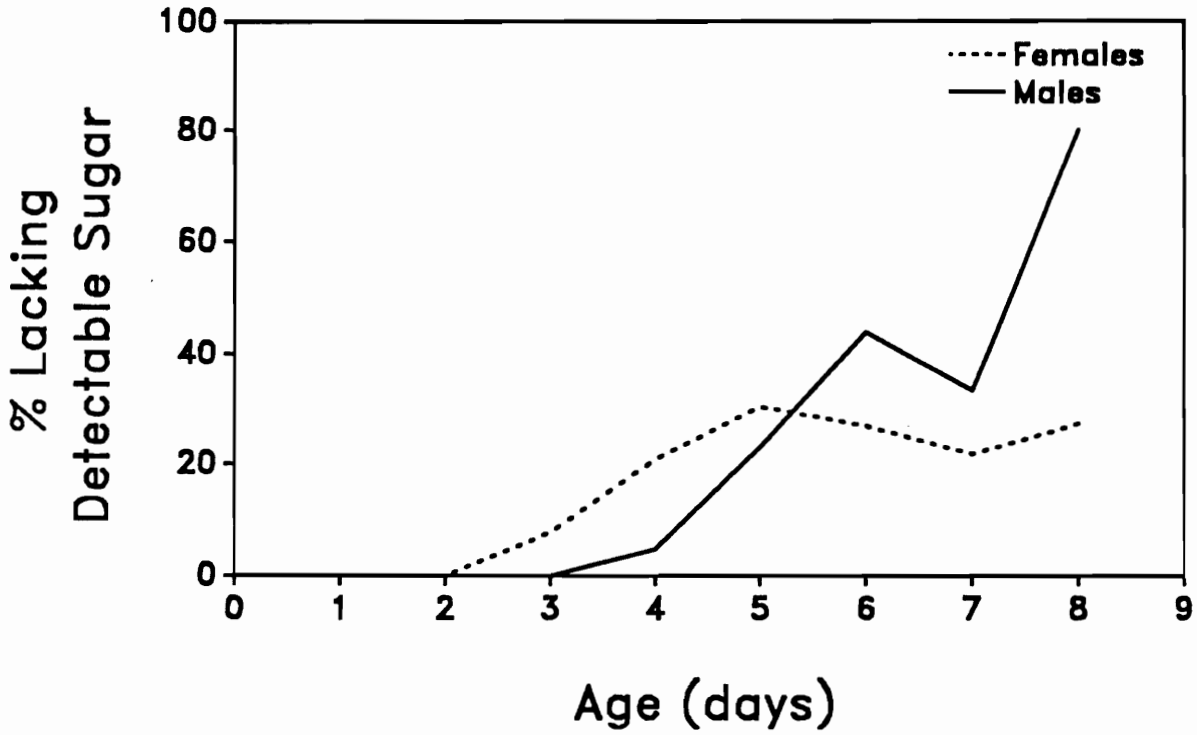


Figure 5. Percent Moths Lacking Detectable Hemolymph Sugars vs Age for Female and Male *Heliothis zea*.

but found the variation too great to make the assay practical and therefore pooled at least 5 crickets/sample. Nettles et al. (1971) determined total trehalose/insect in *H. zea* and also found great variation among individuals, which they attributed to the small number of moths analyzed. This may not necessarily be the case. Many factors may influence the variability of the data including time of last feeding. For example, some insects may feed immediately before being sampled while others may not. This would surely affect their hemolymph sugar concentrations. To minimize this problem, the sucrose was removed one day prior to sampling. Moreover, although all moths were reared following standard procedures, there could have been slight differences in rearing conditions or genetic differences in moths. Each of these factors could have contributed to the observed variability.

### 3.2.4.3 **Basal Levels**

Hemolymph trehalose concentrations for females and males (days 1-6) sampled at two times (0930-1030, 2130-2230) are presented in Figure 6. In relation to flight activity, sampling occurred during the resting phase (day) and during the second peak in activity for males (night). Mean female trehalose titers ranged from 7.4 $\pm$ 1.3 to 12.5 $\pm$ 0.8 ug/ul during the day and between 7.2 $\pm$ 2.2 and 12 $\pm$ 1.8 ug/ul at night. Mean male trehalose titers ranged from 6.0 $\pm$ 1.2 to 15.1 $\pm$ 0.7 ug/ul during the day to 9.0 $\pm$ 1.6 to 15.7 $\pm$ 1.3 ug/ul at night. These values are consistent with others reported in corn earworms: 15 ug/ul, age and sex unspecified (Van Handel 1978) and 10.5 ug/ul, age and sex unspecified (Fell in press). Overall patterns are similar in both sexes, although

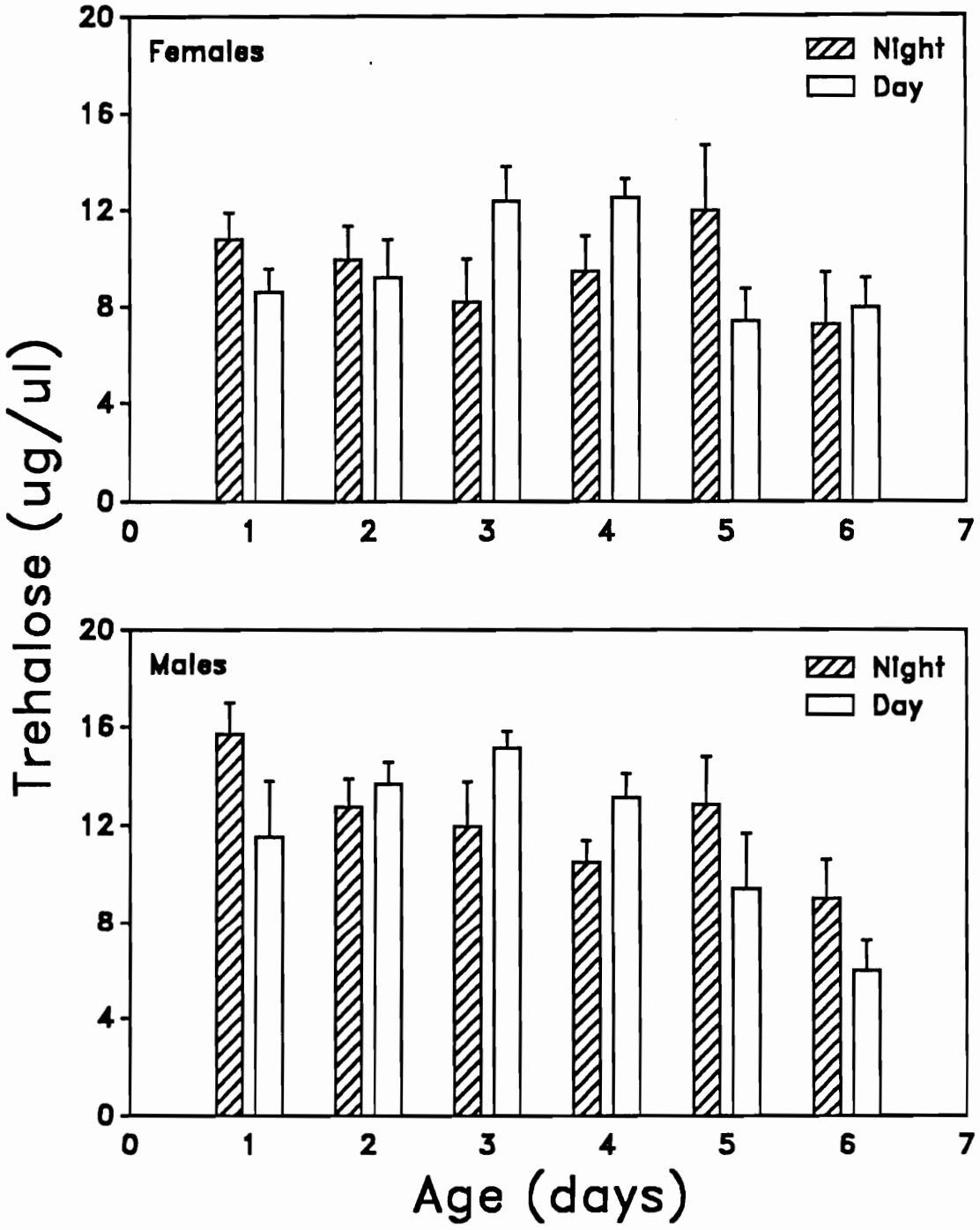


Figure 6. Mean Hemolymph Trehalose Titers by Age and Time of Day for Female and Male Lab-reared *Heliothis zea*. (Night = 2130-2230, day = 0930-1030. Vertical bars = SEM. See 3.2.4.3 for analysis of data. M = male, F = female, N = night, D = day, # = age. n = 7 for FN:6. n = 9 for FD:5,6; MD:5. n = 10 for FN:1,2,3,5,6; FD:2,4; MD:2,3,6. n = 11 for FD:1,3; MD:1. n = 13 for MN:4. n = 14 for FN:4. n = 18 for MN:4.)

females tended to have slightly lower trehalose concentrations than males. During the day, trehalose titers increased and peaked at day 3 in males and days 3-4 in females, decreasing thereafter. At night, titers began decreasing from day 1, then peaked again at day 5 in both sexes, subsequently declining again. In both males and females, night titers were higher than day titers on days 1 and 5.

A two factor ANOVA ($a < 0.1$) on the variables time of day and age resulted in a significant ($p = 0.0005$) effect of age in males. Comparisonwise tests (LSMEANS) indicated that trehalose levels of day 6 moths differed significantly from every other age (maximum $p = 0.0190$) (Figure 7). Day to day and night to night comparisons across ages were made for each sex. Differences in means were identified by LSMEANS (Tables 2-4). For females, no significant differences were found for night to night comparisons ($p = 0.5624$); however, significant differences were observed for day to day comparisons ($p = 0.0134$). Among males, differences for both night to night ($p = 0.0404$) and day to day ($p = 0.0010$) comparisons were observed. Comparison was also made of night to day by each sex and age (t test). Significant differences were observed between night and day trehalose titers for females on days 3 ($p = 0.0872$) and 4 ($p = 0.0791$) and for males on day 4 ($p = 0.0587$).

Biologically, the significance of many of these differences is unclear. The high trehalose titers found in both sexes at night on day 1 seems reasonable in view of the limited flight that occurs. Peak flight activity of males occurs on days 3-4 corresponding to the lower night trehalose titers found on these days. Males 2-3 days old are at an age to be seeking mates (Agee 1969; Callahan 1958) and would thus spend a large portion of time flying. Inexplicably, females peak in

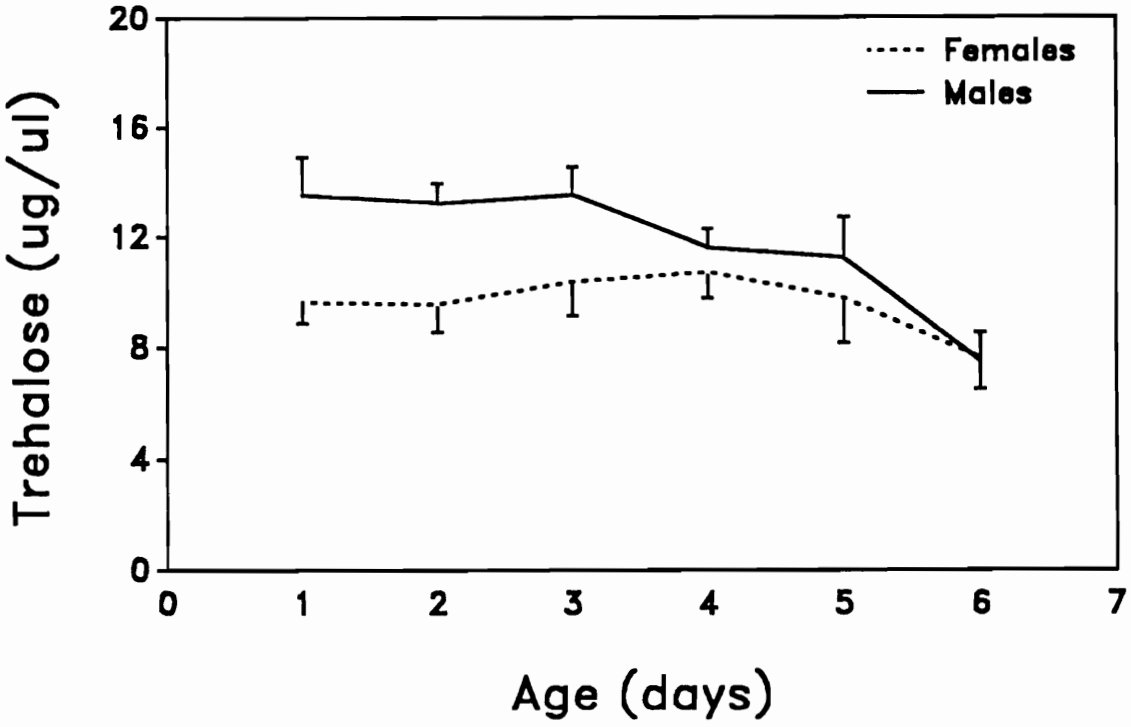


Figure 7. Mean Hemolymph Trehalose Titers by Age for Female and Male Lab-reared *Heliothis zea*. (Vertical bars = SEM. See 3.2.4.3 for analysis of data. M = male, F = female, # = age. n = 16 for F:6. n = 19 for F:5; M:5. n = 20 for F:2; M:2,3,6. n = 21 for F:1,3; M:1. n = 24 for F:4. n = 31 for M:4.)

Table 2. P Values for Female *Heliothis zea* Day to Day Comparisons of Mean Trehalose Levels Across Ages by LSMEANS

| Age | | | Age | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | | 0.7223 | 0.0294 | 0.0273 | 0.4903 | 0.7170 |
| 2 | | | 0.0733 | 0.0672 | 0.3127 | 0.4893 |
| 3 | | | | 0.9322 | 0.0068 | 0.0160 |
| 4 | | | | | 0.0064 | 0.0149 |
| 5 | | | | | | 0.7542 |

Table 3. P Values for Male *Heliothis zea* Day to Day Comparisons of Mean Trehalose Levels Across Ages by LSMEANS

| Age | | | Age | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | | 0.3132 | 0.0946 | 0.4298 | 0.3319 | 0.0104 |
| 2 | | | 0.5076 | 0.7786 | 0.0593 | 0.0007 |
| 3 | | | | 0.3257 | 0.0127 | 0.0001 |
| 4 | | | | | 0.0828 | 0.0008 |
| 5 | | | | | | 0.1240 |

Table 4. P Values for Male *Heliothis zea* Night to Night Comparisons of Mean Trehalose Levels Across Ages by LSMEANS

| Age | | | Age | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | | 0.1697 | 0.0827 | 0.0070 | 0.1830 | 0.0025 |
| 2 | | | 0.7093 | 0.2293 | 0.9661 | 0.0828 |
| 3 | | | | 0.4328 | 0.6779 | 0.1698 |
| 4 | | | | | 0.2115 | 0.4352 |
| 5 | | | | | | 0.0757 |

flight activity on day 5, when trehalose levels are higher at night than during the day. Males exhibit this reversal also. The variableness in whether day or night trehalose titers are higher is not mirrored by diacylglyceride (DG) levels. Judge (1988) found that night DG levels were lower than day DG levels for all ages and sexes except day 4 females. Consistently lower night DG levels would be expected since this is the moths major flight fuel.

