This is to certify that the

thesis entitled

CONSEQUENCES OF INTRASPECIFIC HOST QUALITY ON THE ECOLOGY OF TWO SPECIALIST PHYTOPHAGES, MANDUCA QUINQUEMACULATA AND MANDUCA SEXTA, (LEPIDOPTERA:SPHINGIDAE)

presented by

Janice Lynne Bossart

has been accepted towards fulfillment
of the requirements for

M.S. degree in ENTOMOLOGY

Stuart H Gage
Major professor

Date 5-24-88

O-7639

MSU is an Affirmative Action/Equal Opportunity Institution

CONSEQUENCES OF INTRASPECIFIC HOST QUALITY ON THE ECOLOGY OF TWO SPECIALIST PHYTOPHAGES, *MANDUCA QUINQUEMACULATA* AND *MANDUCA SEXTA*, (LEPIDOPTERA:SPHINGIDAE)

By

Janice Lynne Bossart

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1987

ABSTRACT

CONSEQUENCES OF INTRASPECIFIC HOST QUALITY ON THE ECOLOGY OF TWO SPECIALIST PHYTOPHAGES, *MANDUCA QUINQUEMACULATA* AND *MANDUCA SEXTA*, (LEPIDOPTERA: SPHINGIDAE)

By

Janice Lynne Bossart

Field and laboratory experiments investigated the affect of host plant quality on conspecific host discrimination by two specialist phytophages, *Manduca quinquemaculata* and *Manduca sexta*, the tomato and tobacco hornworm moths (Lepidoptera: Sphingidae), and development of *M. quinquemaculata* larvae and adults. Results of these experiments were utilized to examine the hypothesis that female host preference is correlated with subsequent larval performance.

Higher rates of fertilization produced plants that conferred the highest fitness on larvae reared on foliage from these plants. Increased food efficiency, growth, survival and fecundity were correlated with mean percent total plant nitrogen and soluble nitrates. In addition, moth response, measured as the percent of total eggs deposited and the percent of plants with eggs, was highest on plants treated at higher rates of nitrogen fertilization. Finally, plants most preferred by moths permitted larvae to obtain the highest overall fitness.

This thesis is dedicated to my daughter Allyson. Through her support, patience and cooperation, I have been able to obtain goals that would have otherwise been impossible. Thank you Allyson, I will always remember how much you gave up to make graduate research possible for me.

ACKNOWLEDGEMENTS

First, I would like to express my gratitude to the members of my graduate committee, Drs. Stuart H. Gage, William J. Mattson, James R. Miller and Irvin E. Widders for their assistance and comments throughout this study. I would especially like to acknowledge Dr. Gage for providing the monetary support that enabled me to pursue this particular study. Secondly, I must extend my appreciation to Mike Jackson and the other folks at the Tobacco Research Station in Oxford, NC. Without the assistance of these people and the prompt shipment of hornworm eggs from their lab, this study would not have been possible. Finally, I must acknowledge Bruce Birr, of the USDA Forest Service, and John Dahl and Roger Deason, of the Soil Testing Laboratory at Michigan State University, all of whom assisted in the chemical analysis of foliage collected during this study.

PREFACE

This thesis addresses the interaction between host plant physiology/quality as it relates to total plant nitrogen and soluble nitrates and, subsequently, its affect on herbivore behavior and development. The analysis and discussion of this research are preceeded by a review of the literature presenting information relevant to the investigation of hypotheses regarding herbivore response to variable host quality. The literature review begins with a summary of the biology of *Manduca spp*. and the factors known to induce host selection and ovipositional responses in these insects. It then continues with a discussion of the plant components generally thought to influence host selection in female herbivores and subsequent development of their offspring. The final section of the review presents a summary of the literature pertaining to the affect of nitrogen availability on plant biochemistry and herbivore behavior and fitness.

Results of the research are presented as three separate manuscripts. All manuscripts follow the format guidelines set forth in *Oecologia*. The first paper, "The seasonal occurrence and development of *Manduca quinquemaculata* (Haworth) and *Manduca sexta* (L) (Lepidoptera: Sphingidae) in southwestern Michigan", describes the biology and relative abundance of *Manduca spp.*, at the Kellogg Biological Station in Barry Co., Michigan, in relation to degree day accumulation and investigates differences between the two species. The second manuscript, "Nutritional consequences of variable plant nitrogen: Experiments with plant fertilization and *Manduca quinquemaculata*", investigates the influence of food quality on larval development and survival and subsequent adult emergence and fecundity. The final manuscript, "Conspecific host selection and subsequent larval performance in a specialist phytophage (Lepidoptera: Sphingidae): the influence of plant quality on herbivore behavior and physiology", examines associations between tomato plant quality/ physiology, host preference and larval development and survival. This manuscript also investigates the hypothesis that female moths prefer to oviposit on plants conferring the highest overall fitness on their larvae.

