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BIONOMICS OF *CARDIOCHILES NIGRICEPS* VIERECK, A PARASITE OF TOBACCO BUDWORM

by

Robert Leroy Bertwell

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF ENTOMOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by ROBERT LEROY BERTWELL

entitled BIONOMICS OF CARDIOCHILES NIGRICEPS VIERECK, A PARASITE OF TOBACCO BUDWORM

be accepted as fulfilling the dissertation requirement for the degree of DOCTOR OF PHILOSOPHY

Roger T. Nuber — Dissertation Director — May 5, 1977 — Date

As members of the Final Examination Committee, we certify that we have read this dissertation and agree that it may be presented for final defense.

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Final approval and acceptance of this dissertation is contingent on the candidate's adequate performance and defense thereof at the final oral examination.

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ABSTRACT

Investigations were conducted to determine the feasibility of utilizing the parasite Cardiochiles nigriceps Viereck as a biological control agent of Heliothis virescens (F.) in Arizona. Therefore, the following six areas of interest were investigated: (1) effect of parasitism on host feeding, (2) selection for early parasite emergence, (3) parasite storage capability, (4) parasite fecundity, (5) parasite longevity, and (6) parasite searching capacity. These areas of study were chosen because it was believed that C. nigriceps would probably not become an established parasite of H. virescens in Arizona.

Experiments conducted to determine the effect of parasitism on host feeding, selection for early parasite emergence, and parasite fecundity and longevity were accomplished in an Environator® bioclimatic cabinet programmed for a daily photoperiod of 17 hours of light and 7 hours of darkness. Relative humidity was maintained at 70 ±10% and temperature was programmed for 30°C.

Host larval and fecal weights were used to determine the effect of parasitism on host feeding.

Consumption of food by host larvae fed on lima bean diet or cotton squares and bolls was significantly reduced in parasitized individuals.

Selection for early parasite emergence involved segregating all adult parasites that emerged within 10 days of each other into individual cages where they were allowed to mate. Results indicated that more than 85% of all parasites could be expected to emerge within 12 days of each other and that 53 to 54% would be females.

Parasite fecundity and longevity were examined while being continuously exposed to host larvae for 1- and 24-hour periods. Parasite longevity, the number of host larvae dying, the number of host larvae escaping and percent host parasitism were not significantly different in the two host exposure periods.

Parasite storage capability was accomplished in bioclimatic cabinets in which parasite pupae were held at 5°, 10°, and 15°C in constant darkness. Storage proved feasible at each temperature for 2 weeks or less. Longer periods of storage were not successful because temperatures were not low enough to prevent continued parasite development.

Parasite searching capacity was examined in greenhouse releases where mean temperature extremes ranged from 15.3° to 36.3°C (N:D) and mean humidity ranged from 97.8 to

49.5% (N:D). Preliminary studies were on cotton plants enclosed with nylon organdy cloth with subsequent releases being made in an open greenhouse. Results in both cases indicated that C. nigriceps was not capable of providing the kind of control that would be required in an inundative release program.

INTRODUCTION

The tobacco budworm, Heliothis virescens (F.), is a pest of many crops and has been a serious pest of cotton (Gossypium hirsutum L. and G. barbadense L.) throughout Texas and the southeastern portion of the United States for several years. More recently, the tobacco budworm has risen to pest status in Arizona, culminating in a major pest outbreak in Maricopa County cotton-growing regions in 1976. Problems of growing concern with H. virescens are insecticide resistance (Harris et al. 1972) and insecticide destruction of beneficial insects that play a vital role in reducing pest populations. These have accentuated the need for additional means of control while minimizing the use of insecticides.

Several researchers have examined the potential role of parasitic insects for control of Heliothis species in cultivated crops (Lewis et al. 1971; Knipling and McGuire 1968; Parsons and Ullyett 1966; Lewis and Brazzel 1966a). Little use has been made of these important biological agents against cotton pests, possibly because many feel that parasites are too erratic to provide reliable control. Since there have been many cases where parasitic insects have been successful in control, or have greatly

aided in the control of serious pests of other crops, it is hypothesized that Cardiochiles nigriceps Viereck may have the potential of being an important factor in control of H. virescens (F.) in Arizona.

To utilize C. nigriceps as a biological control agent of H. virescens, an understanding of its biology and the biological relationship to its host is necessary. The objective of this research was to develop such an understanding, and to accomplish this, the following six areas of interest were investigated: (1) effect of parasitism on host feeding, (2) selection for early parasite emergence, (3) parasite storage capability, (4) parasite fecundity, (5) parasite longevity, and (6) parasite searching capacity.

These areas of study were chosen because it was believed that C. nigriceps would probably not become an established parasite of H. virescens in Arizona, in that the host is infrequently present in large enough numbers to support a parasite population. Further doubt existed that the parasite would be adaptable on a long term basis to the rather harsh environmental conditions that are present in Arizona. Subsequent research was directed toward an inundative parasite release approach which would be directed toward reducing the effect of H. virescens populations in cotton. The benefit from such an approach would be to reduce or, in some instances, eliminate the need for insecticide applications used against this pest in cotton.

LITERATURE REVIEW

Cardiochiles nigriceps Viereck

Description

Cardiochiles nigriceps Viereck is a member of the hymenopterous superfamily Ichneumonoidea and family Braconidae, the adult of which has been described by Viereck (1912, pg. 575) in the following manner:

> Female.----Length 7.4mm; head including antenna and palpi, prescutum, scapulae, pleurae, wings, coxae, trochanters, fore and mid femora, hind tarsi, tips of hind tibiae and propodeum mostly black or blackish, elsewhere, excepting fore and mid tibae and tarsi which are dark brown or blackish, mostly reddish. Related to *C. viator* (Say) and *C. seminigra* (Cresson).

Distribution and Economic Importance

C. nigriceps is distributed throughout the southeastern United States where it is recognized as an important parasite of the tobacco budworm, *H. virescens* (F.). Chamberlin and Tenhet (1926) rated this parasite first among several species of parasites that attack the tobacco budworm in the tobacco (*Nicotiana tabacum* L.)-growing sections of Georgia and Florida. Seasonal abundance reportedly remained fairly constant from year to year. During July and August, parasitism by this insect was found frequently to average

from 50 to nearly 100%. These same authors stated that this parasite had also been reported from Alabama and Mississippi, and had been found as far north as Huntsville, Arkansas, and Clarksville, Tennessee. Grayson (1944) observed from 78.6 to 95.2% parasitism of H. virescens by C. nigriceps during the summer of 1943 in tobacco fields near Chatham, Virginia. Neunzig (1963) and Snow, Hamm, and Brazzel (1966) reported that large numbers of H. virescens collected from wild host plants were parasitized by C. nigriceps. Lewis and Brazzel (1966b) found that C. nigriceps was an important parasite of H. virescens collected from several wild and cultivated host plants in Mississippi.

Life History

Several authors have contributed studies to the life history of C. nigriceps. Chamberlin and Tenhet (1926), Lewis and Brazzel (1966a), and Vinson and Lewis (1965) reported on the biology of C. nigriceps in the prepupal, pupal and adult stages. Chamberlin and Tenhet (1926) described the eggs which they removed from the adult female parasite, and described also the general characteristics of the fully developed larva which emerged from its host. Lewis and Vinson (1968) described and illustrated the egg and larval development of C. nigriceps. A summary of each developmental stage follows (Lewis and Vinson, 1968):

Egg----The female inserts the egg into the body cavity of the host, where it remains free in the hemolymph. The egg hatches from 36 to 48 hours after oviposition.

First Instar----During this instar the larva remains free in the body cavity of the host and feeds on body fluids. The first instar lasts about 5 to 6 days.

Second Instar----The midgut of the parasite is very large, filled with host fluids and occupies most of the parasite's body cavity. The second instar lasts 2 to 3 days.

Third Instar----Approximately 2 days after the beginning of the third instar, the parasite larva emerges from the metathoracic region of the host. When the parasite is almost free of the host larva, but is still attached by its posterior end, it begins feeding at the back end of the host's remains. It works toward the anterior end of the host's body and completely consumes all remaining body fluids and tissues leaving only the head capsule and integument. Upon completion of feeding the larva spins a white silken cocoon in a cell in the soil.

Prepupal Period----After completion of the cocoon there is an interval of approximately 4 days

before pupation takes place. The larva is gradually contracting and preparing to pupate.

Pupal Period----On or about the 4th day after cocoon formation, pupation takes place. The length of the pupal period ranges from 8 to 12 days.

Adult Period----Adult longevity under normal conditions has not been established.

Parasite-Host Relationship

Lewis and Brazzel (1966b) found that C. nigriceps attacked the larvae of both Heliothis zea (Boddie) and H. virescens in the field and laboratory but that eggs developed only in H. virescens. They concluded, therefore, that the parasite was host specific to H. virescens. Later, Lewis, Brazzel and Vinson (1967) reported that H. subflexa (Guenee) could also serve as a suitable host.

Vinson and Lewis (1965) found that the active substance eliciting the searching response by the parasite can be extracted from H. zea as well as from the suitable host, H. virescens, but it appeared less concentrated in H. zea than in H. virescens. It was concluded that H. zea has recently evolved a mechanism of defense against C. nigriceps, although this species is still attractive to the parasite.

Lewis and Brazzel (1966b) have further reported that the parasite attacks all instars of H. virescens larvae and that the rates of parasite egg and larval development are

similar for each instar. Additionally, unmated female parasites were observed to produce only male offspring, indicating that this species exhibits facultative parthenogenesis.