Perhaps a more useful way to make sense of the data is by pooling all the day and night samples to determine a mean value for each age. When looked at this way (Figure 7), it is apparent that trehalose concentrations are relatively constant for several days post-emergence but then begin to decline. This decline occurs earlier in males than females and likely coincides with senescence, as previously mentioned (3.2.4.2). Additionally, it was observed (but not quantified) that females lived longer than males, hence the more constant trehalose levels observed for females. These changes in trehalose titers over the first six days contrast with observations by Nettles et al. (1971) on total trehalose/moth. They found that moths fed 10% sucrose for one day sharply increased their trehalose levels, with titers decreasing on the second day, changing only slightly with age thereafter (age up to 8 days). The difference between their data and that presented here might be accounted for by the presence of trehalose sequestered elsewhere, such as in the fat body or flight muscles. Examination of changes in hemolymph trehalose titers will not include these other sources of trehalose.

Mean trehalose titers for wild females and males, days 3 and 5, are presented in Figure 8. Means varied little ranging only from 6.9 $\pm$ 2.9 to 8.9 $\pm$ 1.5 ug/ul in females, and 7.6 $\pm$ 2.3 to 8.6 $\pm$ 3.8 ug/ul in males. All analyses

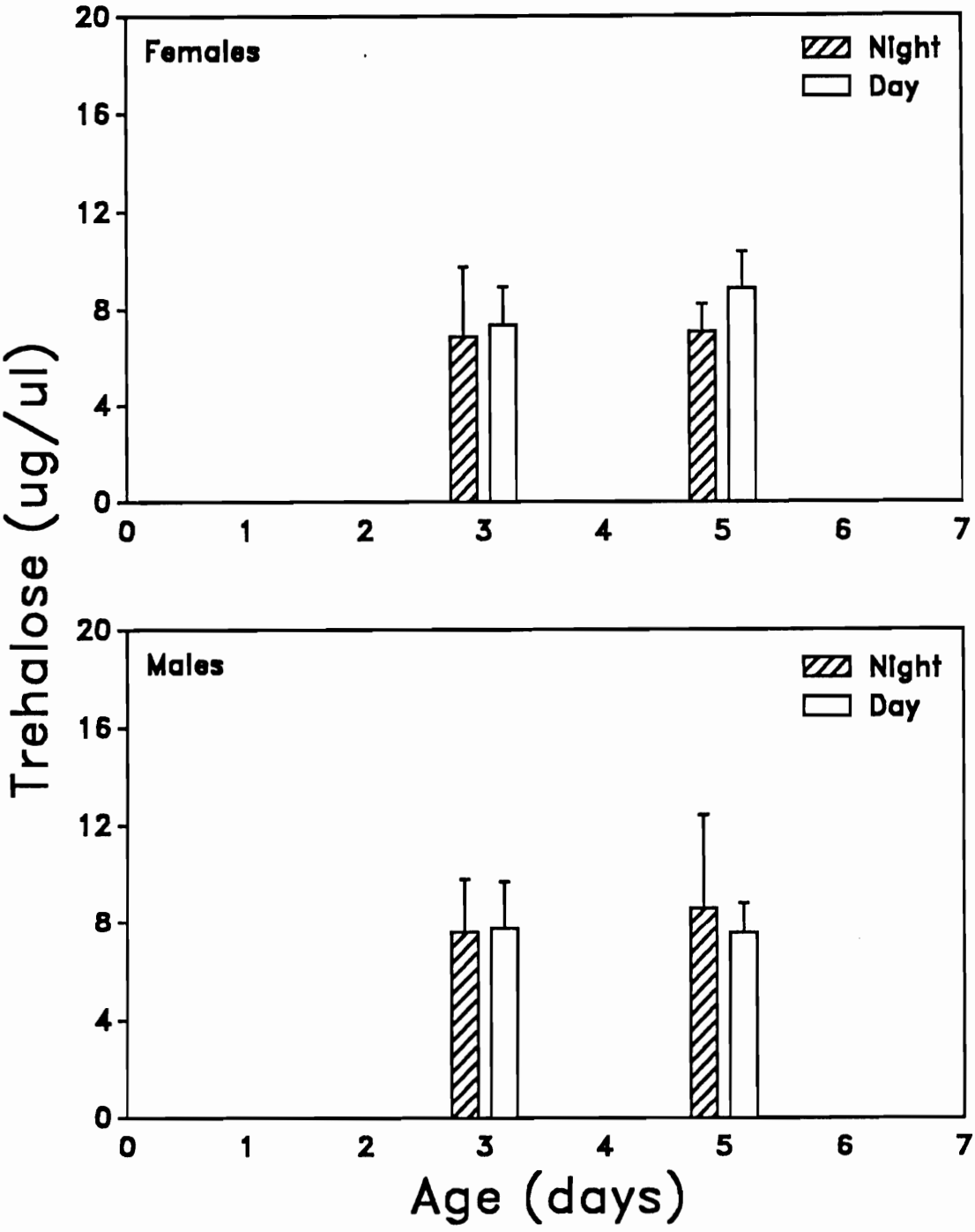


Figure 8. Mean Hemolymph Trehalose Titers by Age and Time of Day for Female and Male Wild *Heliothis zea*. (Night = 2130-2230, day = 0930-1030. Vertical bars = SEM. See 3.2.4.3 for analysis of data. M = male, F = female, N = night, D = day, # = age. n = 5 for FN:3; MN:5. n = 7 for MD:5. n = 8 for MN:3; MD:3. n = 9 for FD:3; FN:5. n = 10 for FD:5.)

performed on lab-reared moths were performed on wild moths, with no significant differences resulting.

Comparison of wild moths to lab-reared moths indicated lower mean trehalose levels at every time except the day of day 5 females. ANOVA on the variables type (wild vs lab), age and time of day resulted in a significant effect of type for both sexes ($p = 0.0673$ for females, $p = 0.0015$ for males). Comparison (t test) of wild to lab-reared moths by sex, age and time of day revealed significant differences between hemolymph trehalose concentrations of wild and lab-reared moths during the day on day 3 (females: $p = 0.0314$, males: $p = 0.0066$). Differences between wild and lab-reared moths are not unusual. For example, Raina et al. (1989) found that inbreeding due to laboratory culture resulted in *H. zea* males that were less discriminating to qualitative differences in pheromone blends than were wild males.

Wild moths may have had lower levels because of population and/or environmental differences. Interestingly, Judge (1988) found that wild moths had higher DG than lab-reared moths, the opposite of the situation found for sugars. Although he sampled different moths, the wild moths used in both studies were collected at the same time and location during the summer of 1987, so they were probably from the same population. It may be that the wild moths were a mixture of immigrants and overwintering moths. Wild moths were observed to fly more in their cages than lab-reared moths. Perhaps many of the wild moths were destined to be migrants, and therefore had high DG levels.

### 3.2.4.4 Diurnal Cycle

Figure 9 shows the trehalose titers in day 4 female and male moths sampled over a 24 hour period. Both sexes exhibited a gradual increase in trehalose levels beginning at 0300 (sunrise) to a peak at 1600 (17.4 $\pm$ 2.4 ug/ul in females,18.0 $\pm$ 2.5 ug/ul in males) one hour before sunset. In females this is followed by a sharp decline to 11.9 $\pm$ 1.4 ug/ul at 1700 (sunset), followed by a second peak of 17.0 $\pm$ 2.9 ug/ul at 1800; whereas in males, trehalose titers declined to 8.8 $\pm$ 1.2 ug/ul at 1900, the lowest point in the cycle, with a subsequent increase after 1900, around the same time that flight activity picks up again. A comparison of resting trehalose levels (0300, 0700 and 1100) vs one hour before sunset indicated significant differences for males ($p = 0.0034$) and females ($p = 0.0161$). This raises the question as to why trehalose decreases from a peak one hour before sunset to a lower point at sunset when moths are not yet flying. Beenakkers (1969) suggested that carbohydrate degradation is important in wing vibration necessary to elevate thoracic temperatures prior to flight, particularly in migratory insects. According to Casey and Joos (1983), preflight warm-up enhances the flight repertoire of many Noctuids. Moths of both sexes were often observed vibrating their wings between 1600 and 1700 although this observation was not quantitated.

Female moths' hemolymph trehalose concentrations continued decreasing throughout the night, unlike those of males. Willers et al. (1987) suggested that females of *Heliothis virescens* preferentially used sugars for flight upon maturity to conserve lipids for egg production. If this were the case in *H. zea*, females might be expected to have a different lipid pattern throughout the

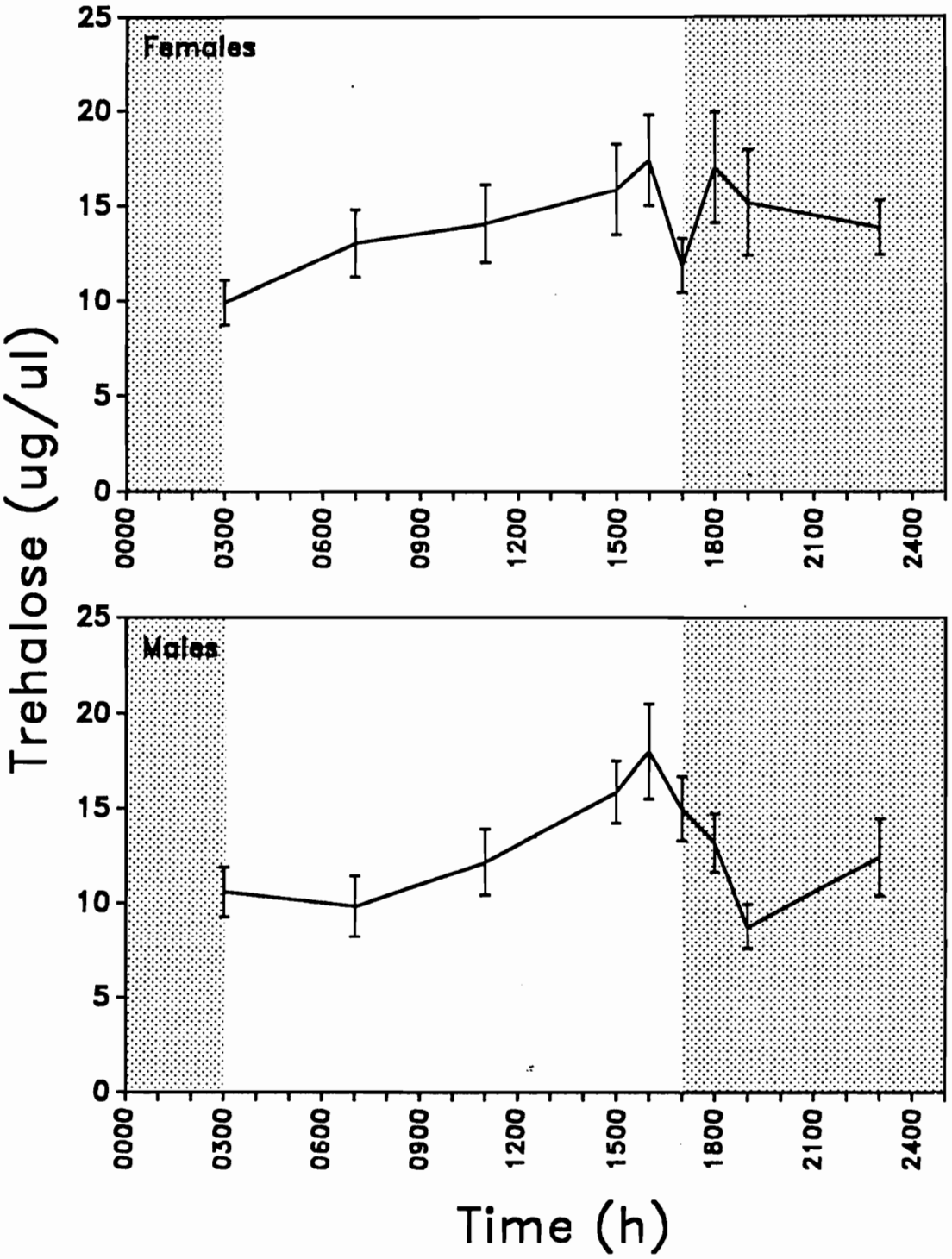


Figure 9. Mean Hemolymph Trehalose Titers for Day 4 Female and Male *Heliothis zea* Sampled Over 24 Hours. (Sunrise = 0300, sunset = 1700. Vertical bars = SEM. See 3.2.4.4 for analysis of data. M = male, F = female, # = time. n = 9 for F:1100,1500,1600,1900. n = 10 for M:1900,2300. n = 11 for F:1800,2300; M:1100,1500,1600. n = 12 for F:1700. n = 13 for M:1700,1800. n = 14 for F:0700. n = 16 for F:0300. n = 17 for M:0300. n = 19 for M:0700.)

night than males; however, lipid utilization follows the same pattern throughout the night in both sexes (Judge 1988). It is possible that the non-mated status of the females prohibits a change in lipid patterns that might otherwise occur; nevertheless, unmated females were observed to oviposit eggs in their cages so being non-mated may not have any effect on egg production. Another possibility may involve the energetics of calling. Calling or receptive females are known to vibrate their wings (Agee 1969; Callahan 1958). The females of this study probably spend more time calling than occurs in the field since they never mate. Mating is known to reduce calling activity (Raina et al. 1986).

It appears that trehalose levels increase gradually over the course of the day from their depleted night time values. How is this increase in trehalose regulated? It may result simply by having the rate of trehalose synthesis exceed the rate of hydrolysis during the resting state as reported by Van Handel (1978). The recently synthesized hypertrehalosemic hormone, Hez-HrTH, of *H. zea* (Jaffe et al. 1988) could also be involved. Trehalose is the only saccharide to respond significantly to Hez-HrTH. Finally, as sunset approaches the neurohormone octopamine may become involved. Octopamine has been demonstrated to cause a 100% increase in hemolymph trehalose within 15 minutes of injection into the hemocoel of American cockroaches (Downer 1979a). It is also thought to increase excitability or arousal (Claassen & Kammer 1986), thus octopamine could increase arousal and increase trehalose at the same time, in preparation for flight.

It is interesting to note the difference in diurnal cycles of males and females from 1600 to 1900. This difference does not appear to be due to a

difference in activity of the sexes at this time. As indicated previously, *H. zea* are crepuscular feeders, with similar feeding rhythms for both sexes (Adler 1987).