The concluding section of the thesis is devoted to a discussion of the overall conclusions and implications of the project. Reference is made to results of the study that were especially interesting to the researcher and those aspects thought to warrant future investigation.

TABLE OF CONTENTS

MANUSCRIPT I. The seasonal occurrence and development of Manduca sexta and Manduca quinquemaculata (Lepidoptera: Sphingidae) in southwestern Michigan.

MANUSCRIPT II. Nutritional consequences of variable plant nitrogen: Experiments with plant fertilization and *Manduca quinquemaculata*.

MANUSCRIPT III. Conspecific host selection and subsequent larval performance in specialist phytophages: the influence of plant quality on herbivore behavior and physiology.

LIST OF TABLES

MANUSCRIPT I

MANUSCRIPT II

Table 5. Correlation of mean percent water content, dry weight nitrogen and soluble nitrates with larval performance parameters calculated from the Detailed Growth Study. Top row of numbers show Pearson's coefficient of correlation. Numbers in parentheses demonstrate level of significance. See text for definition of indices.

MANUSCRIPT III

Table 1. Means and standard errors for percent water content, total plant nitrogen and soluble nitrates calculated during each separate aspect of the study. These values were utilized in all correlation analyses requiring data on the concentration of particular plant substances.

Table 2. Distribtuion of eggs on sampled plants. Data separated by treatment and year. The top row of numbers, within each treatment, represents the frequency of occurrence and the bottom row represents the percent of total.

Table 3. Total number of hornworm eggs sampled during each year of the preference study. The number of eggs found at each nitrogen level was converted to a percent of the total eggs found during both years. See text for explanation of calculations. N = 357 and 576 total plants sampled in 1985 and 1986 respectively.

Table 4. Means (± standards errors) for all measured parameters of larval fitness at each level of nitrogen fertilizer applied.

Table 5. Linear regression statistics of larval fitness study. Numbers depict R^2 value and probability of a greater F-stastic (n.s. = not significant at p = .05).

Table 6. Correlation of mean percent dry weight plant nitrogen and soluble nitrates with measured parameters of insect performance. Top row of numbers show Pearson's coefficients of correlation. Numbers in parentheses demonstrate level of significance (n.s. = not significant at p = .05).

Table 7. Correlation of female preference with larval fitness. Top rows of numbers show Pearson's correlation coefficients. Numbers in parentheses demonstrate level of significance (n.s. = not significant at p = .05).

LIST OF FIGURES

Figure 2. Seasonal trends of percent soluble nitrates. Foliage collected during 1986. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Figure 3. Mean percent plant water at each fertilization level. Foliage collected during Detailed Performance Study. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Figure 4. Linear regression of male pupal dry weights, $p < .0001$, $R^2 = .40$, $N = 9$ at each nitrogen level. Mean values depicted by stars. See text for explanation of statistics.

Figure 5. Linear regression of female pupal dry weights, $p < .0002$, $R^2 = .34$, $N = 9$ at each nitrogen level. Mean values depicted by stars. See text for explanation of statistics.

Figure 6. Percent successful emergence of adult males from larvae reared on tomato foliage treated at four different nitrogen levels. A linear regression of arcsine transformed data produced a slope that was not significantly different from zero ($R^2 = .23$, $p < .46$). See text for explanation of statistics.

Figure 7. Percent successful emergence of adult females from larvae reared on tomato foliage treated at four different nitrogen levels. A linear regression of arcsine transformed data produced a slope significantly different from zero ($R^2 = .97$, $p < .02$). See text for explanation of statistics.

Figure 8. Linear regression of female fecundity. $R^2 = .17$, $p = .009$. $N = 10$ at each nitrogen level. Mean values depicted by stars.

Figure 9. Percent survival of larvae reared from egg hatch to premolt fifth instar on foliage treated at four rates of nitrogen fertilization. A linear regression of arcsine transformed data determined that survival was associated with fertilization level ($R^2 = .95$, $p = .02$). $N = 30$ larvae per treatment.