Vinson and Barras (1970) reported on the effects of C. nigriceps on the growth, development and tissues of H. virescens. They found that both parasitized and control larvae entered the pharate pupal phase at the same time. This indicated that the rate of host larval development to the pharate stage is similar in both parasitized and non-parasitized hosts. There was, however, a reduction in weight gain by parasitized larvae.

Hays and Vinson (1971) examined the acceptance of H. virescens as a host by C. nigriceps. They determined that the antennae of the parasite are involved in host finding, the tarsi aid in orienting the parasite to the host and the ovipositor is involved in final host acceptance.

Lewis, Snow and Jones (1973) examined the use of a pheromone trap suitable for indexing and studying populations of C. nigriceps in tobacco. Traps were baited with five males or five females of C. nigriceps and placed 30.5, 91.4 and 182.9 cm above ground level. They found that females were attracted to both males and other females, with the relative height of the trap being important in determining the sex captured. Traps which were baited with females captured predominately males when placed at 30.5 cm, whereas

they captured mostly females at 91.4 or 182.9 cm which indicated that males remain in the undergrowth while females seek hosts at the mid to upper portions of the tobacco plants where most of the H. virescens larvae occur. Males attracted other males, mostly at the low height, but did not attract females.

The efficiency of C. nigriceps as a parasite of H. virescens was determined by Lewis et al. (1971). A 2-year population density study was made of C. nigriceps on cotton and the corresponding parasitism of H. virescens larvae. They concluded that 80% host parasitism can be achieved with 988 to 1482 C. nigriceps females per hectare.

Heliothis spp.

Importance of Heliothis spp.

Two of the more damaging mid- to late-season pests of cotton throughout the United states are the tobacco budworm, H. virescens, and the cotton bollworm, H. zea. Neunzig (1969) noted that these two species were found throughout most of the Western Hemisphere and he further characterized H. virescens as a major pest of tobacco and cotton. Neunzig reported that corn and cotton were the two primary hosts of H. zea although he observed this species on 16 different cultivated hosts in North Carolina. The presence of H. zea on alfalfa (Medicago sativa L.), soybeans (Glycine max (Merr.) L.), sorghum (Sorghum vicolor (L.) Moench), Okra (Hibiscus

esculentus L.), tomatoes (Lycopersicom esculetum Mill.), and tobacco was sporadic. Graham and Robertson (1970) reported that in Texas, H. virescens was found occasionally on okra, tomatoes, and alfalfa, and H. zea was observed on lettuce (Lactuca sativa L.).

Both species of Heliothis are known to have several wild hosts. Graham and Robertson (1970) found H. zea on burclover, Medicago hispida Baertn., and sunflower, Helianthus annus L., in the spring. They observed both Heliothis species on wild tobacco, Nicotiana repanda Willd., in the spring and on passionflower vine, Passiflora foetida L., in the fall. Pigweed, Amaranthus spp., is also known to be a wild host of H. zea in Arizona in the fall (Fye 1973). Snow and Brazzel (1965), in Mississippi, found 11 wild host species of H. virescens. They indicated, that wild hosts were probably more vital to the biology of H. virescens than to that of H. zea.

Seasonal abundance of Heliothis spp. in cotton has been studied by Henry and Adkisson (1965) in Texas, and by Snow (1964) in Georgia. They observed that H. virescens was the prominent species of Heliothis during June. By early and mid-July H. zea began to predominate, and it continued to increase throughout the rest of the season. Increases of the H. zea population within cotton were credited to an immigration of this species from some of its alternate hosts,

such as corn. In contrast, Cole, Adkisson and Fye (1973) did not find H. virescens in cotton until early July, although H. zea had been present since early June. The H. virescens population rose to 50% of the total Heliothis population by the 2nd week in August and made up 85% of the total population by the 2nd week in October.

Heliothis spp. Life History

Fye and McAda (1972) studied the life history of H. virescens reared on an artificial diet. A series of insects was maintained at four different 24-hour temperature regimes. These programs provided daily mean temperatures of 20°, 25°, 30°, and 33°C, respectively. At 25°C the duration of the five larval instars for males was 3.5, 2.0, 2.0, 2.5, and 7.5 days, respectively. The five instars of females averaged 3.4, 2.0, 2.0, 2.5, and 7.2 days, respectively. Occasionally a few individuals required a 6th larval instar to complete development. Duration of male pupae at 25°C averaged 16.2 days, while females averaged 14.8 days. The total larval-pupal periods averaged 33.9 days for males and 32.2 days for females. Total adult longevity was 19.6 days for males and 20.7 days for females. The minimum preoviposition period was 2 days, and by the 5th day of the adult stage most females had oviposited. Longer oviposition periods were prevalent in the lower temperatures. The higher temperatures resulted in reduced fecundity. Fecundity for the

four temperature programs ranged from a mean of 495 eggs per female at 33°C to 1626 at 20°C. At 25°C, fecundity averaged a total 963 eggs per female. Duration of the egg stage at 25°C was approximately 3.5 days.

Neunzig (1969) studied the life history of H. virescens on tobacco in North Carolina, and determined that during mid summer minimum developmental times of 17 to 18 days for the larval period and 15 to 16 days for the prepupal and pupal period were required. During the earlier months of May and June the larval period was increased by 3 to 4 days, and the prepupal and pupal period averaged about 17 days. The developmental time required during August and September was extended considerably beyond that of even the early season individuals. Both H. virescens and H. zea had a total of four generations per year when all hosts were combined. In Mississippi, Snow and Brazzel (1965) indicated that H. zea passed through 6 to 8 generations per year in Arizona.

Enemies of Heliothis spp.

Lingren, Ridgway and Jones (1968) listed several major insect predators of Heliothis spp. eggs and small larvae; these included Chrysopa, Nabis, Geocoris, Orius, Collops, Zelus, Hippodamia and Scymnus. Whitcomb and Bell (1964) listed about 600 species of predaceous arthropods associated with cotton. In addition to those named above,

they listed many species of spiders, ants, mites, several beetles and a few dipterians and orthopterans as predators of Heliothis spp. All stages of Heliothis spp. were observed to be attacked by at least a few of these predators.

Parasites of Heliothis spp. have been observed by Lewis and Brazzel (1968). Two braconids were the predominant species in Mississippi. Microplitis croceipes (Cress.) parasitized both Heliothis species, and Cardiochiles nigriceps Viereck was a successful parasite of H. virescens only. One other braconid and a few species of Ichneumonidae and Tachinidae were also identified as parasites of Heliothis spp. Six families of egg and larval parasites of H. zea in California were listed by van den Bosch and Hagen (1966).

Watson, Gudauskas and Canerday (1966) encountered several larval parasites of Heliothis spp. in Alabama. Included were two families of Hymenoptera (Braconidae and Ichneumonidae) and one of Diptera (Tachinidae). These parasites and their hosts were : Microplitis croceipes (Cress.) (both H. zea and H. virescens); Cardiochiles nigriceps Viereck (H. virescens), Apanteles marginiventris (Cress.) (H. zea), and Meteorus autographae Mues.(Heliothis spp., undetermined) of the family Braconidae; Archytas marmoratus (Tns.) (H. zea and H. virescens) and Lespesia sp. (H. zea) among the Diptera; and Netelia sp. (H. zea and H. virescens) which belongs to the family Ichneumonidae. Bottrell et al. (1968) reared parasites from Heliothis spp.

collected from cultivated crops in Oklahoma and found 15 parasites representing the families Tachinidae, Braconidae and Ichneumonidae. Microplitis croceipes (Cress.) was the most abundant parasite of the bollworm, H. zea, and the only parasite confirmed from the tobacco budworm, H. virescens (F.). Other parasites frequently reared were Chelonus texanus (Cress.), Eucelatoria armigera (Coquillett), Lespesia archippivora (Riley), and Archytas marmoratus (Townsend). Bibby (1942) reported on the parasites of Heliothis armigera in Texas and, Butler (1958) listed the braconid wasps reared from lepidopterous larvae in Arizona.

Studies of parasite : Heliothis spp. Relationships

Several authors have conducted laboratory and greenhouse studies in which parasite - Heliothis spp. relationships have been examined in order to evaluate the feasibility of parasite introduction as a means of pest control. The majority of these studies have involved the fecundity, longevity, searching capacity, dispersal, patterns of emergence, and host preference of those parasites being examined primarily as inundative control agents.

For example, Fye and Larson (1969) found that Trichogramma minutum Riley would be a poor inundative control agent for Heliothis spp. as well as other lepidopterous pests of cotton because: (1) the rate of dispersal for centralized release stations was slow and the distances the

wasps dispersed was limited; (2) mortality of broadcast parasitized host eggs would be high and the timing difficult; (3) the searching capability of the wasps in complex situations was relatively poor; (4) the longevity and ovipositional periods were short, and the number of eggs laid was small; and (5) high temperature apparently limited the activity of the Trichogramma female.

Bryan, Jackson and Patana (1968, 1969a, -b) conducted laboratory studies of the biology of Lespesia archippivora and Microplitis croceipes which included the time required for parasite development under different temperature regimes and the effect of temperature on parasite progeny production and longevity. In similar studies, Jackson, Bryan and Patana (1969) and Bryan et al. (1972) examined the same areas for Eucelatoria armigera and Eucelatoria sp.

These and similar studies provided the impetus for examining the same or similar areas for Cardiochiles nigriceps as an inundative control agent of Heliothis virescens.