#### 3.2.4.5 **15 Minute Interval Sampling**

In this experiment, trehalose levels during the sunset period were examined more closely by sampling every 15 minutes (Figure 10). In females, the comparison of 1600 to 1700 was significant ($p = 0.0005$); however, 1700 to 1800 was not ($p = 0.8950$). Figure 11 presents the 15 minute sampling data superimposed on the diurnal cycle data. Females still exhibit a peak in trehalose at 1600 ($19.5 \pm 2.0$ ug/ul); however, the lowest point occurs somewhat later, at 1830 ($8.3 \pm 1.9$ ug/ul). Additionally, the second peak is much less pronounced. It may be that this is a more accurate reflection of what is occurring and that the females' trehalose levels actually follow a pattern more similar to that of the males. Among the males, the trend of going from a high point ($17.4 \pm 1.6$ ug/ul) to a low point ($8.2 \pm 1.6$ ug/ul) several hours later is evident, although the peak is one hour earlier at 1500. There are many fluctuations during this time, probably reflecting individual variation as well as population activity and nutritional state.

### 3.2.5 **Conclusions**

The flight metabolism of *H. zea* has not been widely examined. Van Handel (1974) demonstrated that DG are the major fuel for flight. Judge (1988) examined changes in hemolymph lipid concentrations over a 24 hour period and

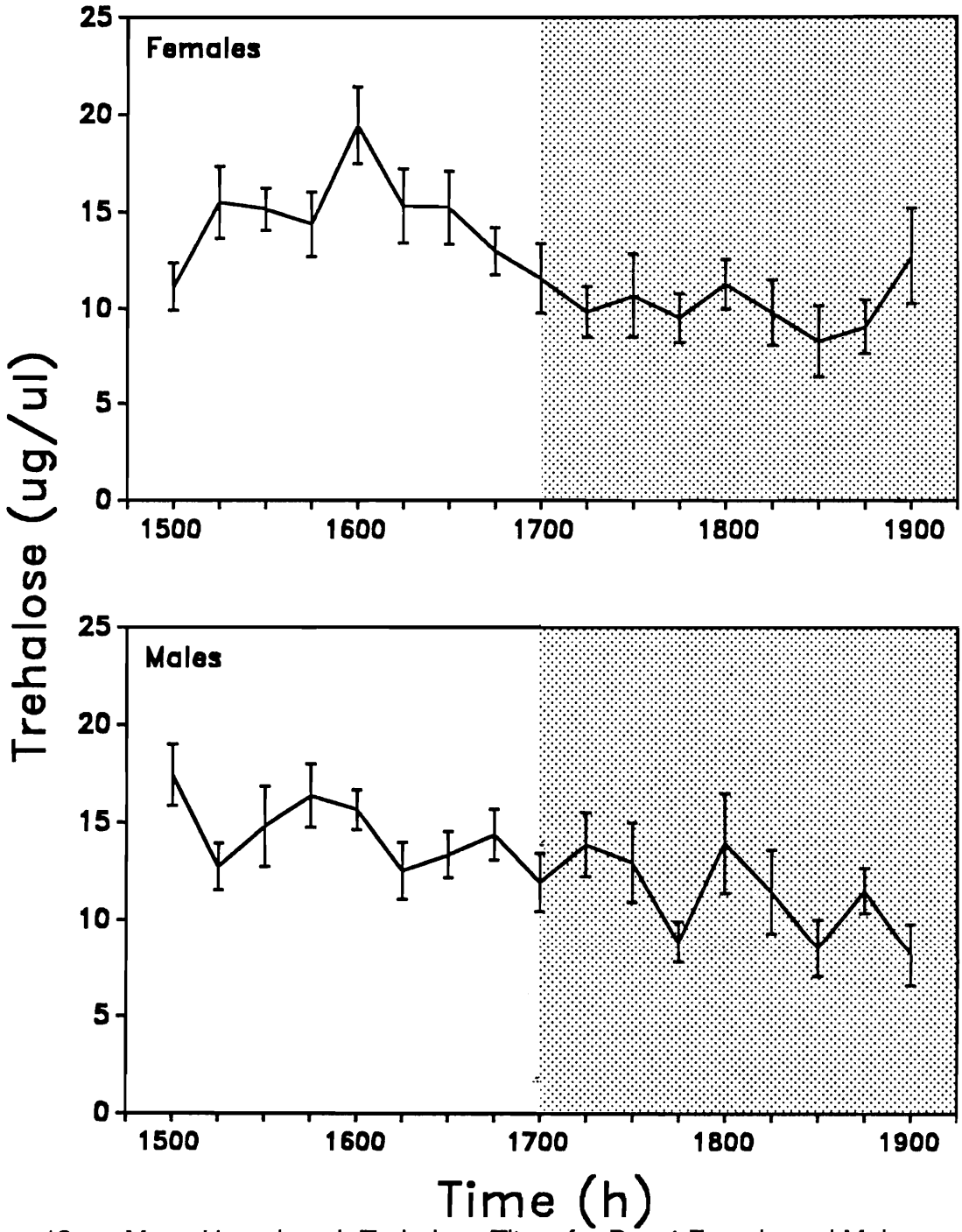


Figure 10. Mean Hemolymph Trehalose Titers for Day 4 Female and Male *Heliothis zea* Sampled Every 15 Minutes from 1500 to 1900.
(Sunset = 1700. Vertical bars = SEM. See 3.2.4.5 for analysis of data. M = male, F = female, # = time. n = 8 for F:1500,1845; M:1900. n = 9 for F:1600,1700,1800. n = 10 for F:1515,1530,1545,1615,1645,1715,1730,1815,1830; M:1500. n = 11 for F:1745,1900. n = 12 for M:1745. n = 13 for M:1815,1830. n = 14 for M:1530,1800. n = 15 for M:1515,1700,1845. n = 16 for M:1545,1600,1615. n = 17 for M:1630. n = 18 for M:1645. n = 19 for M:1730.)

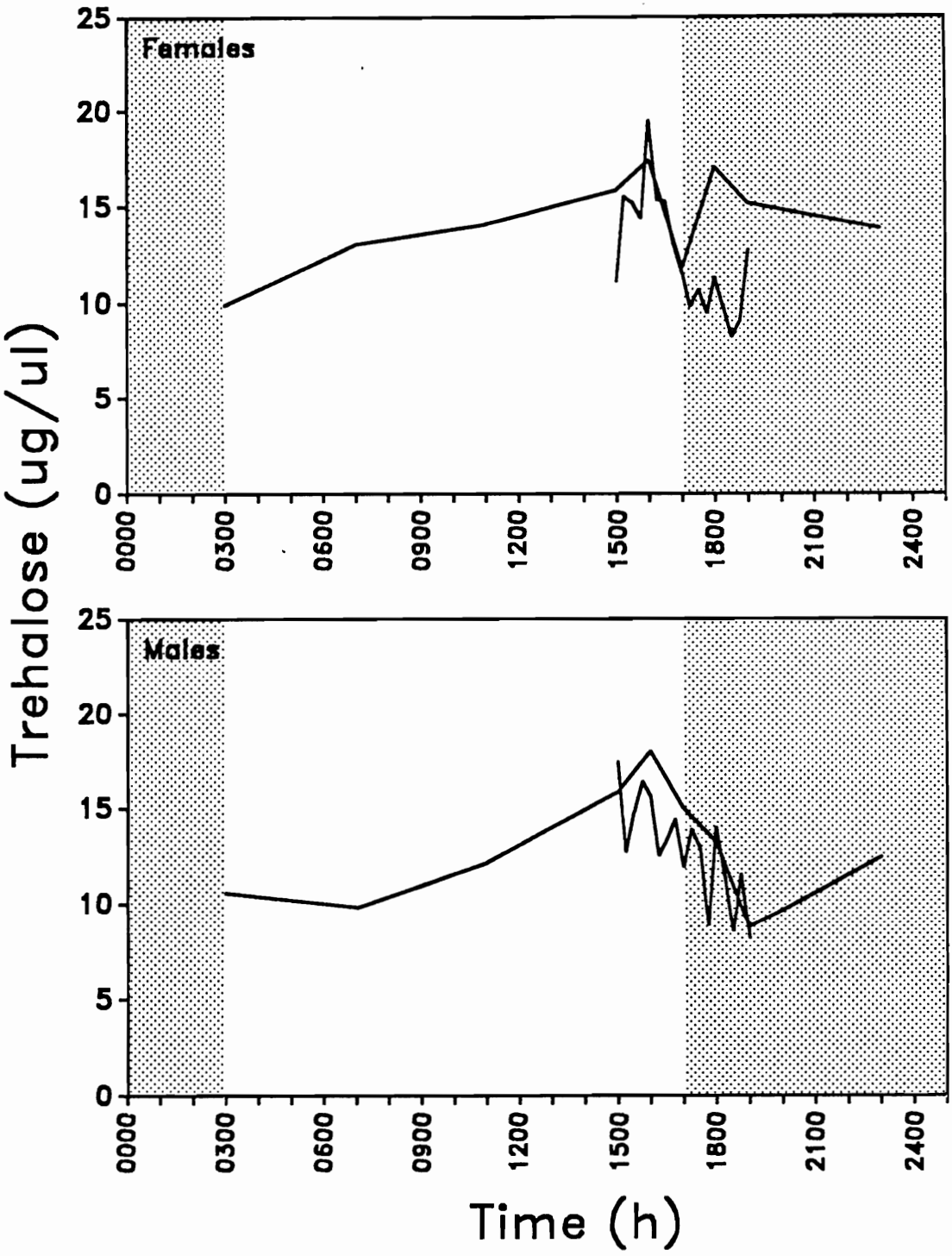


Figure 11. Comparison of 15 Minute Sampling and 24 Hour Sampling for Day 4 Female and Male *Heliothis zea*. (Sunset = 1700.)

found that DG levels were highest before flight, decreasing during flight. A similar pattern was found in both sexes. Interestingly, triacylglyceride (TG) levels were also high. The significance of this finding to flight physiology is as yet undetermined.

The data from this study appear to indicate a role for carbohydrates, specifically trehalose, during warmup and the early stages of flight (Figure 9). This is most apparent in males, where trehalose levels begin decreasing before sunset and continue decreasing during the first peak in flight activity. The situation in females is less clear. The decrease before sunset is present, but strangely, a second peak was found one hour after sunset. This pronounced second peak was not observed when moths were sampled at 15 minute intervals. Perhaps the 15 minute interval data is a more accurate reflection of the true situation, i.e. there actually is no major second peak in females. The females' higher trehalose levels later in the night (1900-2300) could result from the lower flight activity of females compared to males at this time.

It appears that the mobilization of trehalose is related to sunset, and thus, changes in light intensity. How such changes are detected remains unknown. The ocelli have been suggested to play a role in the detection of changes in light intensity: unfortunately, attempts to produce anocellate *H. zea* were unsuccessful, so this aspect was not examined.

Another possible effect on trehalose concentrations is hemolymph volume changes during flight. Changes can result from dehydration or production of metabolic water during flight. At the present, there is no evidence that volume changes account for the observed changes in trehalose levels. Consideration of this question led Judge (1988) to look for changes in levels of

lipids (i.e. cholesterol esters (CE)) that are not thought to be used in flight. Such increases should occur if there are changes in hemolymph volume. No changes in CE were observed throughout the night. Further, the Noctuid *Anticarsia gemmatalis* does not undergo volume changes during flight (Teo et al. 1987).

Trehalose levels were found to be quite variable in both sexes, from unmeasurable to 37.4 ug/ul, even though moths were sampled at the same age and time of day. Binder et al. (1984) observed high variability in trehalose levels of *H. zea* larvae and concluded that this was due to the larvae being at slightly different stages of development although chronologically the same age. Adults of the same chronological age may likewise be at slightly different stages of development and hence have more variable trehalose concentrations. Wild moths had lower trehalose concentrations as well as lower variability than lab-reared moths.

As indicated, large variation in trehalose levels was observed throughout these experiments. Samples for the various experiments were collected over several months. Although standard rearing procedures were followed throughout, some differences may have occurred due to the time of year. Furthermore, the colony appeared to lose vigor the longer it was kept in culture, requiring the addition of new moths. Moths were added as needed, not at a predetermined interval. The consistency of the moths over time may have been improved if the colony had been supplemented on a set schedule. Moths set up at the same time develop at different rates and emerge over a period of days. Moths emerging at the end of the period may be different than those emerging at the beginning. Performance of experiments with moths that had all emerged

on the same day might help to reduce variability. Both of these issues were considered in the design of the experiments discussed in section 4.

Although this research indicates that hemolymph trehalose concentrations are related to flight activity, many questions remain unanswered. The effects of mating on both flight activity and trehalose utilization remain unknown and are likely to impact results, particularly in female moths. An examination of carbohydrate and lipid utilization by the same group of moths would be of value, especially for comparison with other migratory species. Wild moths, reared to adulthood on plants rather than transferred to diet, could be compared to lab-reared moths, and the validity of using lab-reared moths as a model ascertained.

Section 4

# HEMOLYMPH TREHALOSE LEVELS FOLLOWING STRESS OR INJECTION OF OCTOPAMINE IN *Heliothis zea* (Boddie) ADULTS

The regulation of trehalose titers in insect hemolymph and the mobilization of trehalose from fat body tissue is believed to be under hormonal control. Hypertrehalosemic peptide hormones cause an increase in hemolymph trehalose levels by stimulating the conversion of fat body glycogen to trehalose. Stress or injection of the biogenic amine octopamine into cockroaches may also stimulate a hypertrehalosemic response (Downer 1979a). In locusts an increase in octopamine concentration in the blood occurs during the first 10 minutes of flight, decreasing thereafter until it returns to its resting value. Octopamine has been suggested to govern initial mobilization of energy reserves from the fat body during flight (Goosey & Candy 1980).

Taurine, a reputed naturally occurring octopamine antagonist, increases in the hemolymph of cockroaches and locusts after stress (Jabbar & Strang 1985) and has been demonstrated to inhibit release of octopamine from the nervous system of American cockroaches. Hayakawa et al. (1987) proposed taurine may serve as an inhibitory modulator of octopamine release in a dual control mechanism for excitation.

In the previous section, hemolymph trehalose levels in the corn earworm, *Heliothis zea*, were examined. When day 4 adult females were examined over a

24 hour period, two peaks (at 1600 - one hour pre-sunset and at 1800 one hour post-sunset) in sugar titers were found which corresponded to the beginning of flight activity at dusk. Upon closer examination of the sunset period, the second peak was much less apparent, but the first peak remained significant and invites further investigation. The role of the first peak remains unclear. As proposed previously, this increase in sugars might be related to flight preparation. Such an increase could be modulated by octopamine since neurohormones have been suggested to show cycles in hemolymph levels that are correlated with periods of insect activity (Hinks 1967; Bailey et al. 1983; Davenport & Evans 1984). Thus, the objectives of the following study were to determine the effects of octopamine or stress on hemolymph trehalose levels, especially with respect to trehalose concentration increases and to determine the possible antagonistic effects of taurine on these possible octopamine mediated hemolymph trehalose increases.

## 4.1 MATERIALS & METHODS

### 4.1.1 Experimental Animals

Moths used in these experiments were day 4 or 5 males or females, as indicated in each individual experiment, reared and handled as described in 3.1.1, with the following exception: moths were provided tap water *ad libitum* in addition to 10% sucrose until immediately prior to experimentation. In several experiments the response of American cockroaches, *Periplaneta americana*, to stress and octopamine were examined. Adult male cockroaches were obtained

from the laboratory colony maintained at VPI & SU. Cockroaches were housed in aquaria with harborage: water and dog food pellets were supplied *ad libitum*. The laboratory was maintained at 24-27°C with a 10:14 (L:D) light regime. The cockroaches had not been used experimentally before, and were used only once in an experiment. Two days before experimentation, cockroaches were isolated in individual petri dishes with only water supplied *ad libitum*.