Figure 10. Means and standard errors for days to premolt fourth instar. $F = 14.04$, $p < .0001$. $N = 26, 25, 30$ and 30 for 0, 30, 120 and 240 lbs. nitrogen applied/acre respectively.

Figure 11. Linear correlation between ECD and Approximate Digestion. Pearson's correlation coefficient = -.90. N = 15, 18, 20 and 22 for 0, 30, 120 and 240 lbs. nitrogen applied/ acre. See text for definition of indices. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied/acre.

Figure 12. Means and standard errors for days to premolt fifth instar. F = 9.52, $p < .0001$. N = 15, 18, 20 and 22 for 0, 30, 120 and 240 lbs. nitrogen applied/acre respectively.

MANUSCRIPT III

Figure 1. Field design layout for conspecific host preference study. Large dots represent dwarf apple trees, small dots represent tomato plants. See Materials and Methods for further explanation of design.

Figure 2. Plot design depicting fertilization of single block. White circles represent treated tomato plants. Black circles represent untreated tomato plants. See Materials and Methods for further explanation of design.

Figure 3. Seasonal trends of percent dry weight nitrogen. Foliage collected during 1986. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Figure 4. Seasonal trends of percent soluble nitrates. Foliage collected during 1986. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Figure 5. Plot of mean plant volume at each level of nitrogen fertilization. Y-axis represents mean plant volume, X-axis represents date sampling conducted and Z-axis represents nitrogen treatment applied. N = 9 - 21 plants sampled per treatment per day in 1985 and 24 plants sampled per treatment per day in 1986. See Results for further explanation of plots and calculation of plant volume.

Figure 6. Seasonal hornworm egg densities. Mean eggs calculated per half plant. Reference bars denote period of maximum egg deposition. Data combined from field and orchard site. N = 60 and 96 half plants sampled per day in 1985 and 1986 respectively.

Figure 7. Percent tomato plants with eggs at each nitrogen level. Data combined from field and orchard site--1985 (heterogeneity Chi-square = 1.50, $p > .50$). A G-test analysis for independence determined that the proportion of plants with eggs is associated with the level of nitrogen applied ($G = 10.168$, $p < .025$). See text for explanation of statistics.

Figure 8. Percent tomato plants with eggs at each nitrogen level. Data combined from field and orchard site--1986 (heterogeneity Chi-square = 3.03, $p > .40$). A G-test analysis for independence determined that the proportion of plants with eggs is independent of the level of nitrogen applied ($G = 5.02$, $p < .20$). See text for explanation of statistics.

Figure 9. Percent contribution of each nitrogen treatment to total eggs found. Data fit to a quadratic regression, $R^2 = .78$, $p = .02$. Circles = 1985 data, stars = 1986 data. See text for explanation of statistics.

Figure 10. Ovipositional response to treated plants. Correlation of percent nitrogen dry weight with total number of eggs deposited (a) and proportion of plants with eggs (b) at each treatment. Data collected during 1986 only. Total number of eggs deposited = 108. N = 576 total half plants sampled. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Figure 11. Ovipositional response to treated plants. Correlation of percent soluble nitrates with total number of eggs deposited (a) and proportion of plants with eggs (b) at each treatment. Data collected during 1986 only. Total number of eggs deposited = 108. N = 576 total half plants sampled. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Figure 12. Correlation between percent plants with eggs and larval performance potential. Percent plants with eggs based on 144 plants sampled per treatment. See text for definition of larval performance potential. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

REVIEW OF LITERATURE

History and Ecology of Manduca spp.

Manduca sexta (L.), the tobacco hornworm, and Manduca quinquemaculata (Haworth), the tomato hornworm, (Lepidoptera: Sphingidae) are widely distributed throughout North America. The northern range for both species extends into Southern Canada (Madden and Chamberlin 1945, Baumhover 1985). The southern range however, is more variable. For M. quinquemaculata, the limit appears to be Northern Florida and Southern Georgia (Madden and Chamberlin 1945), while M. sexta has been documented as far south as Southern Brazil (Madden and Chamberlin 1945, Baumhover 1985).

The adult moths are crepuscular, i.e., active prior to 9 p.m. and just before dawn, and remain hidden during daylight hours. These moths are large, strong fliers with a wingspan stretching approximately 12 cm. At the beginning of active periods, the moths can be found hovering over the tubular blossoms of jimsonweed, petunia, evening primrose and hollyhock, feeding on the nectar from these flowers. The female oviposits an average of 200 - 300 eggs during her lifetime, the majority of which are generally deposited on the upper 1/3 of the host plant. The spherical, light yellowish-green eggs are usually laid singly on the lower surface of a host leaf. Hatch occurs in approximately four to five days (Madden and Chamberlin, 1945).