MATERIALS AND METHODS

Source of Insects

This study was conducted in the Entomology Research Laboratory of The University of Arizona Agricultural Experiment Station at the Campbell Avenue Farm, located approximately 5 miles north of the university. The initial stock culture of H. virescens was started from eggs and larvae collected from cotton near La Palma, Arizona during July and August of 1972 and 1973. In subsequent years, additional field-collected stock was added to the culture when available. Field-collected Heliothis spp. were reared individually and the adults were identified by the characteristic striped pattern and green color of the wings of H. virescens.

Since C. nigriceps is not native to Arizona, the initial parasite culture in the form of 100 pupae was obtained from Dr. S. Bradleigh Vinson, Texas A & M University, in July 1973. This initial culture was maintained for research purposes until the fall of 1974 when the entire culture succumbed to an unknown pathogen. In January 1975, a second shipment of parasite pupae was received which survived through the completion of this research.

Rearing of *Cardiochiles nigriceps*

The parasitized larval stages of *H. virescens* were maintained in a walk-in type, temperature-controlled chamber at 27.4 ±0.5°C and 64 ±13% relative humidity, with a 15 hr. photoperiod provided by fluorescent lights. All other stages of the parasite culture were maintained in an Environator® bioclimatic cabinet[1] programmed for a daily photoperiod (L:D) of 17 hours of light and 7 hours of darkness which is necessary to reduce diapause to acceptable levels (Vinson et al. 1973). Relative humidity was maintained at 70 ±10% and temperature was programmed for 30°C.

The basic rearing procedures used for *C. nigriceps* were modified from those described by Vinson et al. (1973). Newly-emerged adults were collected daily and caged in transparent plastic storage boxes (34.6 X 27.0 X 8.7 cm). The center portion of the top and two sides were removed and covered with nylon organdy cloth to allow for adequate ventilation. Fewer than 100 insects were placed in any one cage because Vinson et al. (1973) reported that overcrowding reduces mating and adult longevity due to constant agitation. Females were exposed to males for 24 hours and

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1. Environator Corperation Model E 3448 with Partlow temperature control unit and Herrmidifier Company Model 707 SM return-aire humidifier.

allowed to mate before being removed to a separate cage. This served to prevent mated females from being exposed to sexually aggressive males and increased female longevity. Adult parasites were placed in clean cages every 4 to 5 days. All cages were washed with detergent in hot water and sterilized in a 5.25% solution of sodium hypochlorite to prevent contamination.

Adult food consisted of a 5% sugar-distilled water solution absorbed into cotton pads in small petri dishes and placed in the bottom of the cage.

Adult females are not responsive to the courting male at light intensities of less than 2000 foot candles. The best female responses and mating occur between 2000-4000 foot candles (Vinson et al. 1973). Therefore, the maximum fluorescent light output (approximately 2000 fc) of the bioclimatic cabinets was utilized.

For oviposition, 50 late second- or early third-instar H. virescens larvae were placed on a piece of paper towel in the bottom of a 20 X 100 mm petri dish. Five female parasites were then placed with their hosts in the petri dish, and returned to the bioclimatic cabinet for approximately 20 minutes. Under those conditions, the females actively searched for and attacked in excess of 95% of the host larvae.

Following oviposition, host larvae were placed in separate 28g plastic cups containing approximately 14g of lima bean (Phaseolus lunatus L.) diet (Patana 1969). The cups were closed with cardboard tab lids and stored diet end up in the walk-in type, temperature controlled chamber. Nine days later, the pharate pupae were placed in 14g plastic cups containing cotton and placed in the bioclimatic chamber.

The emergence of the parasites from host larvae occurred over a period of 10 to 16 days at which time they consumed the host and spun cocoons. Following cocoon formation, the cocoons were collected and placed in a 20 X 100 mm plastic petri dish to await adult emergence.

Rearing of Heliothis virescens

The larval stages of the H. virescens stock culture were maintained in a walk-in type, controlled temperature chamber at 27.4 ±0.5°C and 64 ±13% RH, with a 15-hour photoperiod provided by fluorescent lights. All other stages of the H. virescens culture were held in the open laboratory, which was maintained at temperatures between 22° and 27°C.

The basic rearing procedures used for H. virescens were adapted from those described by Patana (1969). Adults were caged in 3.8 l, wide mouth glass jars. Two strips of

paper towelling taped to the neck of the jar extended down the inside walls of the jar and a circular piece of towelling covering the mouth of the jar served as oviposition sites. an inverted glass procaine tube protruding through the top sheet of towelling was used as a dispenser for a 5% sugar solution fed to the moths.

At 2-day intervals the sections of towelling containing eggs were removed from the jars. Eggs were surface sterilized by washing them in a 0.3% solution of sodium hypochlorite, then rinsing in 10% sodium thiosulfate, and finally rinsing in distilled water (Ignoffo 1963). Washed egg sheets were allowed to air dry, and then placed in clean 3.8 l glass jars for hatching.

Freshly-hatched first-instar larvae were removed daily from the egg sheet jars, and transferred to larval rearing cups with a brush. These 28g plastic cups were half filled with an artificial lima bean diet (Patana 1969) and closed with cardboard tab lids. Several larvae were introduced into each cup initially, but during the third-instar, they were separated into individual cups. After pupation the pupae were removed and placed in 3.8 l jars for adult emergence.

Effect of Parasitism on Host Feeding

Late second-instar parasitized and non-parasitized H. virescens larvae were placed in 28g plastic cups with

approximately 14g of lima bean diet and closed with cardboard tab lids. The cups were inverted and stored in the walk-in type chamber under the previously described temperature and humidity conditions. When host larvae entered the pharate pupal stage, they and their feces were collected. Pharate pupae were weighed to the nearest 0.0001 of a gram on a Mettler® (Model H6 DIG) balance. Fecal material collected from individual larvae was oven dried at 70°C for 24 to 48 hours and then weighed in the same manner as the pharate pupae.

These same methods were later applied to parasitized and non-parasitized host larvae fed on fresh cotton squares and bolls rather than artificial diet. Host larvae were initially placed in 28g plastic cups with cotton squares. The cups were then inverted and stored but checked on a daily basis to insure a consistantly fresh food supply. When host larvae reached a size where they were consuming more than one large square per day (approximately late third-instar), cotton bolls were used as the food source. However, before host larvae reached the pharate stage and their feces collected, mold destroyed the contents of the cups. The contamination problem, due to excess moisture accumulation and low air circulation, led to a different method of housing host larvae.

Several containers were constructed of paraffin-lined 237 ml squat Dixie® cups. Windows 2.5 X 7 cm were cut in opposite sides and covered with nylon organdy cloth to provide free air circulation. Procaine tubes filled with water were inserted through a small hole in the top of the containers. Stems of squares and bolls were then inserted into the procaine tubes which served to keep them in a fresher condition for a longer period of time. Host larvae were then added and the containers closed with cardboard tab lids. The cups were then stored and checked on a daily basis.

Selection for Early Parasite Emergence

C. nigriceps pupae were collected and held for emergence in a bioclimatic chamber under the reported parasite rearing conditions. The selection process involved segregating all adult parasites that emerged within 10 days of the first parasite emergence into a separate cage where they were allowed to mate. The F_1 and subsequent generations were handled in the same selective manner and changes in emergence patterns and sex ratios were determined.

Parasite Storage Capability

Two different developmental stages of parasite pupae were held in constant darkness under three different

temperature regimes in bioclimatic cabinets to determine the feasibility of parasite storage. Storage periods of 1, 2, 3, 4, 8, 12 and 16 weeks were utilized at 5°, 10°, and 15°C. Parasite pupae designated as "YM", referring to pupae that were yellow in color with brown meconium present, and "B", referring to pupae that were black in color, were subjected to the previously mentioned conditions.

"YM" pupae, if left under normal parasite rearing conditions, would emerge as adults in a period of 4 to 10 days whereas "B" pupae under the same conditions would emerge as adults in 1 to 3 days. At the completion of each storage period, pupae were returned to normal parasite rearing conditions and the pattern of emergence as well as mortality recorded.

Parasite Fecundity and Longevity

This experiment was conducted in an Environator® bioclimatic cabinet under the conditions previously stated for parasite rearing. Newly-emerged parasite adults were allowed to feed and mate for 24 hours before being confined in cages containing H. virescens larvae.

Cages used to confine host larvae and parasites were constructed from clear plastic 20 X 100 mm plastic petri dishes. A 2.5 cm hole was cut in the lid of each cage and covered with nylon organdy cloth to permit air

circulation. Parasite food (5% sugar solution) was initially provided in procaine tubes inserted through a small hole in the lid of each cage. However, preliminary experimentation indicated that food provided in this manner was not accessable enough to the parasite and longevity was reduced. Subsequently, small plastic cups (7 X 20 mm) which contained food absorbed into cotton pads provided greater accessibility and, therefore, increased longevity.

Initially, groups of 25 late second-instar larvae were exposed to mated pairs of parasites for 24 hours. Preliminary results, however, indicated that lengthy exposure to that many larvae may have reduced longevity. Therefore, a different exposure regime was initiated in which 20 host larvae were exposed to either pairs or single female parasites for 1 hour and 24 hours. Host food, provided in the 24 hour exposure regime, was a small (5 X 20 mm) piece of lima bean diet placed in the bottom of the cages. Diet was not provided in the 1 hour regime due to the relatively short exposure time.

Host larvae, after being exposed, were removed from host cages and placed in 28g plastic cups half filled with lima bean diet. The cups were closed with cardboard tab lids and placed in the walk-in controlled-temperature chamber under the conditions described for host rearing.