### 4.1.2 **Sampling Procedure**

Moths were sampled as described previously (3.1.3) with the exception of not being refrigerated before injection or sampling unless otherwise indicated. In later experiments, moths were not chilled because of the delays resulting during injecting and sampling. Additionally, preliminary experiments indicated no significant differences between chilled or unchilled moths. Cockroaches were kept warm until just before sampling and were sampled in a cold room (5°C) to minimize coagulation of hemolymph. Most samples were obtained from the antennae, but in the last experiment, samples were obtained from the mesothoracic coxae. Samples were collected into several different solvents; however, only samples collected comparably to moth samples (i.e. into 70% ETOH) will be considered here. Samples were centrifuged at 9700 RPM and dried under $N_2$. All samples were stored at 0°C until assayed.

### 4.1.3 **Analytical Methods**

#### 4.1.3.1 **Sugar Quantification**

Sugars were separated and quantified by HPTLC as described (3.1.4.1). In some cases sugars were also assayed using a modified anthrone analysis (Trevelyan & Harrison 1952). Biochemicals were obtained from Sigma Chemical Company. All other chemicals (reagent grade) were from Fisher Scientific. Dried hemolymph samples were resuspended in an appropriate volume of 70% ETOH (usually 25 ul) and an aliquot added to $ddH_2O$ (double distilled water) to make up the sample solution. The anthrone reagent was prepared before each assay by adding 0.2 g of anthrone to 100 ml of diluted sulfuric acid (500 ml concentrated $H_2SO_4$:200 ml $ddH_2O$). Five ml aliquots were pipetted into test tubes cooled in an ice water bath. One ml of blank, glucose or trehalose standard (5, 12.5, 25, 50 ug/ml) or sample was layered on top of the acid. The tubes were capped and vortexed, then placed in boiling water for 10 minutes, as indicated in the original procedure. This amount of time was subsequently found to be insufficient for maximal color development; therefore, the heating time was increased to 15 minutes. After they were cooled in an ice water bath, the resulting blue-green product was quantified in a Perkin-Elmer$^R$ Lambda 3B Dual Beam Spectrophotometer at 620 nm.

When moth samples were analyzed by the anthrone method, the volume of anthrone reagent was reduced to 2.5 ml with 0.5 ml sample, standard or blank layered on top.

### 4.1.3.2 Comparison of HPTLC, Anthrone & HPLC Methods

During these experiments, differences in results were obtained using the HPTLC and anthrone techniques. To eliminate the possibility that these differences were due to the method of analysis, a pooled hemolymph sample from *H. zea* adults was analyzed by both techniques as well as HPLC. The procedure is detailed in section 3.1.4.2.

### 4.1.3.3 Accuracy of the Anthrone Procedure

The recovery of the anthrone technique was examined by spikeover analysis of a pooled hemolymph sample from adult *H. zea*. Hemolymph (14 ul) was diluted into 250 ul ETOH (70%). After centrifugation, the supernatant was transferred to a microcentrifuge tube and dried under $N_2$. The sugars were resuspended in 200 ul 70 % ETOH. Aliquots of supernatant were dispensed as indicated (25 ul into tubes 1-6, 10 ul into tubes 7-8) and the tubes again dried under $N_2$. Tubes 1, 3, 5, 7 were resuspended in 1.1 ml of $ddH_2O$, while tubes 2, 4, 6, 8 were resuspended in 1.1 ml $ddH_2O$ + 25 ug/ml trehalose.

## 4.1.4 Experimental Protocols

In preliminary experiments evaluating the effect of octopamine, 2 ul of either saline (control) (Davenport & Wright 1985) or $10^{-4}$ M octopamine (treatment) were injected into the abdominal hemocoels of day 4 female moths with a Becton-Dickinson 250 ul tuberculin syringe fitted into a micrometer. After

a predetermined interval (15, 30 or 60 minutes), 1 ul hemolymph samples were collected and assayed by HPTLC. All experiments were performed between 1200 and 1400, before the time period when trehalose titers reach their peak.

The procedure of the cockroach experiments varied from the above in that larger volumes were injected into the abdomens of male American cockroaches, either 10 ul of saline or 10 ul of the appropriate dose of octopamine. Cockroaches were exercised by running them in a battery jar for 30 seconds after injection in order to obtain a greater consistency in response (Matthews & Downer 1974). Samples were collected immediately before injection and 20 minutes post-injection in 70% ETOH in three experiments to compare the HPTLC and anthrone methods. In two experiments, cockroaches were injected with either saline (control) or $10^{-4}$ M octopamine (treatment). In the third experiment, an additional dosage level of octopamine ($10^{-3}$M) was examined.

The procedure for subsequent moth experiments was modified from the preliminary experiments to insure data comparable to that collected for the cockroach. Hemolymph samples were collected immediately before injection. Moths were then injected with 2 ul of either saline or $10^{-4}$ M octopamine and returned to their cages. Since the moths could not be exercised as the cockroaches were, they were stressed by vigorously shaking the cage by hand for 30 seconds. Hemolymph samples were treated as previously stated (3.1.3, 3.1.4). Samples were assayed by both the HPTLC and anthrone methods.

As indicated previously, increases in hemolymph trehalose in response to stress have been hypothesized to result from the effects of octopamine. Therefore, to investigate the possible effects of octopamine on increasing

trehalose titers from other aspects, several experiments were conducted simultaneously with the above octopamine injection experiments. In the first of these experiments the effects of injecting the suggested octopamine antagonist, taurine, were examined. The premise of this experiment was that if the increase in trehalose at 1600 was octopamine mediated, then an injection of taurine would block this increase. The effects of taurine are reported to persist for more than 15 hours after injection of a physiologic concentration in American cockroaches (Hayakawa et al. 1987). Day 4 female moths were injected with 2 ul of either saline or $2.25 \times 10^{-3}$ mM/ul taurine at least two hours before the start of the experiment. Samples were collected at 1500, 1600 and 1700 and assayed by HPTLC.

Three experiments were performed to examine the effects of stress on hemolymph trehalose concentrations. 1) Hemolymph samples were collected repeatedly from day 5 female moths at intervals over a 24 hour period (time 0 (0830), 30 minutes, 1 hour, 6 hours, 24 hours). All moths were chilled 15 minutes at $5^{o}C$ before sampling. 2) Day 4 male moths were divided into two groups: "unstressed" moths were refrigerated as usual before sampling, "stressed" moths were placed into one cage and shaken vigorously for one minute. All moths were sampled after 15 minutes. 3) Day 4 male moths were divided into five groups. In addition to the two groups in experiment 2, moths were injected with 2 ul of either saline or $2.25 \times 10^{-3}$ mM/ul taurine and shaken one minute or moths were sampled without refrigeration. All moths were sampled after 15 minutes.

### 4.1.5 **Data Analysis**

Data were analyzed by the Students t test and ANOVA using the Statistical Analysis System (SAS 1985a, b). Tests were considered significant at $a < 0.1$.

## 4.2 RESULTS & DISCUSSION

### 4.2.1 **Comparison of HPTLC, Anthrone & HPLC**

The results of the comparison of three methods of sugar analysis (HPTLC, anthrone, HPLC) indicate good agreement between all techniques, with the anthrone technique giving slightly higher values than either HPTLC or HPLC, section 3.2.2). The nonspecific nature of the anthrone method (i.e. reactive with any reducing substance present) could explain the slightly higher values resulting from analysis by that technique. Any other reducing substances (including other sugars) present in the hemolymph (3.2.4.1) would also be included in the anthrone quantification.

### 4.2.2 **Accuracy of the Anthrone Method**

Good recovery of hemolymph sugars is indicated. The mean difference ($n = 4$) between the unspiked and spiked tubes was $24.5 \pm 1.0$ ug/ml, with the expected difference 25.0 ug/ml, representing a 2% underestimate.

### 4.2.3 **Octopamine**

#### 4.2.3.1 **Preliminary Experiments**: *Heliothis zea* & *Periplaneta americana*

The effects of octopamine injection in the initial *H. zea* experiments were variable, with increases in trehalose levels ranging from 3.7 to 16.7%. In one case, trehalose titers decreased 15.8 %. Octopamine has been reported to cause a 100 % increase in trehalose levels in the American cockroach (Downer 1979a) and similar results had been expected in the corn earworm. The discrepancy between the expected and actual experimental results led to testing the HPTLC technique on American cockroaches. The results obtained were similar to those for the previous octopamine studies with the moths. At this point it was speculated that the differences in these results may be due to variations in the methods of sugar analysis.

A series of experiments were conducted on American cockroaches where samples were analyzed by both the HPTLC and anthrone methods. In each experiment the HPTLC method gave consistently lower trehalose concentrations than the anthrone method (Table 5). Both techniques gave highly variable responses with changes in sugar concentration ranging from decreases of 3% to increases up to 69%. Interestingly, both magnitude and direction of change varied with the method. Although the anthrone method measures total carbohydrates, this does not account for the lower HPTLC readings. The amount of glucose present in cockroach hemolymph ranged from undetectable levels to about 2.0 ug/ul and was most often present in post-injection samples. T tests comparing pre- and post-injection samples indicated

Table 5. Comparison of Mean Hemolymph Trehalose Concentrations by HPTLC (ug/ul ± SE) and Mean Hemolymph Anthrone Positive Concentrations by Anthrone (ug/ul trehalose units ± SE) Pre- and Post-injection of Saline or Octopamine in *Periplaneta americana*

| | HPTLC | | Anthrone | |
|---|---|---|---|---|
| | pre | post | pre | post |
| Expt. 1 | | | | |
| C | 12.9 ± 1.0 | 13.0 ± 1.0 | 18.7 ± 1.3 | 22.6 ± 1.6 |
| O ($10^{-4}$M) | 11.7 ± 1.0 | 13.3 ± 1.5 | 17.3 ± 0.9 | 22.8 ± 3.2 |
| Expt. 2 | | | | |
| C | 13.8 ± 1.2 | 13.7 ± 1.2 | 16.9 ± 0.8 | 20.5 ± 1.6 |
| O ($10^{-4}$M) | 14.6 ± 1.1 | 15.3 ± 1.7 | 17.7 ± 1.3 | 21.7 ± 1.6 |
| O ($10^{-3}$M) | 12.1 ± 0.9 | 13.5 ± 1.2 | 17.2 ± 0.7 | 19.5 ± 0.9 |
| Expt. 3* | | | | |
| C | 12.1 ± 0.7 | 11.7 ± 1.0 | 16.0 ± 6.7 | 24.9 ± 6.7 |
| | | | 17.5 ± 6.4 | |
| O ($10^{-4}$M) | 12.6 ± 1.2 | 13.9 ± 1.2 | 15.8 ± 5.1 | 26.8 ± 6.9 |
| | | | 16.3 ± 4.9 | 27.5 ± 6.5 |

C = saline injected
O = octopamine injected
*Anthrone means were calculated twice in cases where sample values were below the limit of detection. Lower means include these points as equal to the lower limit of detection (2.5 ug/ul). Higher means exclude these points.
n = 8 for Expt. 1; Expt. 2, O $10^{-4}$M. n = 9 for Expt. 2, O $10^{-3}$ M. n = 10 for Expt. 2, C; Expt. 3.

no significant differences by HPTLC for any experiment, whereas significant differences were found for the anthrone procedure in experiments 1 and 2 (experiment 1: control $p = 0.0807$; experiment 2: control $p = 0.0593$, octopamine $10^{-4}$ $p = 0.0649$, octopamine $10^{-3}$ $p = 0.0655$).

The differences between the anthrone data and the HPTLC data were probably due to the insufficient heating of the anthrone assays. This problem was only discovered after the completion of the cockroach experiments. Since the samples were rather dilute, they could have completely reacted with the anthrone in the reaction time (10 minutes) whereas the much more concentrated standards would not have completed the reaction. In this scenario, the standards, although linear, had fainter color and lower absorbancies, thereby increasing the difficulty in detecting slight changes between the samples. Evidence supporting this idea is provided by later moth experiments, where similarly dilute samples produced absorbancies similar to those of the cockroach samples, while the standard absorbancies were much higher. Good agreement was found between both techniques in these experiments. Another possibility is that the cockroach samples contained some other reducing substance, resulting in apparently higher levels of trehalose than were actually present. In some cases, other reducing substances may produce color early in the reaction, only to appreciably fade by its completion (Koehler 1952). If the samples were not sufficiently heated, such a substance may have contributed to the resulting color, leading to higher trehalose estimates.

### 4.2.3.2 *Heliothis zea* - **Fed**

Corn earworm experiments were resumed after Downer (pers. comm.) indicated that increases in hemolymph trehalose levels were not always obtained following injection of octopamine into cockroaches. Samples were assayed by both the HPTLC and anthrone methods. In the first experiment, moths were given access to sucrose solutions until immediately before the injection procedure. The data collected for the study were analyzed by t test comparing pre- and post-injection samples of each group i.e. control pre- to post-samples or octopamine pre- to post-samples. HPTLC data yielded no change in the controls (21.8 $\pm$ 1.0 vs 21.7 $\pm$ 1.2 ug/ul) and an apparent 6.9% decrease in the treated group (20.2 $\pm$ 1.4 vs 18.8 $\pm$ 1.4 ug/ul), while anthrone data indicated an apparent 3.5% increase in controls (22.6 $\pm$ 1.1 vs 23.4 $\pm$ 2.6 ug/ul) and no change in the treated group (20.5 $\pm$ 1.4 vs 20.6 $\pm$ 1.3 ug/ul) (Figure 12). When tested statistically, however, no significant differences were found in any cases ($p = 0.9916$, $p = 0.5076$ respectively for HPTLC, $p = 0.7536$, $p = 0.9663$ respectively for anthrone). Unlike the earlier cockroach experiments, the HPTLC and anthrone techniques resulted in quite similar hemolymph sugar concentrations, perhaps resulting from the longer heating time.