The larval stage generally consists of five instars although, six instars are not unknown. The two species are readily distinquishable by the third instar. _M. sexta_ larvae are characterized by a scarlet horn and a soft, pubescent covering on their body. _M. quinquemaculata_ have a black horn and lack pubescence. Larvae are fully developed in approximately 20 days and reach a final weight of about 8 - 9 grams and final length of approximately 75 - 85 mm (Gilmore 1938). The mature larvae leave the plant, burrow into the ground and hollow out an earthen cell (Gilmore 1938, Madden and Chamberlin 1945). Pupation occurs in the soil at a depth of 10 - 12 centimeters (Madden and Chamberlin 1945). Eclosion takes place, during the night, approximately 17 - 25 days after the onset of pupation. Both species overwinter as diapausing pupae.

M. sexta and _M. quinquemaculata_ are both host specific, utilizing only members of the Solanaceous family--which includes, among others, tobacco, tomato, eggplant, potato, jimsonweed and nightshade. Laboratory reared hornworms can be induced to feed on some non-solanaceous species (= non-host species) (Waldbauer and Fraenkel 1961, Waldbauer 1962, 1964), thus suggesting that development can occur on a much wider range of plants. It is interesting to note however, that natural larval populations are almost entirely restricted to tobacco and tomato (Gilmore 1938, Yamamoto et. al. 1969). Since it has already been determined that larvae tend to remain on the same plant selected by the adult female (McFadden 1968), it appears that egg deposition occurrs almost exclusively on these tobacco and tomato plants.

Captive adults are known to exhibit three types of flight behavior (Yamamoto et. al. 1969). The first, dispersal flight, is characterized by being a highly erratic and accelerated flight. Following this flight is a normal foraging flight characterized by moths flying in a circular path at mid-level in the cage. During this stage, objects may be approached and landed upon. The third type of flight, low altitude foraging flight, occurs at or near ground level. Again, acceptable objects are approached and landed upon. The first two types of behavior have also been noted during experiments conducted during this study at the Kellogg Biological Station (KBS) in Hickory Corners, Michigan.

The moths approach objects of various physical attributes nearly equally from a distance but do not land on them equally. Instead, they tend to veer away from non-host plants at distances of from 4 to 15 cm (Yamamoto et. al. 1969). This observation suggests that host searching is comprised of random or directed random movements until the host odor stimulus is encountered by the moth (a close-range stimulant). Upon contacting this odor, the moth is stimulated to approach the host and, if an appropriate "pattern" of stimuli is present, is induced to land. Once a suitable plant is located, the hovering female places her fore tarsi on the upper surface of the ovipositional site and deposits a single egg on the lower leaf surface (Sparks 1973).

Single-choice tests, conducted by Yamamoto and Fraenkel (1960a), determined that moths highly prefer solanaceous plants over non-solanaceous ones. In fact, moths failed to oviposit at all on the non-solanaceous plants. In addition, results from a two-choice test demonstrated that tomato plants were significantly preferred over other choices, including other solanaceous plants. These results led to the hypothesis that at least two different chemical stimuli influence host selection and ovipositional responses by the moth. The first stimulus serves to orient the moth to the plant (=orientational stimulus) and appears to be particular for a given plant species. The other stimulus, an ovipositional stimulus, appears to be common to all solanaceous plants.

Further experimentation was conducted to elucidate the chemical components that elicited these behaviors (Yamamoto and Fraenkel 1960b). Ethanol extracts of tomato leaves, which were known to contain the "ovipositional stimulus", were tested for their ability to generate a response by the moths. Since egg deposition occurred only in those areas in which the ethanol extract had been sprayed, it was concluded that this stimulus was perceived upon contact.

Steam distillants of leaves were examined to determine if the stimulatory chemicals could be detected through olfactory processes. Results demonstrated that volatile components did, indeed, act as "attractants" and served to orient the moth to the host. It was also determined that the steam distillant and ethanol extract used together elicited a synergistic effect, dramatically increasing the number of eggs deposited. As a result of these studies, Yamamoto and Fraenkel (1960b) proposed that these chemical stimuli must act in sequence, i.e., the steam distillable or volatile component attracts the moth to the host and the ethanol extractable component induces the moth to oviposit.