After 15 to 20 days, hosts were removed from the chamber and the total parasitized, pupated, and dead larvae recorded. Percent host parasitism was calculated using the formula:

$$\% \text{ Parasitism} = \frac{\text{No. Parasitized}}{\text{No. Parasitized + No. Pupated + No. Dead}} \times 100$$

Percent host mortality was calculated using the formula:

$$\% \text{ Mortality} = \frac{\text{No. Parasitized + No. Dead}}{\text{No. Parasitized + No. Pupated + No. Dead}} \times 100$$

Adult parasite longevity was also recorded as mortality occurred.

Parasite Searching Capacity

This study was conducted in a 2.8 X 3.7 m Aluminex Jr.® greenhouse cooled by a Gaffers and Sattler® 85.0 to 424.8 CMM side-discharge cooler. Initially, six cotton plants in the squaring stage of development were each infested with two late second-instar H. virescens larvae. Host larvae were allowed to establish themselves and feed for 2 days. Individual cotton plants were then enclosed with nylon organdy cloth and one, 2-day old female parasite was released into each bagged plant.

An open greenhouse release was then conducted in which two rows of cotton plants in the late square-early

boll stage of development were utilized. The total length of both rows (14 total plants) approximated 1/4000th of a hectare. Plant spacing was approximately 23 cm and row width was 101.6 cm. Three releases were conducted in which plants were infested with two late second-instar host larvae each. The larvae were allowed to establish themselves and feed for 2 days. Two, 2-day old C. nigriceps females were then released in the greenhouse. An additional release was made using four host larvae per plant and three female parasites.

In both open and bagged plant releases, host larvae were collected from the plants after 24 hours, placed in larval rearing cups and stored in the walk-in chamber to complete their development. Approximately 15 days later, host insects were removed and the number parasitized, pupated and dead was recorded.

All of the previously mentioned parasite releases were made in the late afternoon. Temperature and humidity conditions in the greenhouse were monitered by a hygrothermograph for 27 days while releases were being conducted.

Data Analyses

One-way Analysis of variance for both equal and unequal sample sizes, where applicable, was used to evaluate the data for parasite storage capability and the effect of

parasitism on host feeding. The Least Significant Different test (LSD) was then utilized to inspect differences between pairs of means. Parasite fecundity and longevity means were examined with the Student's "t" test in order to determine if statistical differences existed.

All data were analyzed on a Hewlett-Packard® 25C programmable calculator with the appropriate statistical methodology taken from Snedecor and Cochran (1967) and Little and Hills (1972).

RESULTS AND DISCUSSION

The Effect of Parasitism on Host Feeding

Vinson and Barras (1970) reported a reduction in weight gain by parasitized host larvae and that the rate of larval development and number of moulting cycles between parasitized and non-parasitized controls was similar. Host larvae in their experiment were reared on an artificial medium.

When H. virescens are parasitized by C. nigriceps, host larvae remain on the plant where they continue to feed and develop until the pharate pupal stage is reached. At this point, parasitized host larvae are destroyed by the emergence of larval parasites.

This experiment was devised to compare the actual reduction in host feeding caused by parasitism of host larvae. Comparisons were made between parasitized (n=26) and non-parasitized (n=26) host larvae fed on lima bean diet or cotton squares and bolls during a 6-week period in the spring of 1974. Larval and fecal weights were the criteria used to determine differences.

Table 1 shows the total larval weight gain of parasitized and non-parasitized H. virescens larvae reared on both types of diet. A comparison of mean weights

Table 1. Comparison of total larval weight gain (g) of parasitized and non-parasitized Heliothis virescens larvae reared on two different food sources.

	Food Source			
	Cotton squares and bolls		Lima bean diet	
	Parasitized n=13	Non-parasitized n=13	Parasitized n=13	Non-parasitized n=13
$\bar{X}$ *	0.0526	0.2166	0.0981	0.2897
s	0.0115	0.0398	0.0194	0.0476

* all means are significantly different at 0.05 level with LSD=0.0259

for 13 host larvae examined in each treatment showed all to be significantly different from each other at the 0.05 level. Of greater importance, however, was the actual observed differences between the means. Parasitized H. virescens larvae reared on cotton squares and bolls weighed approximately 24.3% of non-parasitized hosts reared on the same food. A similar comparison made with host larvae reared on lima bean diet indicated that parasitized larvae weighed nearly 33.8% of the non-parasitized controls. Appendix A illustrates the individual larval weight gains (g) observed and the statistical analyses for this portion of the experiment.

Table 2 compares the total weight of feces produced by parasitized (n=26) and non-parasitized (n=26) larvae reared on both diets. Once again, all means were significantly different at the 0.05 level. The observed differences between means are similar to those previously reported for larval weight differences. For example, fecal material produced by parasitized H. virescens larvae reared on cotton squares and bolls was 24.6% of non-parasitized hosts reared in the same manner. When host larvae reared on lima bean diet are compared, fecal material produced by parasitized host larvae was 36.9% of non-parasitized hosts. Appendix B shows the amount of feces (g)

Table 2. Comparison of total fecal weight (g) produced by parasitized and non-parasitized Heliothis virescens larvae reared on two different food sources.

	Food Source			
	Cotton squares and bolls		Lima bean diet	
	Parasitized n=13	Non-parasitized n=13	Parasitized n=13	Non-parasitized n=13
$\bar{X}$ *	0.1073	0.4367	0.2186	0.5924
s	0.0273	0.1135	0.0402	0.1216

* all means are significantly different at 0.05 level with LSD=0.0683

produced by individual larvae and the statistical analyses for this portion of the experiment.

These results illustrate several important points. First, the relative magnitude of mean differences in larval and fecal weights for individuals reared on the same food source are similar. Second, the magnitude of differences of means between larval and fecal weights of host larvae reared on the different food sources can undoubtedly be attributed to nutritional differences of the respective diets because all host larvae were reared under similar environmental conditions and an adequate food supply was always present. Finally, the effect of parasitism on host feeding is great enough to warrant the re-examination of current Heliothis spp. economic thresholds. Because parasitized larvae remain in the population and continue to develop at a normal rate but consume approximately 30% less food than their non-parasitized counterparts, cotton plants should be able to withstand larger populations of parasitized than non-parasitized H. virescens. Therefore, the reduced larval consumption and subsequent potential reduction in the number of squares and bolls fed upon by parasitized larvae could be applied in conjunction with observed values of percent parasitism to determine the effect of a given H. virescens population on cotton.

The complexity of developing a dynamic threshold for H. virescens in cotton with C. nigriceps present would be magnified when populations of H. zea are also present. In Arizona, H. zea would serve as the only detractant to C. nigriceps. H. subflexa, which serves as the only other alternate host (Lewis et al. 1967), does not occur in this state. As previously stated, Henry and Adkisson (1965), Snow (1964) and Cole et al. (1973) found population levels of H. virescens and H. zea to be prominent at different times of the growing season but overlap of populations did occur. Similar relationships occur in Arizona in that populations of both species are usually present at the same time. This, coupled with the fact that the economic threshold currently used in Arizona includes both H. virescens and H. zea indicates that to develop a dynamic threshold that includes the effect of C. nigriceps, one should consider the relative magnitude of both populations and the percent parasitism of H. virescens by C. nigriceps. The reduction of larval feeding by H. virescens would then need to be incorporated with the information relative to numbers present and amount of parasitism in order to obtain a more realistic dynamic economic threshold. Actual increases in the economic threshold may be small when a large proportion of the population is H. zea. On the other hand, the opposite may be true when the population is

predominately H. virescens and could conceivably lead to eliminating one or more insecticide treatments that would normally be considered necessary.

Selection for Early Parasite Emergence

A record of emergence patterns of adult C. nigriceps was kept from the time initial parasite culturing began. In September of 1973, 84.5% of 190 total parasites emerged within 12 days of each other (Fig. 1). However, by the end of October, only 71% of 129 total parasites emerged within 12 days (Fig. 2) and by December, less than 41% of 108 total parasites were emerging within a period of 12 days (Fig. 3).

The decline in early emergence, i.e., the leveling of the emergence curve can best be explained by the way the parasites were previously cultured. Vinson et al. (1973), may have inadvertently selected for early emerging parasites in his mass rearing program by first, having a large number of adults emerging daily and second, by segregating those parasites into groups of approximately 50 per cage. This would have had the effect of preventing most of the later emerging parasites from contributing a great deal to the gene pool of the culture.

While C. nigriceps were cultured for this study, all adults regardless of emergence time, were indiscriminately caged together. This, probably more than any

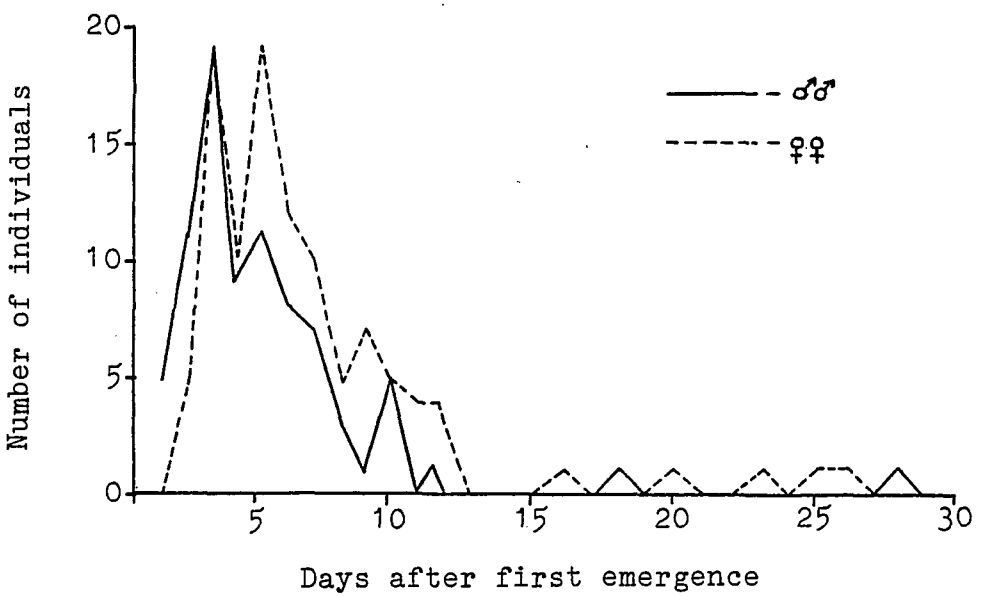

Fig. 1 Emergence pattern of Cardiochiles nigriceps in generations 1 to 5, September, 1973.