### 4.2.3.3 *Heliothis zea* - **Starved**

The above procedure was then repeated on moths that had been starved for two days before the experiment to provide data similar to the previously

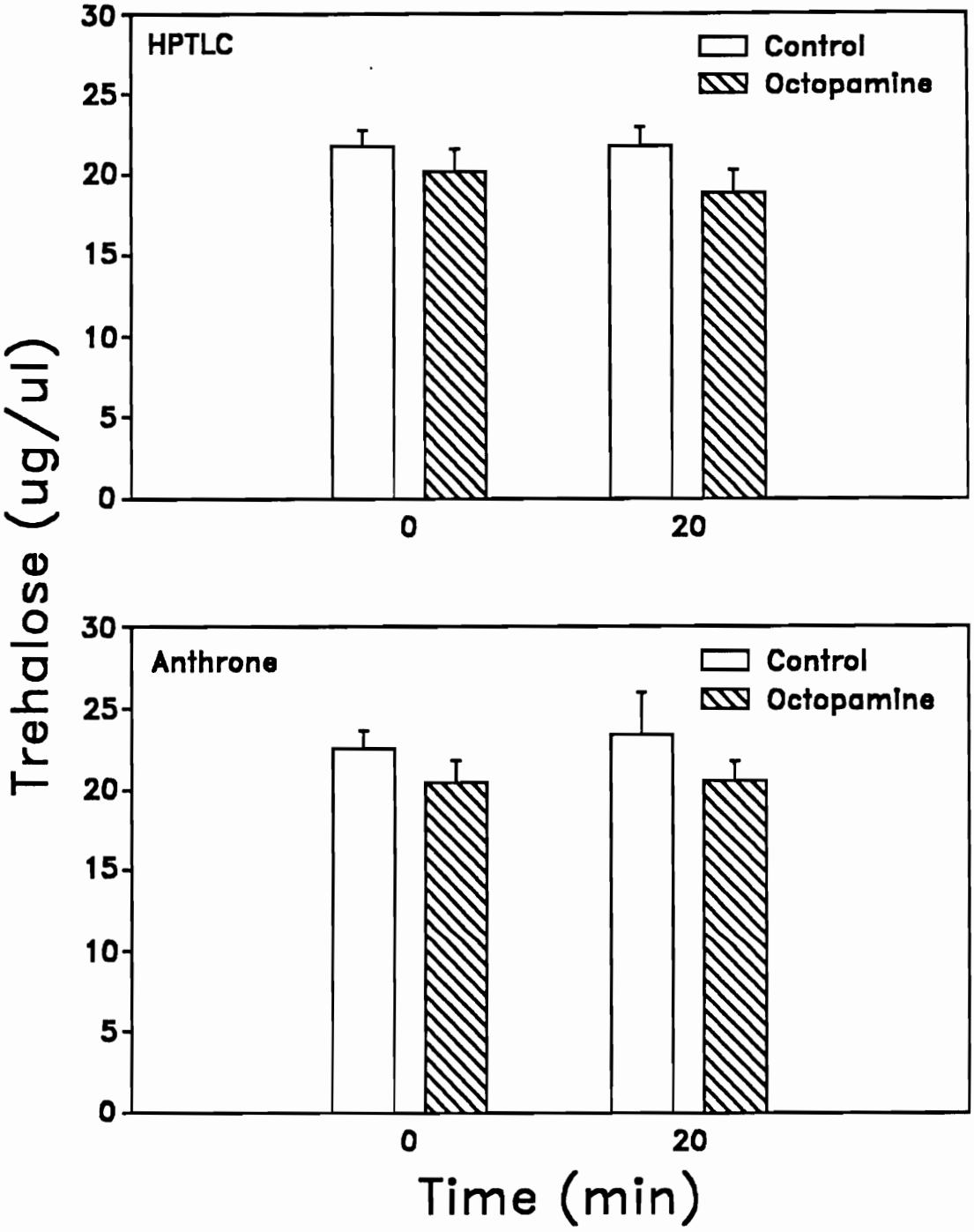


Figure 12. Hemolymph Trehalose Levels Pre-injection (Time 0) and Post -injection (20 Minutes Later) of 2 ul Saline (Control) or $10^{-4}$ M Octopamine in Fed Day 4 Female *Heliothis zea* by HPTLC and Anthrone. (Anthrone concentrations = trehalose units (ug/ul). Vertical bars = SEM. T test of pre- and post-injection means indicated no significant differences. # = Time, A = anthrone, H = HPTLC, C = control, O = octopamine: n = 19 for A: C0, O0, C20. n = 20 for A: O20; H:C0, O0, C20, O20.)

performed cockroach experiments. Samples were assayed and data analyzed as indicated. Trehalose concentrations were much lower than in the previous experiment because the moths had been starved (Figure 13), with the anthrone technique again yielding similar sugar concentrations to the HPTLC technique. Once more, no significant differences were found (t test, $p = 0.7310$, $p = 0.6058$ by HPTLC, $p = 0.6681$, $p = 0.6692$ by anthrone) even though there appeared to be slight increases or decreases. Based on these results it seems reasonable to conclude that the lack of increase in trehalose titers is not a result of the method of analysis, but rather the absence of a measurable octopamine effect in moths. Injection of octopamine into worker honeybees resulted in significant increases in trehalose levels only after two hours (Bounias 1986). Thus, it appears unlikely that octopamine is exhibiting a direct effect. As mentioned earlier, Downer (pers.comm.) has indicated that hypertrehalosemia does not always occur after injection of octopamine into American cockroaches and that several other laboratories have also had difficulty obtaining this response.

### 4.2.4 **Taurine**

When taurine was injected into moths, a decrease in trehalose levels of taurine treated moths occurred at 1600 (Figure 14); however, the uninjected controls did not exhibit the formerly observed peak at 1600 (Figure 9, 3.2.4.4), making these results difficult to interpret. Possibly these moths were different than those previously used as the colony had lost vigor and was re-established with new moths from Gainesville, FL. Moths in the previous experiment had lower basal trehalose levels than those of this experiment (1500: 15.9 ug/ul,

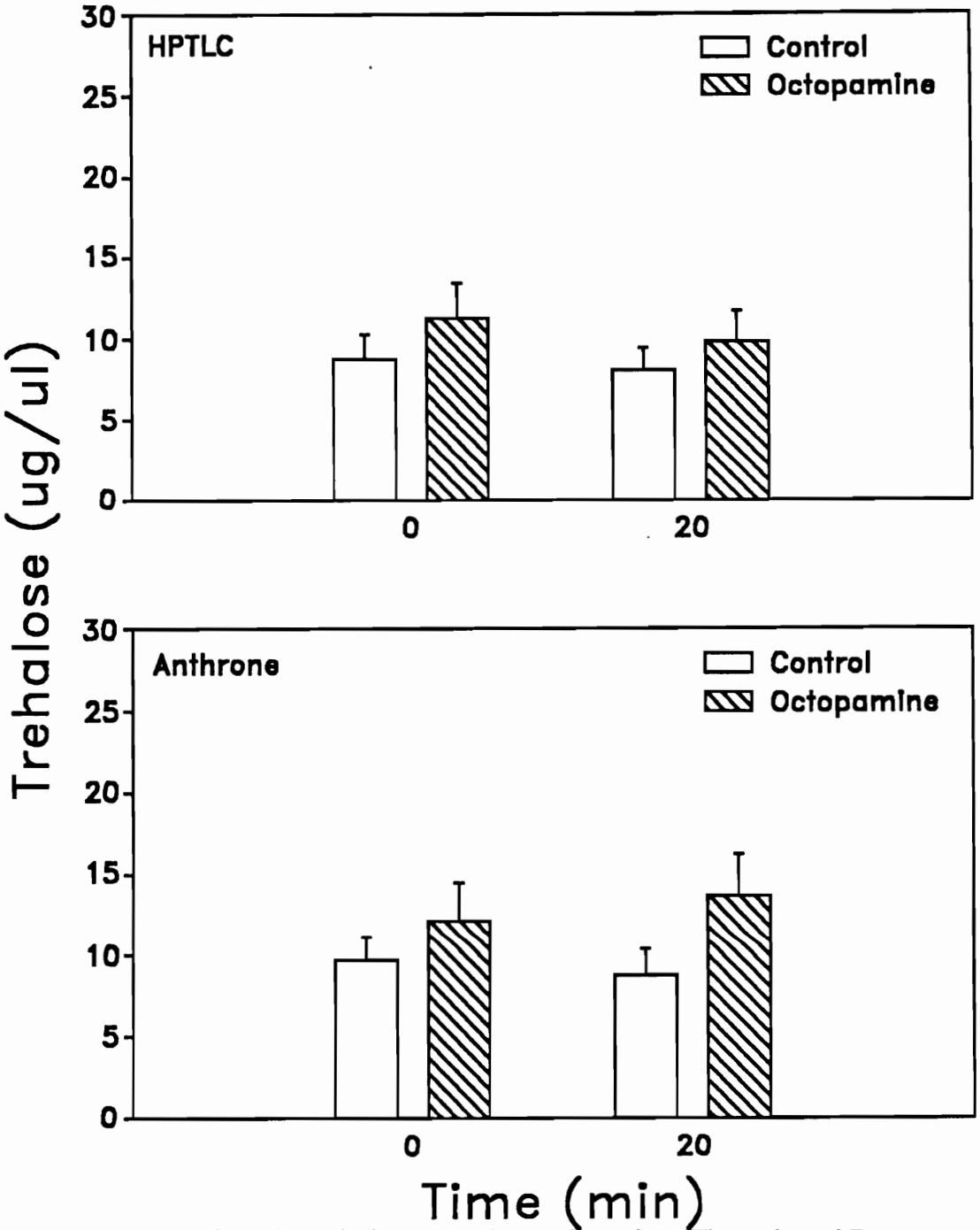


Figure 13. Hemolymph Trehalose Levels Pre-injection (Time 0) and Post -injection (20 Minutes Later) of 2 ul Saline (Control) or $10^{-4}$ M Octopamine in Starved Day 4 Female *Heliothis zea* by HPTLC and Anthrone. (Anthrone concentrations = trehalose units (ug/ul). Vertical bars = SEM. T test of pre- and post-injection means indicated no significant differences. # = Time, A = anthrone, H = HPTLC, C = control, O = octopamine: n = 9 for H: C20, O20; A: O20. n = 10 for A: C20. n = 11 for H: C0, O0; A: C0, O0.)

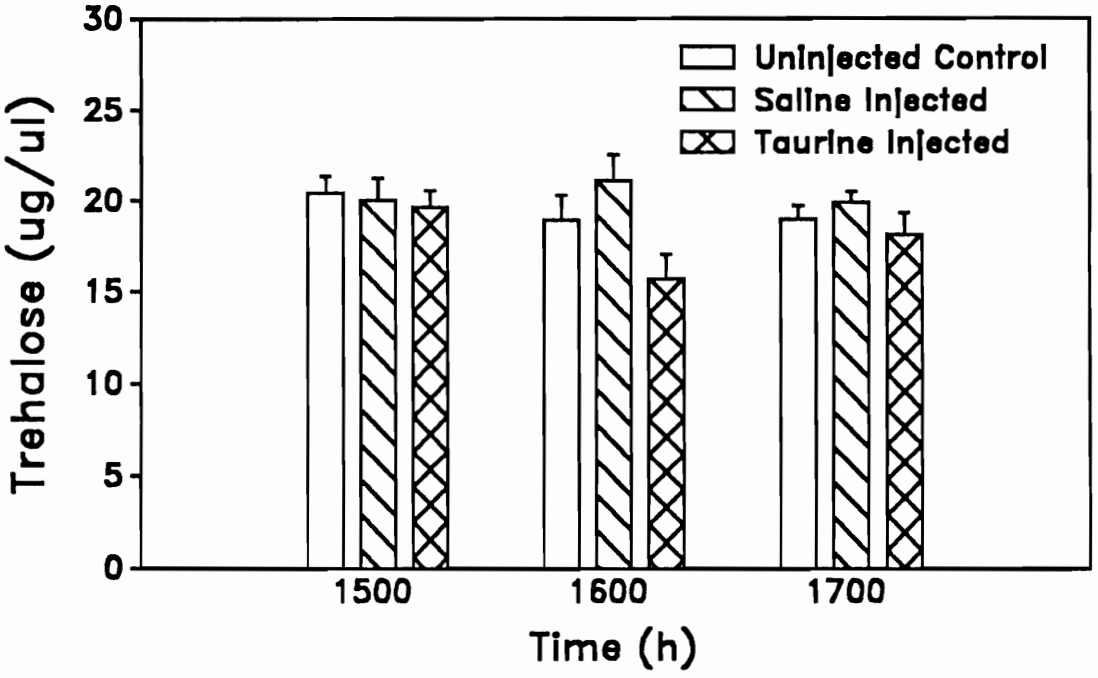


Figure 14. Hemolymph Trehalose Levels at Three Times (1500, 1600, 1700) in Female Day 4 *Heliothis zea*, Uninjected or Following Injection of 2 ul Saline or $2.25 \times 10^{-3}$ mM/ul Taurine. (Sunset = 1700. Vertical bars = SEM. See 4.2.4 for analysis. # = time, C = uninjected control, S = saline injected, T = taurine injected. n = 13 for 1600: C. n = 14 for 1500: C, S. n = 15 for 1500: T; 1700: C. n = 16 for 1600: S; 1700: S, T. n = 17 for 1600: T.)

1600: 17.4 ug/ul, 1700: 11.9 ug/ul vs 1500: 20.4 ug/ul, 1600: 18.9 ug/ul, 1700: 18.9 ug/ul).

Comparison of the three groups at 1600 indicated significant differences only between the saline and taurine injected moths (LSMEANS, $p = 0.0068$) not between uninjected and saline injected moths ($p = 0.2913$) or uninjected and taurine injected moths ($p = 0.1152$). Additionally, the 1600 taurine injected moths differed significantly from the 1500 taurine injected moths (LSMEANS, $p = 0.0225$). It appears that the taurine is having an effect at 1600 but exactly how this occurs is unclear.

### 4.2.5 **Stress**

Stressed cockroaches exhibit an increase in trehalose levels which has been suggested to be caused by octopamine. To see if a similar phenomenon might occur in moths, a variety of stresses were examined, including handling, sampling, shaking, and injection of saline or taurine. When moths were stressed by resampling them over a 24 hour period no significant differences were found (LSMEANS) between any of the groups (Figure 15). Thus, octopamine does not appear to be performing the same function in moths as in cockroaches. Slight increases (high of 9.4% at 30 minutes) and decreases (low of 8.9% at 24 hours) were found when the groups were compared to the baseline measurement at time 0. Matthews and Downer (1974) found a maximum increase in cockroaches at 10 minutes (nearly 15%), with a drop at 20 minutes (to 7.5%) and a subsequent increase at one hour (about 10.8%). Interestingly, the moths exhibited a drop of 6.5% at one hour. It is possible that the increase at 30

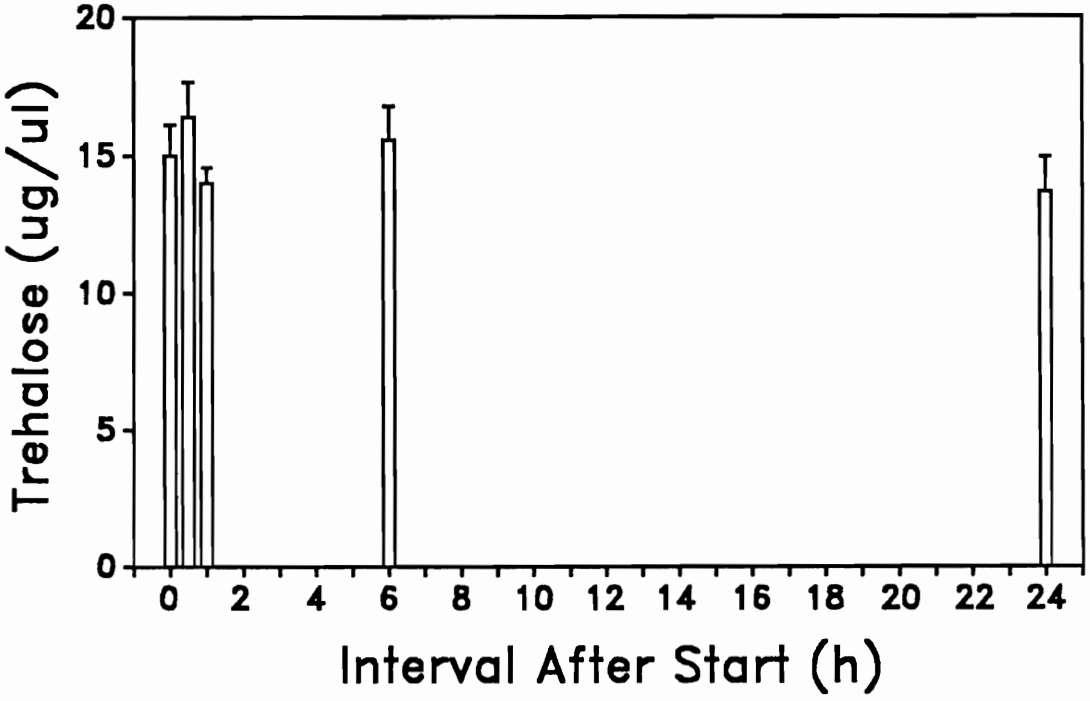


Figure 15. Hemolymph Trehalose Levels at Various Times After Handling and Sampling in Day 5 Female *Heliothis zea*. (Vertical bars = SEM. No significant differences indicated by LSMEANS. # = h after start. n = 7 for 1, 6. n = 9 for 24. n = 10 for 1, 0.5.)

minutes may represent a stress response, even though statistically non-significant. The increase at six hours (1430) is expected since trehalose levels were previously shown to increase over the course of the day (3.2.4.4). These moths had high trehalose concentrations when compared to those previously observed for day 5 or 6 females (3.2.4.3).

In the second experiment moths were provided with a more severe form of stress (shaking) than just handling and sampling. Again, no significant differences were found between stressed and unstressed moths (t test, $p = 0.4749$). Finally, in the third experiment moths were divided into five treatments 1) unstressed, 2) stressed, 3) taurine injected, 4) saline injected and 5) unchilled (Figure 16). No significant differences were found between any of the groups (LSMEANS, lowest $p = 0.1365$). There were no effects of taurine (unlike 4.2.4). It appears that *H. zea*, unlike the American cockroach (Downer 1979a) does not significantly elevate its hemolymph sugars in response to stress. Woodring et al. (1988) reported an increase in hemolymph sugars in response to stress in the house cricket, but did not indicate whether this increase was significant. Thus, the absence of a measurable response in trehalose concentrations of *H. zea* is perhaps not surprising. American cockroaches generally fly short distances utilizing muscle glycogen, with fat body stores of minor importance (Downer & Matthews 1976). As indicated previously, *H. zea* is a migratory insect, and probably more closely resembles the migratory locust, utilizing carbohydrate initially and then switching to lipid utilization as flight continues. If this is the case, hemolymph trehalose titers may be sufficiently high for initial flight, and mobilization once flight has begun may be brought about by hypertrehalosemic hormone (recently synthesized for *H. zea*) as in the locust.

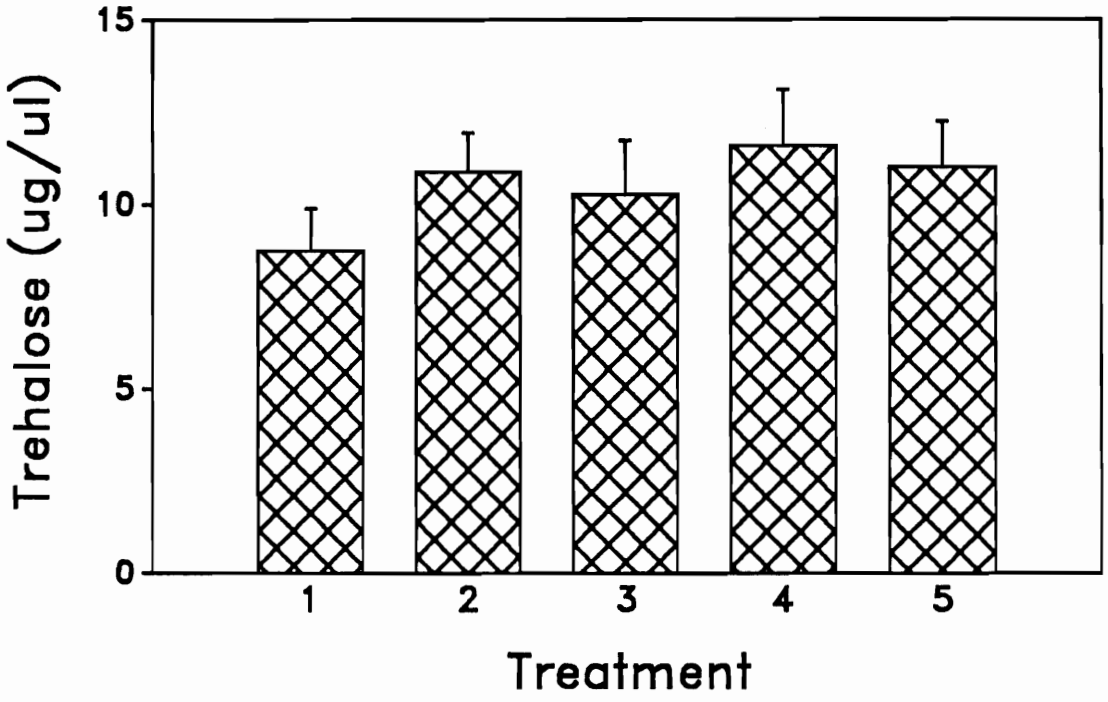


Figure 16. Hemolymph Trehalose Levels After Various Forms of Stress in Day 4 Male *Heliothis zea*. (Treatment: 1 = saline injected, 2 = taurine injected, 3 = shaken, 4 = chilled, 5 = unchilled. Vertical bars = SEM. No significant differences indicated by LSMEANS. n = 5.)

### 4.2.6 **Conclusions**

Originally, it was hypothesized that flight activity and trehalose concentrations were related, a theory that was examined from several aspects simultaneously. Having demonstrated this relationship in section 3, it became of interest to try to determine the cause of trehalose mobilization one hour before sunset. The biogenic amine octopamine was investigated because of its reported role in the elevation of trehalose during the stress response in cockroaches (Downer 1979a) and its ability to increase glycogenolysis in cockroach fat body (Downer 1979b). The expectation was that octopamine would perform a similar function in *H. zea*.

The results of this research do not support the hypothesis of octopamine involvement in elevating trehalose concentrations in the corn earworm. Injection of octopamine into the hemocoel of day 4 female moths resulted in no significant differences from saline injected control females. The nutritional state of the moths did not appear to be a factor as both the moths allowed to feed and those deprived of food for two days before experimentation showed a similar absence of response to octopamine, although the latter group had lower hemolymph trehalose levels. These results were verified by both HPTLC and anthrone analyses. Additionally, the role of octopamine in increasing American cockroach hemolymph trehalose concentrations could not be confirmed.

The possibility of an octopamine effect was also examined indirectly: 1) by applying various forms of stress to moths and 2) by injecting a suspected octopamine antagonist, taurine. If moths responded in a manner similar to

cockroaches, then stress should have resulted in significant increases in hemolymph trehalose levels. Instead, no significant differences were found in any experiments, whether moths were repeatedly sampled, chilled or shaken (all of which cause increases in cockroach trehalose concentrations). The injection of taurine should prevent an increase in trehalose concentration if octopamine is responsible for causing an increase in trehalose. The results of this experiment are equivocal. Significantly lower trehalose concentrations were detected among treated moths at 1600 (one hour before sunset) as anticipated, but control moths did not exhibit the formerly observed peak. This fact along with the lack of an octopamine effect in any other experiments make it unlikely that taurine is inhibiting an octopamine response. Further, taurine had no effect when injected into subsequently stressed moths.

These experiments improved on those of section 3 by utilizing moths reared from the same cohort in each experiment. Additionally, the moths in each replicate had all emerged on the same day. Both of these procedures appeared to improve the consistency of the data, although variability was still great. Unfortunately, data from experiments separated by a large space of time (e.g. 4.2.4) may be quite different, making comparisons difficult.

Other ways of examining the trehalose peak at 1600 could include monitoring octopamine levels in the hemolymph. Monitoring of octopamine in hemolymph has been performed for other insects (Goosey & Candy 1980; Davenport & Evans 1984). This could be done by sampling moths at times surrounding this period. Regrettably, hemolymph samples would have to be pooled since there is no method currently available with adequate sensitivity to detect octopamine in 1 ul hemolymph samples. Should the results of such a

study prove negative, hypertrehalosemic hormone or turnover rates of trehalose might be scrutinized.

Section 5

## SUMMARY

A unique feature of nocturnal moths, including *Heliothis zea*, is the phenomenon whereby populations of moths become active at approximately the same time every day near dusk. Weather permitting, activity begins as soon as the moths are dark adapted, a process that occurs during sunset. Indications are that ocelli play a role in the detection of changes in light intensity, aiding in the regulation of arousal and hence, the onset of activity. Energy requirements increase with the beginning of flight activity. Increases in metabolism provide the needed energy and appear to be regulated by hormonal, including neurohormonal, changes. Octopamine is a multifunctional neurohormone which may function in the control of both increased arousal and increased metabolism. The interrelationships of these three factors, photoreceptors, neurohormonal systems and locomotor related metabolism, are poorly understood.

This interrelationship was studied by examining the influence of flight activity and octopamine injection on hemolymph trehalose concentrations in *H. zea*. Attempts to examine the role of ocelli were discontinued because the thermocoagulation technique for producing anocellate moths was unsuccessful in *H. zea*. Although lipids are known to be the major fuel for flight (Van Handel 1974), changes in trehalose levels were studied because it was suspected that *H. zea* might also use carbohydrates, in the form of trehalose, for flight as many other demonstrated migratory insects do. If trehalose is used, it might be

mobilized before flight actually begins, and this mobilization may be controlled neurohormonally.

HPTLC was the chosen method of analysis because of the specificity, rapidity and relative inexpense of the method compared to other methods. The validity and accuracy of the technique on *H. zea* samples were confirmed by both HPLC and anthrone. Analysis of a pooled *H. zea* hemolymph sample resulted in good agreement between all three methods although the anthrone technique gave a somewhat higher concentration. This may have been due to the nonspecific nature of the anthrone technique as well as working at the low end of the standard curve. Further, spikeover experiments using the HPTLC method indicated good recovery of trehalose although variation among samples was observed. Under the parameters routinely employed for HPTLC, the lower limit of detection was 1.0 ug sugar/ul hemolymph.

Trehalose was indicated by HPTLC as the predominant form of carbohydrate in *H. zea* (82.2-100%), a finding confirmed by HPLC. Other known sugars detected by HPTLC included glucose and sucrose. Small amounts of glucose are to be expected since glucose is involved in the synthesis and breakdown of trehalose, while sucrose probably reflects the diet of the moths. Evidence exists for the presence of additional carbohydrates, however these were not identified. One such possible sugar appears to be a trisaccharide and was observed most often in newly emerged moths. Pooled samples of day 4 moths indicated its presence only in females.

Because samples were examined individually and not pooled, large variations in hemolymph trehalose concentrations were observed, ranging from undetectable (< 1 ug/ul) to 37.4 ug/ul. Such large variations may mask

genuine differences between experimental groups. Interestingly, the absence of detectable trehalose began at day 3 and coincided with observed mortality. Since it could not be clearly determined whether a lack of trehalose indicated a moribund condition (Anelli & Friedman 1986), or if such moths occurred normally in the population, these moths were eliminated from all analyses. Lab-reared moths had higher trehalose titers and were more variable than field collected moths, possibly due to environmental and/or genetic factors.

Flight began shortly before sunset (1700 EST). Males exhibited a bimodal flight pattern with the first peak in activity just after sunset (1700-1900), followed by a second peak between 2000 and 2400. Females were continuously active throughout the night. Males tended to cease flying near sunrise, before females. Little flight occurred on day 1, the day of eclosion. Males flew most on days 3 and 4, whereas females flew most on days 4 and 5. Males flew more than females at every age, probably a result of differing behavioral activities. Females would not be flying when calling to males or ovipositing eggs (whether fertile or not), while males would be flying in search of females.

Mean trehalose concentrations are rather constant for several days after emergence, then begin declining. This decline is significant only on day 6 in males. The decrease in trehalose levels coincides with observed increases in mortality. When day to night trehalose levels are compared over age and sex, some differences are seen but their biological significance in not clear. A diurnal cycle was observed for day 4 moths of both sexes. Presumably, this cycle is present at other ages as well. Trehalose concentrations gradually increase during the day, when flight is minimal, and peak one hour before sunset (1600). This increase in trehalose could occur in several ways: 1) as a result of a greater

rate of synthesis over hydrolysis of trehalose during the day (Van Handel 1978), 2) from the action of hypertrehalosemic hormone or 3) from the action of the neurohormone octopamine. Trehalose decreased between 1600 and 1700. Flight preparation (e.g. wing vibration) was hypothesized to account for the decrease as preflight warm-up is known to enhance the flight repertoire of many Noctuids (Casey & Joos 1983). Examination of the sunset period at 15 minute intervals indicates a general decrease from the peak at 1600 to a low point between 1800 and 1900. Such a decrease correlates well with the peak in flight activity exhibited by males between 1700 and 1900. This finding differs from that of the diurnal study where females had a second peak at 1800 whereas males did not. Which of these is the correct depiction of events during sunset is unknown.

Octopamine was hypothesized to be the causative agent in trehalose mobilization at 1600. Octopamine has been linked to increased trehalose levels in American cockroaches (Downer 1979a, b) and was anticipated to have a similar role in moths. No significant increases in trehalose titers of *H. zea* resulted from direct injection of octopamine into the hemocoel or indirectly by the stress response. The absence of a response was not related to nutritional state or method of sugar analysis (HPTLC or anthrone). Various forms of stress, demonstrated to cause an increase in trehalose titers in American cockroaches, were applied to moths. Moths exhibited no significant increases whether they were chilled, handled or shaken. Injection of taurine, a suspected octopamine antagonist, had no effect in moths subsequently stressed, while in moths sampled at 1600 it caused a significant decrease in trehalose levels. The relevance of the latter response is unclear, since control moths in this

experiment did not exhibit the formerly observed peak in trehalose at 1600. Based on these results, octopamine does not appear to be involved in trehalose mobilization in *H. zea*.

Section 6

## LITERATURE CITED


Adler, P.H. 1987. Temporal feeding patterns of adult *Heliothis zea* (Lepidoptera: Noctuidae) on pigeonpea nectar. Environ. Entomol. 16:424-427.

Agee, H.R. 1969. Mating behavior of bollworm moths. Ann. Entomol. Soc. Am. 62:1120-1122.