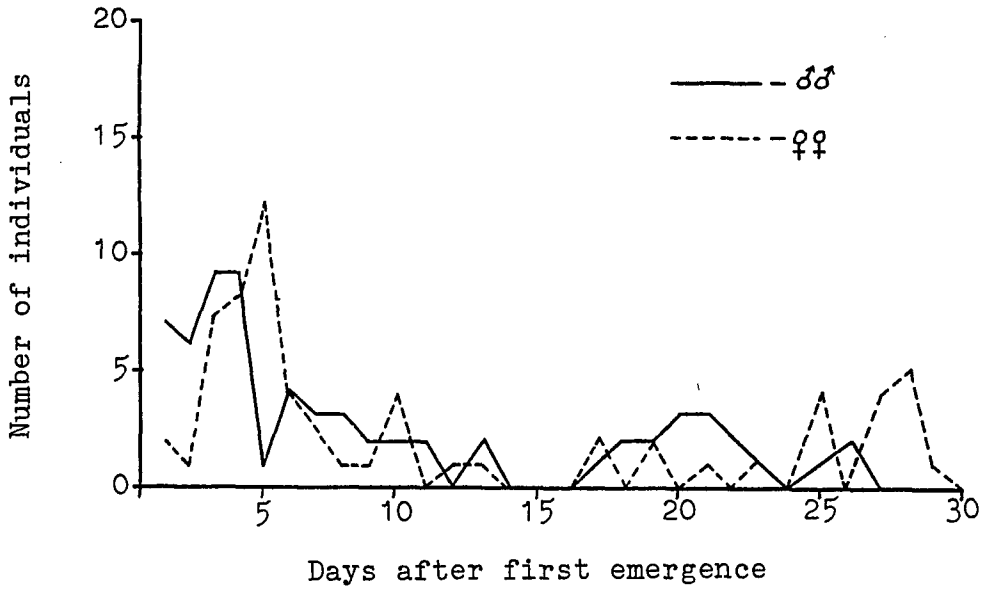

Fig. 2 Emergence pattern of Cardiochiles nigriceps in generations 11 to 15, October, 1973.

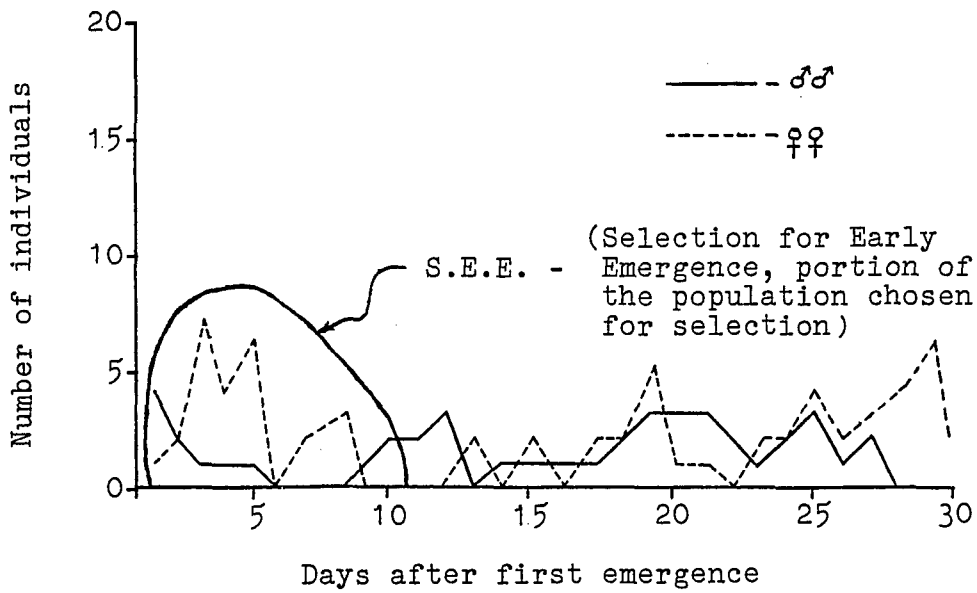

Fig. 3 Emergence pattern of Cardiochiles nigriceps in generations 16 to 20, December, 1973 and portion of parasite population chosen for selection.

other factor, was responsible for changes noted in emergence patterns.

The observed changes in parasite emergence patterns would seem to indicate that a genetic factor is involved in determining when parasites emerge. The premise for this study was, therefore, that selection for early-emerging parasites could be accomplished and in time, provide a maximum number of parasite adults that would be available at any given time for inundative releases.

The merit of having a large population of parasites available within a short period of time is obvious in an inundative release program. Therefore, in January of 1974, a parasite selection program was begun. The selection process, referred to as SEE (Selection for Early Emergence) in Fig. 3, involved segregating all adult parasites that emerged within 10 days of each other, i.e., the encircled part of the population, into individual cages. These individuals were allowed to mate and then females were confined with H. virescens larvae for parasitization. F_1 and subsequent generations were selected and handled in the same manner and the results are illustrated in Fig. 4.

In the relatively short period of 3 months time, 74% of 148 total parasites were emerging within 12 days of each other. Additionally, the emergence trend illustrated in Fig. 4 strongly resembles the emergence trend in Fig. 2,

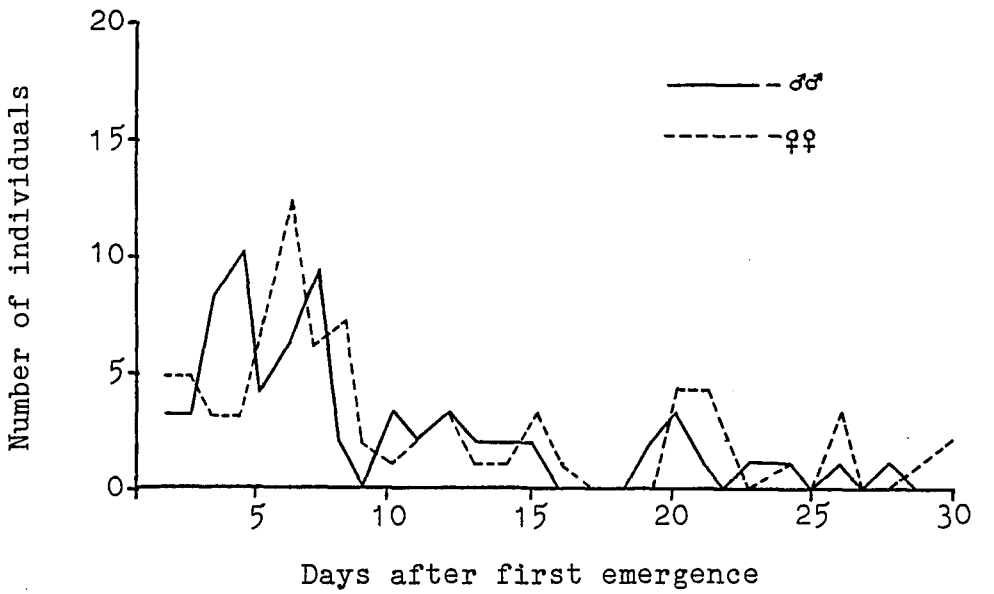

Fig. 4 Emergence pattern of Cardiochiles nigriceps in generations 11 to 15 after selection, March, 1974.

indicating that the selection process was reversing almost exactly the previous leveling trend of the emergence curve. These results indicate that selection for early emergence can be accomplished and that ultimately, in excess of 85% of all parasites could be expected to emerge within 12 days of each other.

The significance of knowing the sex ratio of C. nigriceps would be most evident in an inundative release program utilizing pupae rather than adults. The advantage of this type of release over an adult release program would be from the standpoint of handling and transport of the parasites.

Vinson et al. (1973) reported that a sex ratio of 1:1 is normal for C. nigriceps. In the course of this study, however, the trend was generally toward more females than males (Table 3). Differences in the mean sex ratio noted were not significant at the 0.05 level between individuals that emerged within the first 12 days or those that emerged over the entire 30-day period indicating that the sex ratio remains fairly constant over the entire emergence period.

Since females of this species are the only relevant individuals from a control point of view, the sex ratio of the parasite must be known in order to provide the desired number of females to be released per unit area.

Table 3. Observed sex ratios of the parasite Cardiochiles nigriceps.

	Period of Emergence			
	Days 1-12		Days 1-30	
	Sex ratio (%)		Sex ratio (%)	
	Males	Females	Males	Females
Fig. 1	45	55	44	56
Fig. 2	52	48	52	48
Fig. 3	40	60	40	60
Fig. 4	49	51	47	53
$\bar{X}$ a/	47a	53a	46a	54a

a/ Means in the same row followed by the same letter are not significantly different at the 0.05 level (Student's "t" test).