Anelli, C.M. & S. Friedman. 1986. Presence and regulation of trehalose in the body of adult *Phormia regina*. Arch. Insect Biochem. & Physiol. 3:381-395.

Applebaum, S.W. & H.M. Schlesinger. 1973. Regulation of locust fat body phosphorylase. Biochem. J. 135:37-41.

Arnesen, S.J. & R.F. Olivo. 1988. The effects of serotonin and octopamine on behavioral arousal in the crayfish. Comp. Biochem. Physiol. 91C:259-263.

Bailey, E. 1975. Biochemistry of insect flight, In: *Insect Biochemistry and Function*, D.J. Candy & B.A. Kilby, editors, Chapmen and Hall, London, pp. 89-176.

Bailey, B.A., R.J. Martin & R.G.H. Downer. 1983. Haemolymph octopamine levels during & following flight in the American cockroach, *Periplaneta americana* L. Can. J. Zool. 62:19-22.

Bedford, J.J. 1977. The carbohydrate levels of insect haemolymph. Comp. Biochem. Physiol. 57A:83-86.

Beenakkers, A.M.Th. 1969. Carbohydrate and fat as a fuel for insect flight, a comparative study. J. Insect Physiol. 15:353-361.

Beenakkers, A.M.Th., J.H. Dewaide, P.Th. Henderson & A. Lutgerhorst. 1967. Fatty acid oxidation and some participating enzymes in animal organs. Comp. Biochem. Biophysiol. 22:675-682.

Beenakkers, A.M.Th., D.J. Van der Horst & W.J.A. Van Marrewijk. 1978. Regulation of release and metabolic function of the adipokinetic hormone in insects, In: *Comparative Endocrinology*, P.J. Gaillard & H.H. Boer, editors, Elsevier North Holland, Amsterdam, pp. 481-494.

Beenakkers, A.M.Th., D.J. Van der Horst & W.J.A. Van Marrewijk. 1981a. Role of lipids in energy metabolism, In: *Energy Metabolism in Insects*, R.G.H. Downer, editor, Plenum Press, New York, pp. 53-100.

Beenakkers, A.M.Th., D.J. Van der Horst & W.J.A. Marrewijk. 1981b. Metabolism during locust flight (minireview). Comp. Biochem. Physiol. 69B:315-321.

Beenakkers, A.M.Th., D.J. Van der Horst & W.J.A. Van Marrewijk. 1984. Insect flight muscle metabolism. Insect Biochem. 14:243-260.

Beenakkers, A.M.Th., D.J. Van der Horst & W.J.A. Van Marrewijk. 1985. Biochemical processes directed to flight muscle metabolism, In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 10, G.A. Kerkut & L.I. Gilbert, editors, Pergamon Press, Oxford, pp. 451-486.

Beutler, R. 1936. Uber den Blutzucker der Bienen. Z. Vergl. Physiol. 24:71-115.

Binder, R.G., T.C. Meikle & B.G. Chan. 1984. Growth and hemolymph trehalose levels of *Heliothis zea* larvae fed diets containing various sugars. Comp. Biochem. Physiol. 79A:251-254.

Bounias, M. 1980. *N*-(napthyl)ethylenediamine dihydrochloride as a new reagent for nanomole quantification of sugars on thin-layer plates by a mathematical calibration process. Anal. Biochem. 106:291-295.

Bounias, M. 1986. Etude cinetique de la glycemie de l'Abeille apres injection de dl-octopamine *in vivo*. C. R. Soc. Biol. 180:512-519.

Brady, J. 1974. The physiology of insect circadian rhythms. Adv. Insect Physiol. 10:1-115.

Brown, J.J. & G.M. Chippendale. 1974. Migration of the monarch butterfly, *Danaus plexippus*: energy sources. J. Insect Physiol. 20:1117-1130.

Brown, S.E. & H.M. Mazzone. 1977. Electrophoretic studies on proteins in the egg and hemolymph of the gypsy moth with reference to isoenzymes. J. N. Y. Entomol. Soc. 85:26-35.

Bursell, E. 1963. Aspects of the metabolism of amino acids in the tsetse fly, *Glossina* sp. (Diptera). J. Insect Physiol. 9:439-452.

Bursell, E. 1981. The role of proline in energy metabolism, In: *Energy Metabolism in Insects*, R.G.H. Downer, editor, Plenum Press, New York, pp. 135-154.

Callahan, P.S. 1958. Behavior of the imago of the corn earworm, *Heliothis zea* (Boddie), with special reference to emergence and reproduction. Ann. Entomol. Soc. Am. 51:271-281.

Candy, D.J. 1978. The regulation of locust flight muscle metabolism by octopamine and other compounds. Insect Biochem. 8:177-181.

Candy, D.J. 1981. Hormonal regulation of substrate transport and metabolism, In: *Energy Metabolism in Insects*, R.G.H. Downer, editor, Plenum Press, New York, pp. 19-52.

Candy, D.J. 1989. Utilization of fuels by the flight muscles, In: *Insect Flight*, G.J. Goldsworthy & C.H. Wheeler, editors, CRC Press, Inc., Boca Raton, Fl., pp. 305-319.

Candy, D.J. & B.A. Kilby. 1961. The biosynthesis of trehalose in the locust fat body. Biochem. J. 78:531-536.

Casey, T.M. 1989. Oxygen consumption during flight, In: *Insect Flight*, G.J. Goldsworthy & C.H. Wheeler, editors, CRC Press, Inc., Boca Raton, Fl., pp. 257-272.

Casey, T.M. & B.A. Joos. 1983. Morphometrics, conductance, thoracic temperature, and flight energetics of Noctuid and Geometrid moths. Physiol. Zool. 56:160-173.

Chen, P.S. 1985. Amino acid and protein metabolism, In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 10, G.A. Kerkut & L.I. Gilbert, editors, Pergamon Press, Oxford, pp. 177-217.

Childress, C.C., B. Sacktor, I.W. Grossman & E. Bueding. 1970. Isolation, ultrastructure, and biochemical characterization of glycogen in insect flight muscle. J. Cell Biol. 45:83-90.

Chino, H. 1981. Lipid transport by hemolymph lipoprotein - a possible multiple role of diacylglyerol-carrying lipoprotein, In: *Energy Metabolism in Insects*, R.G.H. Downer, editor, Plenum Press, New York, pp. 155-167.

Claassen, D.E. & A.E. Kammer. 1986. Effects of octopamine, dopamine, and serotonin on production of flight motor output by thoracic ganglia of *Manduca sexta*. J. Neurobiol. 17:1-14.

Clegg, J.S. & D.R. Evans. 1961a. Blood trehalose and flight metabolism in the blowfly. Science. 134:54-55.

Clegg, J.S. & D.R. Evans. 1961b. The physiology of blood trehalose and its function during flight in the blowfly. J. Exp. Biol. 38:771-792.

Crabtree, B. & E.A. Newsholme. 1975. Comparative aspects of fuel utilization and metabolism by muscle, In: *Insect muscle*, P.N.R. Usherwood, editor, Academic Press, New York, pp. 405-500.

Cullen, J.M. & T.O. Browning. 1978. The influence of photoperiod and temperature on the induction of diapause in pupae of *Heliothis punctigera*. J. Insect Physiol. 24:595-601.

Davenport, A.P. & P.D. Evans. 1984. Stress-induced changes in the octopamine levels of insect haemolymph. Insect Biochem. 14:135-143.

Davenport, A.P. & D.J. Wright. 1985. Physiological saline for the larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) based on an analysis of the hemolymph. J. Econ. Entomol. 78:1151-1153.

Dingle, H. 1972. Migration strategies of insects. Science. 175:1327-1335.

Domroese, K.A. & L.I. Gilbert. 1964. The role of lipid in adult development and flight-muscle metabolism in *Hyalophora cecropia*. J. Exp. Biol. 41:573-590.

Downer, R.G.H. 1979a. Induction of hypertrehalosemia by excitation in *Periplaneta americana*. Insect Physiol. 25:59-63.

Downer, R.G.H. 1979b. Trehalose production in isolated fat body of the American cockroach, *Periplaneta americana*. Comp. Biochem. Physiol. 62C:31-34.

Downer, R.G.H. 1985. Lipid metabolism, In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 10, G.A. Kerkut & L.I. Gilbert, editors, Pergamon Press, Oxford, pp. 77-113.

Downer, R.G.H. & J.R. Matthews. 1976. Glycogen depletion of thoracic musculature during flight in the American cockroach, *Periplaneta americana* L. Comp. Biochem. Physiol. 55B:501-502.

Downer, R.G.H. & G.H. Parker. 1979. Glycogen utilisation during flight in the American cockroach, *Periplaneta americana* L. Comp. Biochem. Physiol. 64A:29-32.

Downer, R.G.H., G.L. Orr, W.D. Gole & I. Orchard. 1984. The role of octopamine & cyclic amp in regulating hormone release from corpora cardiaca of the American cockroach. J. Insect Physiol. 30:457-462.

Dreisig, H. 1980. The importance of illumination level in the daily onset of flight activity in nocturnal moths. Physiol. Entomol. 5:327-342.

Eaton, J.L. 1985. Actograph studies of light effects on *Trichoplusia ni* flight activity, In: *Insect Locomotion*, M. Gewecke and G. Wendler, editors, Paul Parey, Berlin, pp. 233-240.

Eger, J.E., J.A. Witz, A.W. Hartstack & W.L. Sterling. 1982. Survival of pupae of *Heliothis virescens* and *Heliothis zea* (Lepidoptera: Noctuidae) at low temperatures. Can. Entomol. 114:289-301.

Ellington, J.J. & A. El-Sokkari. 1986. A measure of the fecundity, ovipositional behavior, and mortality of the bollworm, *Heliothis zea* (Boddie) in the laboratory. Southwest. Entomol. 11:177-193.

Evans, P.D. 1985. Octopamine, In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 11, G.A. Kerkut & L.I. Gilbert, editors, Pergamon Press, Oxford, pp. 499-530.

Farrer, R.R. & J.R. Bradley. 1985. Within-plant distribution of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs and larvae on cotton in North Carolina. Environ. Entomol. 14:205-209.

Farrow, R.A. & J.C. Daly. 1987. Long-range movements as an adaptive strategy in the genus *Heliothis* (Lepidoptera: Noctuidae): a review of its occurrence and detection in four pest species. Aust. J. Zool. 35:1-24.

Fell, R.D. In press. The qualitative and quantitative analysis of insect hemolymph sugars by high performance thin-layer chromatography. Comp. Biochem. Physiol.

Fitt, G.P. 1987. Ovipositional responses of *Heliothis* spp. to host plant variation in cotton (*Gossypium hirsutum*), In: *Insects-Plants*. Proc. 6th Int. Symp. Insect-Plant Relat., Pau, France, 1986, Dordrecht, the Netherlands: Junk, pp. 289-294.

Fitt, G.P. 1989. The ecology of *Heliothis* species in relation to agroecosystems. Ann. Rev. Entomol. 34:17-52.

Fried, B. & J. Sherma. 1986. Thin-Layer Chromatography. Marcel Dekker, Inc., New York, 394 pp.

Friedman, S. 1967. The control of trehalose synthesis in the blowfly, *Phormia regina*. J. Insect Physiol. 13:397-405.

Friedman, S. 1978. Trehalose regulation, one aspect of metabolic homeostasis. Ann. Rev. Entomol. 23:389-407.

Friedman, S. 1985. Carbohydrate metabolism, In: *Comprehensive Insect Physiology, Biochemistry and Pharmocology*, Vol. 10, G.A. Kerkut & L.I. Gilbert, editors, Pergamon Press, Oxford, pp. 43-76.

Fye, R.E. & W.C. McAda. 1972. Laboratory studies on the development, longevity and fecundity of six lepidopterous pests of cotton in Arizona. US Dep. Agric. Agric. Res. Serv. Tech. Bull. Vol. 1454. 72 pp.

Gilbert, L.I. 1967. Lipid metabolism and function in insects. Adv. Insect Physiol. 4:69-211.

Goldsworthy, G.J. 1970. The action of hyperglycaemic factors from the corpus cardiacum of *Locusta migratoria* on glycogen phosphorylase. Gen. Comp. Endocrinol. 14:78-85.

Goldsworthy, G.J. & W. Mordue. 1974. Neurosecretory hormones in insects. J. Endocrinol. 60:529-558.

Goosey, M.W. & D.J. Candy. 1980. The d-octopamine content of the haemolymph of the locust, *Shistocerca americana gregaria* and its elevation during flight. Insect Biochem. 10:393-397.

Hansen, S.A. 1975. Thin-layer chromatographic method for the identification of mono-, di- and trisaccharides. J. Chromatogr. 107:224-226.

Hansford, R.G. & R.N. Johnson. 1975. The nature and control of the tricarboxylate cycle in beetle flight muscle. Biochem. J. 148:389-401.

Hartstack, A.W., J.D. Lopez, R.A. Muller, W.L. Sterling, E.G. King, et al. 1982. Evidence of long range migration of *Heliothis zea* into Texas and Arkansas. Southwest. Entomol. 7:188-201.

Hayakawa, Y., R.P. Bodnaryk & R.G.H. Downer. 1987. Effect of taurine on excitation-induced changes in brain and hemolymph octopamine levels in *Periplaneta americana*. Arch. Insect Biochem. Physiol. 5:129-137.

Hendrix, W.H., T.H. Mueller, J.R. Phillips & O.K. Davis. 1987. Pollen as an indicator of long-distance movement of *Heliothis zea* (Lepidoptera: Noctuidae). Environ. Entomol. 61:1148-1151.

Henneberry, T.J. & A.N. Kishaba. 1966. Cabbage loopers, In: *Insect colonization and mass production*, C.N. Smith, editor, New York, Academic Press, pp. 461-478.

Hinks, C.F. 1967. Relationship between serotonin and the circadian rhythm of some nocturnal moths. Nature. 214:386-387.

Hsiao, H.S. 1978. Circadian activity of *Heliothis zea* at different temperatures studied with an acoustic actograph. Ann. Entomol. Soc. Am. 71:115-120.

Isely, D. 1935. Relation of hosts to abundance of cotton bollworm. Arkansas Agric. Exp. Stn. Bull. Vol. 320. 30 pp.

Jabbar, A. & R.H.C. Strang. 1985. The effects of chemical and physical stress on the concentrations of amino compounds in the haemolymph and nervous system of locusts and cockroaches. J. Insect Physiol. 31:359-370.

Jaffe, H., A.K. Raina, C.T. Riley, B.A. Fraser, T.G. Bird, C. Tseng, Y. Zhang & D.K. Hayes. 1988. Isolation and primary structure of a neuropeptide hormone from *Heliothis zea* with hypertrehalosemic and adipokinetic activities. Biochem. Biophys. Res. Comm. 155:344-350.

Jahagirdar, A.P., R.G.H. Downer, & T. Viswanatha. 1984. Influence of octopamine on trehalase activity in muscle and hemolymph of the American cockroach, *Periplaneta americana* L. Biochim. Biophys. Acta. 801:177-183.

Johnson, C.G. 1969. Migration and dispersal of insects by flight. Methuen, London. 763 pp.

Johnson, M.W., R.E. Stinner & R.L. Rabb. 1975. Ovipositional response of *Heliothis zea* to its major hosts in North Carolina. Environ. Entomol. 4:291-297.

Jones, J., J.V. Stone & W. Mordue. 1977. The hyperglycaemic activity of locust adipokinetic hormone. Physiol. Entomol. 2:185-187.

Judge, D.N. 1988. Flight activity and hemolymph diacylglyceride concentrations in *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). Masters Thesis, Virginia Polytechnic Institute and State University. 110 pp.

Jutsum, A.R. & G.J. Goldsworthy. 1976. Fuels for flight in *Locusta*. J. Insect Physiol. 22:243-249.

Kammer, A.E. & B. Heinrich. 1978. Insect flight metabolism. Adv. Insect Physiol. 13:133-228.

Kennedy, J.S. 1961. A turning point in the study of insect migration. Nature. 189:785-791.

King, E.G. & R.J. Coleman. 1989. Potential for biological control of *Heliothis* species. Ann. Rev. Entomol. 34:53-75.

Koehler, L.H. 1952. Differentiation of carbohydrates by anthrone reaction rate and color intensity. Anal. Chem. 24:1576-1579.

Kozhantschikov, I. 1938. Carbohydrate and fat metabolism in adult Lepidoptera. Bull. Entomol. Res. 29:103-114.

Kravitz, E.A., B. Beltz, S. Glusman, M. Goy, R. Harris-Warrick, M. Johnston, M. Livingstone & T. Schwarz. 1984. The well-modulated lobster: the roles of serotonin, octopamine, and proctolin in the lobster nervous system. Pesticide Biochem. Physiol. 22:133-147.

Leppla, N.C., E.W. Hamilton, R.H. Guy & F.L. Lee. 1979. Circadian rhythms of locomotion in six Noctuid species. Ann. Entomol. Soc. Am. 72:209-215.

Lingren, P.D., W.B. Warner, J.R. Raulston, M. Kehat, T.J. Henneberry, et al. 1988. Observations on the emergence of adults from natural populations of corn earworm, *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). Environ. Entomol. 17:254-258.

Lopez, J.D., A.W. Hartstack & R. Beach. 1984. Comparative pattern of emergence of *Heliothis zea* and *H. virescens* (Lepidoptera: Noctuidae) from overwintering pupae. J. Econ. Entomol. 77:1421-1426.

Matthews, J.R. & R.G.H. Downer. 1973. Hyperglycaemia induced by anaesthesia in the American cockroach, *Periplaneta americana*. Can. J. Zool. 51:395-397.

Matthews, J.R. & R.G.H. Downer. 1974. Origin of trehalose in stress-induced hyperglycaemia in the American cockroach, *Periplaneta americana*. Can. J. Zool. 52:1005-1010.

Matthews, J.R., R.G.H. Downer & P.E. Morrison. 1975. Estimation of glucose in the haemolymph of the American cockroach, *Periplaneta americana*. Comp. Biochem. Physiol. 53A:165-168.

Maurizio, A. 1965. Untersuchungen uber das Zuckerbild Hamolymphe der Honigbiene (*Apis mellifica* L.). I. Das Zuckerbild des Blutes Erwachsener Bienen. J. Insect Physiol. 11:745-763.

Mayer, R.J. & D.J. Candy. 1969. Control of hemolymph lipid concentration during locust flight: an adipokinetic hormone from the corpora cardiaca. J. Insect Physiol. 15:611-620.

Mordue, W. & G.J. Goldsworthy. 1969. The physiological effects of corpora cardiaca extracts in locusts. Gen. Comp. Endocrinol. 12:360-369.

Mullins, D.E. 1985. Chemistry and physiology of the hemolymph, In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 3, G.A. Kerkut & L.I. Gilbert, editors, Pergamon Press, Oxford, pp. 355-400.

Nayar, J.K. & E. Van Handel. 1971a. Flight performance and metabolism of the moth *Spodoptera frugiperda*. J. Insect Physiol. 17:2475-2479.

Nayar, J.K. & E. Van Handel. 1971b. The fuel for sustained mosquito flight. J. Insect Physiol. 17:471-481.

Nettles, Jr., W.C., B. Parro, C. Sharburgh & C.L. Mangum. 1971. Trehalose and other carbohydrates in *Anthonomus grandis*, *Heliothis zea*, and *Heliothis virescens* during growth and development. J. Insect Physiol. 17:657-675.

Normann, T.C. & H. Duve. 1969. Experimentally induced release of a neurohormone influencing hemolymph trehalose level in *Calliphora erythrocephala* (Diptera). Gen. Comp. Endocrinol. 12:449-459.

Nowosielski, J.W. & R.L. Patton. 1964. Daily fluctuation in the blood sugar concentration of the house cricket, *Gryllus domesticus* L. Science. 144:180-181.

Orchard, I. 1982. Octopamine in insects: neurotransmitter, neurohormone, and neuromodulator. Can. J. Zool. 60:659-669.

Orchard, I. & A.B. Lange. 1983. The hormonal control of haemolymph lipid during flight in *Locusta migratoria*. J. Insect Physiol. 29:639-642.

Orchard, I. & B.G. Loughton. 1981. Is octopamine a transmitter mediating hormone release in insects? J. Neurobiol. 12:143-153.

Orchard, I. J.A. Carlisle, B.G. Loughton, J.W.D. Gole & R.G.H. Downer. 1982. *In vitro* studies on the effects of octopamine on locust fat body. Gen. Comp. Endocrinol. 48:7-13.

Palmer, J.K. 1979. Sugars, sugar alcohols, and sacchrin, In: *GLC and HPLC Determination of Therapeutic Agents*, Part 3, K. Tsuji, editor, Marcel Dekker, Inc., New York, pp. 1317-1340.

Provost, M.W. 1952. The dispersal of *Aedes taeniorhynchus* I. Preliminary studies. Mosq. News. 12:174-190.

Quaintance, A.L. & C.T. Brues. 1905. The Cotton Bollworm. US Dep. Agric. Tech. Bull. Vol. 50. 155 pp.

Raina, A.K., J.A. Klun & E.A. Stadelbacher. 1986. Diel periodicity and effect of age and mating on female sex pheromone titer in *Heliothis zea* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 79:128-131.

Raina, A.K., E.A. Stadelbacher & R.L. Ridgway. 1989. Comparison of sex pheromone composition and pheromone-mediated male behavior of laboratory-reared and wild *Heliothis zea* (Lepidoptera: Noctuidae). J. Chem. Ecol. 15:1259-1265.

Roach, S.H. & P.L. Adkisson. 1970. Role of photoperiod and temperature in the induction of pupal diapause in the bollworm, *Heliothis zea*. J. Insect Physiol. 16:1591-1597.

Rowan, A.N. & E.A. Newsholme. 1979. Changes in the contents of adenine nucleotides and intermediates of glycolysis and the citric acid cycle in flight muscle of the locust upon flight and their relationship to the control of the cycle. Biochem. J. 178:209-216.

Rummel, D.R., K.C. Neece, M.D. Arnold & B.A. Lee. 1986. Overwintering survival and spring emergence of *Heliothis zea* (Boddie) in the Texas Southern High Plains. Southwest. Entomol. 11:1-9.

Sacktor, B. & E.C. Hurlbut. 1966. Regulation of metabolism in working muscle *in vivo*. J. Biol. Chem. 241:632-634.

Saito, S. 1963. Trehalose in the body fluid of the silkworm, *Bombyx mori* L. J. Insect Physiol. 9:509-519.

SAS. 1985a. SAS user's guide: basics. SAS Institute Inc., Cary, N.C.

SAS. 1985b. SAS user's guide: statistics. SAS Institute Inc., Cary, N.C.

Saunders, D.S. 1982. Insect Clocks. Pergamon Press, New York. 409 pp.

Schneider, J.C., J.H. Benedict, F. Gould, W.R. Meredith, M.F. Schuster, et al. 1986. Interaction of *Heliothis* with its host plants. In: *Theory and tactics of* Heliothis *population management. I. Cultural and biological control*, Vol. 316, S.J. Johnson, E.G. King & J.R. Bradley, editors, South. Coop. Ser. Bull., pp. 3-21.

Southwood, T.R.E. 1962. Migration of terrestrial arthropods in relation to habitat. Biol. Rev. 37:171-214.

Sprint, M.M. & J.L. Eaton. 1987. Flight behavior of normal and anocellate cabbage loopers (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 80:468-471.

Stadelbacher, E.A. & T.R. Pfrimmer. 1972. Winter survival of the bollworm at Stoneville, Mississippi. J. Econ. Entomol. 65:1030-1034.

Steele, J.E. 1961. Occurrence of a hyperglycaemic factor in the corpus cardiacum of an insect. Nature. 192:680-681.

Steele, J.E. 1963. The site of action of insect hyperglycemic hormone. Gen. Comp. Endocrinol. 3:46-52.

Steele, J.E. 1976. Hormonal control of metabolism in insects. Adv. Insect Physiol. 12:239-323.

Steele, J.E. 1981. The role of carbohydrate metabolism in physiological function, In: *Energy Metabolism in Insects*, R.G.H. Downer, editor, Plenum Press, New York, pp. 101-133.

Stevenson, E. 1968. Carbohydrate metabolism in the flight muscle of the southern armyworm moth, *Prodenia eridania*. J. Insect Physiol. 14:179-198.

Storey, K.B. 1985. Metabolic biochemistry of insect flight, In: *Circulation, Respiration and Metabolism*, R. Gilles, editor, Springer-Verlag, Berlin, pp. 193-207.

Teo, L., H.W. Fescemyer, J.P. Woodring & A.M. Hammond. 1987. Carbohydrate and fatty acid titres during flight of the migrant Noctuid moth, *Anticarsia gemmatalis* Hubner. Insect Biochem. 17:777-781.

Thompson, S.N. 1986. Effect of the insect parasite *Hyposoter exiguae* (Viereck) on the carbohydrate metabolism of its host, *Trichoplusia ni* (Hubner). J. Insect Physiol. 32:287-293.

Trevelyan, W.E. & J.S. Harrison. 1952. Studies on yeast metabolism 1. Fractionation and microdetermination of cell carbohydrates. Biochem. J. 50:298-302.

Van der Horst, D.J., J.H.M. Abbink, J.M. Van Marrewijk & A.M.Th. Beenakkers. 1983. Glycerol dynamics and metabolism during flight of the locust, *Locusta migratoria*. Insect Biochem. 13:45-55.

Van der Horst, D.J., N.M.D. Houben & A.M.Th. Beenakkers. 1980. Dynamics of energy substrates in the haemolymph of *Locusta migratoria* during flight. J. Insect Physiol. 26:441-448.

Van der Horst, D.J., J.M. Van Doorn & A.M.Th. Beenakkers. 1978. Dynamics in the haemolymph trehalose pool during flight of the locust, *Locusta migratoria*. Insect Biochem. 8:413-416.

Van Handel, E. 1974. Lipid utilization during sustained flight of moths. J. Insect Physiol. 20:2329-2332.

Van Handel, E. 1978. Trehalose turnover and the coexistence of trehalose and active trehalase in cockroach haemolymph. J. Insect Physiol. 24:151-153.

Van Handel, E. & J.K.Nayar. 1972a. Turn-over of diglycerides during flight and rest in the moth *Spodoptera frugiperda*. Insect Biochem. 2:8-12.

Van Handel, E. & J.K. Nayar. 1972b. Direct use of carbohydrates during sustained flight in the moth, *Spodoptera frugiperda*. Insect Biochem. 2:203-208.

Van Marrewijk, W.J.A., A.Th.M. Van den Broek & A.M.Th. Beenakkers. 1980. Regulation of glycogenolysis in the locust fat body during flight. Insect Biochem. 10:675-679.

Van Marrewijk, W.J.A., A.Th.M. Van den Broek & A.M.Th. Beenakkers. 1988a. Isolation and partial characterization of three forms of glycogen phosphorylase from fat body of *Locusta migratoria*. Insect Biochem. 18:37-44.

Van Marrewijk, W.J.A., A.Th.M. Van den Broek, P.N.E. de Graan & A.M.Th. Beenakkers. 1988b. Formation of partially phosphorylated glycogen phosphorylase in the fat body of the migratory locust. Insect Biochem. 18:821-827.

Vejbjerg, K. & T.C. Normann. 1974. Secretion of hyperglycaemic hormone from the corpus cardiacum of flying blowflies, *Calliphora erythrocephala*. J. Insect Physiol. 20:1189-1192.

Weeda, E., C.A.D. De Kort & A.M.Th. Beenakkers. 1979. Fuels for energy metabolism in the Colorado potato beetle, *Leptinotarsa decemlineata* Say. J. Insect Physiol. 25:951-955.

Weins, A.W. & L.I. Gilbert. 1967. Regulation of carbohydrate mobilization and utilization in *Leucophaea maderae*. J. Insect Physiol. 13:779-794.

Wheeler, C.H. 1989. Mobilization and transport of fuels to the flight muscles, In: *Insect Flight*, G.J. Goldsworthy & C.H. Wheeler, editors, CRC Press, Inc., Boca Raton, Fl., pp. 273-303.

Willers, J.L., J.C. Schneider & S.B. Ramaswamy. 1987. Fecundity, longevity and caloric patterns in female *Heliothis virescens*: changes with age due to flight and supplemental carbohydrate. J. Insect Physiol. 33:803-808.

Woodring, J.P., O.W. Meier & R. Rose. 1988. Effect of development, photoperiod, and stress on octopamine levels in the house cricket, *Acheta domesticus*. J. Insect Physiol. 34:759-765.

Wyatt, G.R. 1967. The biochemistry of sugars and polysaccharides in insects. Adv. Insect Physiol. 4:287-360.

Ziegler, R. 1979. Hyperglycaemic factor from the corpora cardiaca of *Manduca sexta* (L.) (Lepidoptera: Sphingidae). Gen. Comp. Endocrinol. 39:350-357.

Ziegler, R. 1985. Metabolic energy expenditure and its hormonal regulation, In: *Environmental Physiology and Biochemistry of Insects*, K.H. Hoffman, Springer-Verlag, New York, pp. 95-118.

Ziegler, R. & M. Schulz. 1986a. Regulation of lipid metabolism during flight in *Manduca sexta*. J. Insect Physiol. 32:903-908.

Ziegler, R. & M. Schulz. 1986b. Regulation of carbohydrate metabolism during flight in *Manduca sexta*. J. Insect Physiol. 32:997-1001.

## VITA

The author, Deborah Ann Davidson, was born on 19 October 1955 in Baltimore, Maryland. After graduating from Northern High School, she attended Goucher College and Towson State University, receiving a B.A. in Biology from the latter in 1979. During the last course of her B.A. she developed an interest in entomology which she decided to pursue by entering the Masters program at Towson State University in the Summer of 1980. Deb completed a project on the systematics of bee flies of San Salvador Island and was awarded her M.S. in Biology in August of 1982. Subsequently, she performed allergy research for the Division of Allergy and Immunology at the Johns Hopkins School of Medicine in Baltimore. In the Fall of 1985 she came to VPI & SU to pursue a Ph.D. in Entomology, and is currently a 1990 candidate for the degree.