In general, these results indicate that 53 to 54% of pupae reared for release will be females. And, for example, if a goal of 1976 actively searching females per hectare is sought, then 3659 to 3728 pupae released per hectare should provide the desired number.

Parasite Storage Capability

Experimentation with parasite storage was conducted from May to September, 1974, when results from the early-parasite-emergence studies proved favorable. The ability to store C. nigriceps on at least a short term basis would ease many of the rearing problems normally associated with producing large numbers of parasites for an inundative release program. Also, parasite storage coupled with parasites selected for early emergence would insure that a large number of early-emerging parasites could be accumulated in time.

As previously reported, two different developmental stages of pupae were used, i.e., black stage, "B", which if left under normal rearing conditions would have emerged in 1 to 3 days and yellow-meconium stage, "YM", which if left under normal rearing conditions would have emerged as adults in 4 to 10 days. "YM" and the more developed "B" pupal stages were selected for use in this experiment because the presence of a meconium in the "YM" stage is the first visual evidence that pupae are not in diapause.

Non-diapausing pupae were felt to be a necessity in order to realistically determine time of adult emergence.

Table 4 summarizes the results of up to 4 weeks storage at three different temperatures with the "B" designated parasite pupae. Table 5 illustrates the same information for the "YM" group. Additionally, Appendices C and D offer a detailed illustration of the pattern of emergence and longevity for "B"- and "YM"-type pupae. Originally, storage periods of up to 6 months were planned, however, excessive mortality in periods longer than 4 weeks did not warrant continuation beyond that time. Three replications of three parasite pupae were utilized in each of the four storage periods at each of the three temperatures for both stages. Therefore, n=9 for each temperature within each storage period and n=108 was the total number of pupae observed in each stage.

Mean emergence times for "B"-type C. nigriceps (Table 4) fell within a 2.5 day period with 2- and 3-week storage periods at 15°C emerging within 2 hours after being returned to normal rearing conditions. Statistically, the mean emergence times at 5° and 10°C and those at 5° and 15°C were not significantly different from each other but the mean emergence time at 10°C was significantly longer than at 15°C. In the 4 week storage period at 10°C, the first sign of major mortality became evident when all

Table 4. Storage capability of black "B"-type Cardiochiles nigriceps pupae held at three different temperature regimes.

Storage Period (weeks)	Temperature (°C)	Mean Emergence Time (days) 1/	Mean Longevity (days) 5/ Males	Females	Percent Survival
1	5	1.0ab	24.0a	15.5a	100
	10	1.8a	26.0a	24.0a	100
	15	0.8b	23.5a	14.0a	100
2	5	2.0a	13.5b	17.0a	100
	10	2.5a	12.5b	15.5a	89
	15	0.0a 2/	19.0a	28.0a	100
3	5	1.0	8.0	10.0	56
	10	2.0	6.0	8.0	22
	15	0.0 2/	1.0	8.0	56
4	5	2.0	6.0	10.0	67
	10	--- 3/	---	---	0
	15	--- 4/	---	---	--

1/ Emergence time after pupae were returned to normal rearing conditions. Means followed by the same letter are not significantly different at the 0.05 probability level (LSD).

2/ All emerged within 2 hours after return to normal rearing conditions.

3/ All pupae held under this temperature-storage regime died.

4/ All surviving emerged an average of 19.7 days after storage began.

5/ Longevity measured from time of parasite emergence. Means followed by the same letter are not significantly different at the 0.05 probability level (LSD).

Table 5. Storage capability of yellow meconium "YM"-type *Cardiochiles nigriceps* pupae held at three different temperature regimes.

Storage Period (weeks)	Temperature (°C)	Mean Emergence Time (days)[1/]	Mean Longevity (days)[3/] Males	Females	Percent Survival
1	5	7.3a	22.0a	27.0a	100
	10	7.3a	30.5a	20.0a	100
	15	7.0a	27.7a	23.0a	100
2	5	5.0a	8.0a	20.0a	33
	10	6.0a	10.0a	20.0a	67
	15	4.3a	13.0a	24.5a	100
3	5	8.0	5.0	8.0	33
	10	-- [2/]	----	----	0
	15	3.0	6.5	10.0	33
4	5	-- [2/]	----	----	0
	10	7.0	25.0	8.0	33
	15	1.0	5.0	7.0	33

1/ Emergence time after pupae were returned to normal rearing conditions. Means followed by the same letter are not significantly different at the 0.05 probability level (LSD).

2/ All pupae died.

3/ Longevity measured from time of parasite emergence. Means followed by the same letter are not significantly different at the 0.05 probability level (LSD).

parasites stored failed to survive. Additionally, pupae held for 4 weeks at 15°C emerged in less than 20 days after being put in storage, i.e., emergence occurred in storage. This would indicate that 15°C does not retard pupal development sufficiently beyond 3 weeks to prevent parasite emergence. In addition, percent survival was significantly lower at all temperatures beginning with the 3-week storage period and, for this reason, statistical comparisons were not attempted beyond the 2-week storage period.

A comparison of emergence times for "YM"-type C. nigriceps (Table 5) indicates that all parasites emerged within an average period of 8 days. Mean emergence times for all temperatures within the 1- and 2-week storage periods were not significantly different. Major mortality occurred at 3 weeks in the 10°C treatment and at 4 weeks in the 5°C chamber; however, a noticeable reduction in percent survival was observed in the 2-week storage period at 5° and 10°C.

Longevity for males and females was observed for both pupal types in each storage regime, however, in an inundative release program utilizing C. nigriceps, only female longevity is of major importance. As previously stated, these parasites are known to exhibit facultative parthenogenesis and mating, therefore, is not essential

to their success as an agent in an inundative release program because their establishment is not required. Female longevity of "B"-type (Table 4) and "YM"-type pupae (Table 5) indicate that storage does not begin to adversely affect longevity for periods up to 2 weeks.

Male and female longevity of "B"-type pupae were not significantly affected by the three temperature regimes in the 1-week storage period. Female longevity was also not significantly different in the 2-week storage period but males showed significantly greater longevity at 15°C. Longevity of male and female "YM"-type pupae was not significantly different in the three temperature regimes stored for 1- and 2-week periods. Beginning with the 3-week storage regime, reduction in longevity became evident, therefore, statistical analysis beyond the 2-week storage period was not attempted.

The results of this experiment indicate that short-term storage is feasible for at least 2 weeks for "B"-type pupae and for at least 1 week for "YM"-type C. nigriceps pupae. Storage beyond these time periods resulted in either lower survival rates, reduced female longevity or both. From a practical standpoint, however, storage of "B"-type pupae for 2 weeks at 15°C (Table 4) offers the greatest potential as a result of high survival rate, good female longevity and almost immediate emergence.

Low survival rates and reduced longevity in the longer exposure periods are indicative that pupal development, under the temperature regimes utilized, continued at a faster rate than would normally be desired. Therefore, lowering storage temperatures to 0°C or lower may serve to increase the effective storage periods.

Parasite Fecundity and Longevity

Investigations on the fecundity and longevity of C. nigriceps were conducted in order to determine their maximum values under normal laboratory rearing conditions. Also, the effect of 1 and 24 hour host-exposure periods on fecundity and longevity was examined.

As previously reported, preliminary testing with five mated C. nigriceps pairs yielded poor results. A summary of the fecundity and longevity data for the initial experimentation is illustrated in Table 6. Parasites were exposed to 25 H. virescens larvae continuously for 24 hours. The results were short parasite longevity, a low percentage of host parasitism and low mortality of host larvae. This was partially attributable to first; utilizing procaine tubes to feed the parasites which reduced longevity due to starvation and, second; the stress placed on the parasites by the lengthy exposure to 25 host larvae. As a result, subsequent experimentation utilized small plastic cups (7 X 20 mm) with food absorbed in cotton pads which

Table 6. Summary of fecundity and longevity data for *Cardiochiles nigriceps* exposed continuously to 25 *Heliothis virescens* larvae per day for 24 hours.

	Parasite Longevity (days)		Host Larvae (number)						
	Male	Female	Exposed	Parasitized	Pupated	Died	Escaped	Percent Parasitism	Percent Mortality
$\bar{X}$	5.0	4.6	110.0	37.8	38.4	20.8	13.0	36.6	56.2
s	2.4	1.5	33.5	22.8	13.4	13.9	7.5	20.8	26.9

provided greater accessibility and, therefore, increased longevity. The number of host larvae exposed during a given time period was reduced from 25 to 20 and, a second treatment was initiated in which the parasite-host exposure period was 1 hour daily. In order to provide a means for determining the effect of the long host exposure period on parasite longevity and overall efficiency, the 24 hour parasite-host exposure period was continued as before. Host larvae were supplied and collected daily from parasite cages in each exposure period. Recording of data was accomplished 16 to 20 days after exposure.

Table 7 compares the mean fecundity of C. nigriceps exposed to 20 host larvae per day in both parasite-host exposure regimes. Additionally, Appendices E (1 hour exposure) and F (24 hour exposure) illustrate the source of mean values shown in Table 7. The mean number of larvae exposed in 16 total replications of both regimes was n= 186.25. Mean differences in the number of host larvae parasitized, number of host larvae pupating and percent mortality were not significantly different between the two host exposure periods. Although mean differences in the number of host larvae dying, and percent parasitism were not significantly different, at the 0.05 level, large differences did occur.

Table 7. Comparison of mean fecundity of *Cardiochiles nigriceps* exposed continuously to 20 *Heliothis virescens* larvae per day for 1 and 24 hours.

	Host exposure a/ period (hours)		
	1	24	t
Mean number of host larvae parasitized b/	113.4a	119.8a	-0.56
Mean number of host larvae pupated b/	20.4a	12.2a	0.66
Mean number of host larvae died b/	48.3a	30.3a	2.09
Mean number of host larvae escaped b/	4.0a	21.1a	-5.95
Percent parasitism	62.4a	74.1a	-2.07
Percent mortality	88.8a	93.4a	-0.67

a/ Means in the same row followed by the same letter are not significantly different at the 0.05 level (Student's "t" test).

b/ Mean number of 8 replications in each exposure period.

The reason more host larvae died in the 1-hour parasite-host exposure regime may relate primarily to observed behavior of the parasites in both regimes. Females in the 1-hour exposure period were observed to vigorously attack and parasitize host larvae throughout the exposure period. On the contrary, those females in the 24-hour regime appeared much more sedate in their approach to host larvae, perhaps due to the constant exposure. Therefore, the observed differences in number of hosts dying were probably caused by the effect of multiple parasite attacks and oviposition on host larvae.

The differences noted in the number of escaped host larvae was due to the ability of host larvae in time to extracate themselves from the cages utilized in this study.

The most relevant of the observed differences in terms of the experiment itself, was in the percent parasitism produced by the two exposure regimes. The reason for higher percent parasitism in the 24-hour exposure period was undoubtedly due to the longer exposure period and hence the greater opportunity for parasitism.

A comparison of mean longevity of C. nigriceps in both host-exposure regimes is presented in Table 8. Neither male nor female longevity was significantly different at the 0.05 level although males did live

Table 8. Comparison of mean longevity of *Cardiochiles nigriceps* exposed continuously to 20 *Heliothis virescens* larvae per day for 1 and 24 hours.

Host exposure period (hours)	Number of host larvae	Longevity (days)a/ Males	Females	t
1	20	11.2a	9.9a	0.52
24	20	12.1a	8.8a	1.15
t		-0.25	0.60	

a/ Means in the same rows and columns followed by the same letter are not significantly different at the 0.05 level (Student's "t" test).

approximately 1 day longer in the 1-hour exposure period and nearly 3 days longer in the 24-hour exposure period. Appendix G illustrates the longevity of males and females observed in each replicate for both exposure periods.

Although the results of this experiment indicate that parasite fecundity and longevity were not significantly effected by 1- and 24-hour exposure periods, the data presented may still be applied toward determining the most efficient method of mass rearing parasites for an inundative release. The 24-hour exposure period shows a distinct advantage over the 1-hour exposure period in most areas examined but, particularly in terms of percent parasitism (Table 7). The magnitude of the difference (nearly 12%) more than compensates for the slight reduction in female longevity (Table 8). Therefore, exposing 20 H. virescens larvae to female C. nigriceps for 24 hours will provide the most efficient mass rearing procedure.

Parasite Searching Capacity

Attempts to determine the searching capacity of C. nigriceps were initiated with greenhouse releases conducted during July and August of 1976 under environmental conditions similar to those that would be encountered in the field in Arizona. Observations on the searching ability of this parasite have been well documented by Lewis et al. (1971) in their native southeastern habitat.

In their work, C. nigriceps were characterized as being highly efficient in locating their larval hosts on the plant and they suggested that 80% parasitism can be achieved with 988 to 1482 female C. nigriceps per hectare.

Preliminary examination into the searching capacity of C. nigriceps in this study was initiated on cotton plants enclosed with nylon organdy cloth. Table 9 summarizes the results of six female releases conducted on six different plants infested with two host larvae each. Results indicated that the parasitism was inferior to what might have been expected. Parasitism of host larvae was recorded in only half of the releases and only 50% parasitism was recorded in each. No female parasite used in these releases was observed to live longer than 24 hours.

Table 10 summarizes the results from a subsequent study in which open greenhouse releases of C. nigriceps were conducted. Four separate releases were made, three in which two host larvae per plant were utilized and one with four host larvae. Two female parasites, which corresponded to 4940 parasites per hectare, were utilized in all releases. Again, these results indicate that the parasites were not capable of providing the kind of control necessary in an inundative release program. Again, no female parasite utilized in these releases was observed to live longer than 24 hours. This, when compared with the observations on

Table 9. Summary of *Cardiochiles nigriceps* releases conducted in a greenhouse on enclosed cotton plants.

Cotton plant number	Number of host larvae	Number of host larvae recovered	percent parasitism
1	2	2	50
2	2	1	0
3	2	2	50
4	2	2	0
5	2	2	0
6	2	2	50

Table 10. Summary of the searching capacity of *Cardiochiles nigriceps* under greenhouse conditions.

Release	Number of host larvae	Host larvae per plant	Host larvae recovered	Percent host mortality	Percent host pupation	Percent host parasitism
1	28	2	6	50	50	0
2	28	2	12	75	16.7	8.3
3	28	2	7	28.6	57.2	14.2
4	56	4	32	9.4	78.1	12.5

parasite mortality in the enclosed plant releases, strongly indicates that they are not capable of withstanding the environmental extremes present in this study. Greenhouse mean temperature extremes ranged from 15.3 to 36.3°C (N:D) and mean humidity extremes ranged from 97.8 to 49.5% (N:D) while releases were being conducted. In the parasites' native habitat, they rarely if ever are faced with the environmental extremes which occur in Arizona. This, coupled with the fact that parasites utilized in this experiment may have been weakened genetically by being laboratory reared for a number of years, provides some insight into the apparent ineffectiveness displayed by C. nigriceps during these releases. Therefore, in order to reach a definitive conclusion regarding the parasites potential in Arizona, this portion of the study should be repeated utilizing recently collected individuals from field populations.

SUMMARY

Research was conducted at Tucson, Arizona, from 1973 through 1976 to determine the feasibility of utilizing Cardiochiles nigriceps Viereck as a biological control agent of Heliothis virescens (F.) in Arizona. The following six areas of interest were examined in order to develop a better understanding of parasite capability and parasite-host biological relationships: (1) the effect of parasitism on host feeding, (2) selection for early parasite emergence, (3) parasite storage capability, (4) parasite fecundity, (5) parasite longevity, and (6) parasite searching capacity.

A parent culture of H. virescens was started from eggs and larvae collected from cotton near La Palma, Arizona in 1972 and 1973. Additional field-collected stock was added to the culture when available. C. nigriceps, which are not native to Arizona, were obtained from Texas A & M University in 1973 and again in 1975.

Host larval and fecal weights were used to determine the effect of parasitism on host feeding. Consumption of food by host larvae fed on lima bean diet or cotton squares and bolls was significantly reduced in parasitized individuals. Therefore, the effect of parasitism on host feeding may warrant the re-examination of current Heliothis

spp. economic thresholds for cotton when C. nigriceps is present.

Selection for early parasite emergence was examined in order to determine the maximum number of parasites available at any given time for inundative releases. The selection process involved segregating all adult parasites that emerged within 10 days into individual cages where they were allowed to mate. The results indicated that ultimately, more than 85% of all parasites could be expected to emerge within 12 days of each other and that 53 to 54% would be females.

Parasite storage was feasible at 5°, 10°, and 15°C for 2 weeks or less. Successful storage for longer periods of time may have been possible had lower temperatures been examined. Longer periods of storage at 5°, 10°, and 15°C were probably not successful because these temperatures were not low enough to prevent continued parasite development.

Parasite fecundity and longevity were examined under normal parasite rearing conditions while being exposed to host larvae for 1- and 24-hour periods. Mean longevities of male and female parasites was not significantly different in the two periods of host exposure. Also, differences in the number of host larvae dying, number of

host larvae escaping and percent parasitism were not significantly different in the two host exposure periods.

Attempts to determine the searching capacity of C. nigriceps were initiated with greenhouse releases conducted under environmental conditions similar to field conditions encountered in Arizona. Preliminary studies were with enclosed cotton plants with subsequent releases being made in an enclosed greenhouse. Results indicated that C. nigriceps was not capable of providing the kind of control that would be required in an inundative release program. This may have been due to the environmental extremes present in this study and/or because the parasites had been weakened genetically through laboratory rearing for a number of years.

APPENDIX A

LARVAL WEIGHT GAINS (g) AND ANALYSES FOR *HELIOTHIS VIRESCENS* FED ON COTTON SQUARES AND BOLLS AND LIMA BEAN DIET

	H. virescens larvae			
	Squares and Bolls		Lima Bean Diet	
	parasitized	control	parasitized	control
	0.0451	0.2442	0.0719	0.2238
	0.0432	0.2081	0.0789	0.2897
	0.0521	0.2258	0.1182	0.2546
	0.0612	0.2331	0.0885	0.2770
	0.0653	0.2356	0.1058	0.2849
	0.0293	0.2473	0.1377	0.2640
	0.0752	0.1182	0.0851	0.3048
	0.0525	0.2809	0.0828	0.4274
	0.0519	0.2236	0.1197	0.2632
	0.0472	0.2127	0.0940	0.2790
	0.0598	0.1675	0.1136	0.2914
	0.0447	0.2008	0.0953	0.3162
	0.0563	0.2180	0.0834	0.2902
Total	0.6838	2.8158	1.2749	3.7662
$\bar{X}$	0.0526a/	0.2166a/	0.0981a/	0.2897a/
X^2	0.0376	0.6289	0.1296	1.1182
$(\Sigma X)^2/n$	0.0360	0.6099	0.1250	1.0911
x^2	0.0016	0.0190	0.0046	0.0271
d.f.	12	12	12	12

pooled s^2 = 0.0011

$S_{\bar{D}}$ = 0.0129

$t_{0.05, 48 d.f.}$ = 2.010

LSD = 0.0259

a/ All means significantly different at 0.05 level.

APPENDIX B

FECAL WEIGHTS (g) AND ANALYSES FOR *HELIOTHIS VIRESCENS* FED ON COTTON SQUARES AND BOLLS AND LIMA BEAN DIET

	H. virescens feces			
	Squares and Bolls		Lima Bean Diet	
	parasitized	control	parasitized	control
	0.0898	0.2844	0.2097	0.7979
	0.0864	0.3343	0.2340	0.5950
	0.1042	0.4269	0.2483	0.5661
	0.1236	0.5353	0.2312	0.5574
	0.1344	0.3076	0.2816	0.8524
	0.0575	0.4895	0.2137	0.6049
	0.1717	0.4360	0.2013	0.5122
	0.1054	0.4101	0.1671	0.5838
	0.1129	0.3531	0.2238	0.6337
	0.0973	0.4264	0.1190	0.5442
	0.1121	0.7002	0.2358	0.4122
	0.0878	0.4175	0.2268	0.4428
	0.1119	0.5552	0.2462	0.5983
Total	1.3950	5.6765	2.8385	7.7009
$\bar{X}$	0.1073[a]	0.4367[a]	0.2183[a]	0.5924[a]
X^2	0.1586	2.6333	0.6391	4.7392
$(\Sigma X)^2/n$	0.1497	2.4787	0.6198	4.5618
x^2	0.0089	0.1546	0.0193	0.1774
d.f.	12	12	12	12

$$\text{pooled } s^2 = 0.0075$$
$$S_{\bar{D}} = 0.0340$$
$$t_{0.05,\ 48\text{d.f}} = 2.010$$
$$LSD = 0.0683$$

[a] All means significantly different at 0.05 level.

APPENDIX C

STORAGE CAPABILITY FOR "B"-TYPE *CARDIOCHILES NIGRICEPS* PUPAE HELD AT 5°, 10° AND 15°C

Temperature C	Emergence (days) after return to normal conditions						Longevity (days) after emergence						Number Dead
	Rep 1		Rep 2		Rep 3		Rep 1		Rep 2		Rep 3		
	M	F	M	F	M	F	M	F	M	F	M	F	
1 Week Storage Period													
5	1	0	0	0	1	3	20	17	18	12	26	19	
	--	1	--	2	1	--	--	14.5	--	15	32	--	0
10	2	0	3	1	2	2	26	26	23	19	28	29	
	--	2	1	--	3	--	--	22	24	--	29	--	0
15	1	0	0	0	2	2	20.5	10	22	12	28	17	
	--	0	--	1	--	1	--	15	--	14	--	16	0
2 Week Storage Period													
5	5	4	0	3	1	2	10	19	16	20	13	13	
	3	--	--	0	--	0	15	--	--	16	--	17	0
10	5	2	3	4	3	0	9	14	12	12	16	18	
	--	--	--	2	1	--	--	--	--	18	13	--	1
15	0	0	0	0	0	0	17	32	23	27	21	34	
	--	0	--	0	0	--	--	25	--	22	15	--	0

Temperature C	Emergence (days) after return to normal conditions						Longevity (days) after emergence						Number Dead
	Rep 1		Rep 2		Rep 3		Rep 1		Rep 2		Rep 3		
	M	F	M	F	M	F	M	F	M	F	M	F	
3 Week Storage Period													
5	0	--	2	0	--	--	6	--	8	7	--	--	
	1	--	--	2	--	--	10	--	--	13	--	--	4
10	1	--	--	3	--	--	6	--	--	8	--	--	
	--	--	--	--	--	--	--	--	--	--	--	--	7
15	0	--	--	0	0	0	1	--	--	7	1	9	
	--	--	--	--	--	--	--	--	--	--	--	--	4
4 Week Storage Period													
5	--	0	1	3	4	2	--	6	4	12	8	14	
	--	--	--	2	--	--	--	--	--	7	--	--	3
10	--	--	--	--	--	--	--	--	--	--	--	--	
	--	--	--	--	--	--	--	--	--	--	--	--	9
15*	--	26	--	11	22	--	--	--	--	--	--	--	
	--	--	--	--	--	--	--	--	--	--	--	--	6

*All emerged in storage

APPENDIX D

STORAGE CAPABILITY FOR "YM"-TYPE *CARDIOCHILES NIGRICEPS* PUPAE HELD AT 5°, 10° AND 15°C

Temperature C	Emergence (days) after return to normal conditions						Longevity (days) after emergence						Number Dead
	Rep 1		Rep 2		Rep 3		Rep 1		Rep 2		Rep 3		
	M	F	M	F	M	F	M	F	M	F	M	F	
1 Week Storage Period													
5	11	8	5	10	8	3	23	29	26	35	22	28	
	5	--	--	7	--	9	17	--	--	23	--	30	0
10	10	6	6	8	4	10	36	2	28	15	32	25	
	--	7	--	7	8	--	--	19	--	20	26	--	0
15	5	6	8	4	9	11	30	23	32	28	25	18	
	--	5	7	--	8	--	--	23	28.5	--	23	--	0
2 Week Storage Period													
5	--	--	3	7	--	5	--	--	8	18	--	22	
	--	--	--	--	--	--	--	--	--	--	--	--	6
10	3	4	8	5	--	--	7	18	3	18	--	--	
	--	--	--	10	--	--	--	--	--	24	--	--	3
15	4	7	3	5	2	4	10	18	16	30	13	28	
	--	2	--	2	--	10	--	26	--	21	--	24	0

Temperature C	Emergence (days) after return to normal conditions						Longevity (days) after emergence						Number Dead
	Rep 1		Rep 2		Rep 3		Rep 1		Rep 2		Rep 3		
	M	F	M	F	M	F	M	F	M	F	M	F	
3 Week Storage Period													
5	--	--	5	9	10	--	--	--	3	8	7	--	
	--	--	--	--	--	--	--	--	--	--	--	--	6
10	--	--	--	--	--	--	--	--	--	--	--	--	
	--	--	--	--	--	--	--	--	--	--	--	--	9
15	2	--	--	--	4	3	8	--	--	--	5	10	
	--	--	--	--	--	--	--	--	--	--	--	--	6
4 Week Storage Period													
5	--	--	--	--	--	--	--	--	--	--	--	--	
	--	--	--	--	--	--	--	--	--	--	--	--	9
10	5	--	--	7	--	9	25	--	--	10	--	6	
	--	--	--	--	--	--	--	--	--	--	--	--	6
15	--	1	1	--	--	1	--	9	5	--	--	5	
	--	--	--	--	--	--	--	--	--	--	--	--	6

APPENDIX E

FECUNDITY OF *CARDIOCHILES NIGRICEPS* EXPOSED CONTINUOUSLY TO 20 *HELIOTHIS VIRESCENS* LARVAE PER DAY FOR 1 HOUR

	1 Hour Exposure (Mean number)					
Replicate	Parasitized	Pupated	Died	Escaped	Percent Parasitized	Percent Mortality
1	107.40	6.21	68.29	3.10	59	97
2	130.38	3.33	48.56	3.33	72	98
3	137.82	4.66	40.35	3.10	75	98
4	83.81	20.95	82.07	.58	45	89
5	67.05	102.44	11.18	5.59	37	43
6	129.04	2.67	53.21	1.33	70	99
7	121.06	4.66	54.71	5.82	67	97
8	130.38	18.62	27.94	9.31	74	89
Total	906.94	163.54	386.31	32.16	499	710
$\bar{X}$	113.37	20.44	48.29	4.02	62.38	88.75

APPENDIX F

FECUNDITY OF *CARDIOCHILES NIGRICEPS* EXPOSED CONTINUOUSLY TO 20 *HELIOTHIS VIRESCENS* LARVAE PER DAY FOR 24 HOURS

	24 Hour Exposure (Mean number)					
Replicate	Parasitized	Pupated	Died	Escaped	Percent Parasitized	Percent Mortality
1	146.21	26.08	21.42	11.18	75	87
2	81.27	16.93	49.10	22.01	57	91
3	101.27	5.82	30.27	25.61	76	98
4	134.51	24.83	15.52	10.35	77	86
5	117.57	9.31	27.94	31.43	76	94
6	121.06	10.64	37.25	17.30	72	94
7	125.05	1.33	31.93	27.94	79	99
8	131.31	2.79	28.87	23.28	81	98
Total	958.25	97.73	242.30	169.10	593	747
$\bar{X}$	119.78	12.22	30.29	2. .14	74.13	93.38

APPENDIX G

LONGEVITIES OF *CARDIOCHILES NIGRICEPS* EXPOSED CONTINUOUSLY TO 20 *HELIOTHIS VIRESCENS* LARVAE PER DAY FOR 1 AND 24 HOURS

	C. nigriceps Longevity (days)			
	1 Hour Exposure		24 Hour Exposure	
Replicate	Males	Females	Males	Females
1	15	15	11	10
2	20	14	23	11
3	16	12	26	8
4	11	16	12	9
5	10	5	6	8
6	7	7	4	7
7	3	8	10	7
8	8	2	5	10
Total	90	79	97	70
$\bar{X}$	11.2	9.9	12.1	8.8

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