In a more recent paper, Tichenor and Seigler (1980) assayed chemical fractions of tomato and tobacco leaves for electroantennogram (EAG) activity. Steam distillable portions of both plant species were found to be EAG active. An acid-base extraction demonstrated that the volatiles could be separated into an acidic and neutral fraction both of which generated strong, positive EAG responses. The basic fraction of this extraction did not give a positive EAG response. Tests to examine ovipositional responses demonstrated a strong response to both the acidic and basic fractions but showed almost no response to the neutral fraction. These results suggest that the two-step process of host selection and oviposition as described by Yamamoto and Fraenkel (1960b) holds true. Certain chemical components serve as "attractants" and orient the gravid females to the host (steam distillable portion and neutral fraction) while other components elicit ovipositional behaviors in the moths (basic fraction) and are probably perceived upon contact.

At the Tobacco Insect Investigation Laboratory at Oxford, North Carolina, Sparks (1973) performed a study to examine the influence of free water, water vapor, touch and host-plant odor on ovipositional behavior in Manduca. Results of a two-choice test demonstrated that both odor and moisture induce ovipositional behaviors and that a combination of these two factors is significantly more attractive to the moth than either component separately. Further testing revealed that contact of the ovipositor with a bristly surface elicited a greater response than contact of the ovipositor with a smooth surface. The results of these tests strongly suggest that physical factors, as well as chemical factors, influence behavioral responses in Manduca spp.

Overview of Host Selection

A number of factors are involved in the selection of a host species. These behavioral stimuli can generally be divided into those that are important during the prealighting (host searching) phase and those that exert an influence during the post-alighting (host testing) phase.

During the pre-alighting phase, behavioral responses to olfactory and visual stimuli enable the insect to selectively approach a particular plant species. Visual cues, such as plant height and size and leaf shape, area and color (Rausher 1978, Myers 1985) may provide the insect with a "search image" of the preferred host (Rausher 1978, 1983). Females responding positively to particular visual stimuli will preferentially alight on plants matching this image (Rausher 1978, Courtney 1983), thus increasing the probability that they will alight on the "true" host species.

Certain insects perceive the chemical odors produced by their host plant from a distance (Hawkes 1974, Thibout et. al 1982, Visser and Thiery 1985). For these species, long range odoriferous compound induce an anemotactic response, i.e., molecules of "attractive" chemicals stimulate the insect to fly upwind into an odor plume until reaching the source of the odor (Rausher, 1983). Kennedy and Moorhouse (1969) reported that locusts moved upwind in response to wind borne grass odor. Hawkes (1974) determined that the cabbage fly, Delia brassicae, was able to detect and subsequently locate crucifer crops from distances up to 15 meters upwind.

In other species, host searching is largely made up of random and directed random movements. For these insects, non-hosts are approached at the same rate as hosts species. However, once the insect enters the area immediately surrounding a host plant and encounters the associated chemical stimuli, subsequent movement becomes directed towards the odor source. *Manduca sexta* is known to approach objects of various physical attributes nearly equally, but tends to veer away from non-hosts at distances of from 4 to 15 cm (Yamamoto et. al. 1969).

It is well known that plant chemicals also exert an influence during the post-alighting phase of the selection process (see Rausher, 1983). These stimuli are often received through chemoreceptors located on the tarsi or on the proboscis (Stadler 1984) and provide the insect with information on the suitability of a plant as an ovipositional site. *Pieris brassicae* females have been noted "drumming" the host plant with their front legs prior to ovipositing (Ilse, 1956). *Delia antiqua* exhibits pre-ovipositional behaviors that include a number of "test runs" on the onion plant (Miller and Harris 1985). *Manduca spp*. are known to place their front two pairs of tarsi on the upper leaf margin of the host before curling the abdomen around to the lower surface and depositing an egg (Sparks 1973). These behavioral responses suggest that females "test" potential hosts for the proper array of chemical and physical stimuli prior to oviposition.

Chemical Components of Host-Selection

A vast amount of research has been devoted to determining the major chemical constituents that enable an insect to distinguish among potential hosts. Two basic theories have been proposed concerning the mechanisms that mediate host-plant selection. During the 1950's and 60's, each of these theories was debated quite extensively and, while not as intensely argued today, still remain a source of controversy.

Secondary Chemicals: Many phytophagous insects are associated with particular groups of plants. For these specialists, acceptable host plants are generally limited to species that are taxomonically related and contain similar classes of chemical compounds (Fraenkel et. al. 1962, Schoonhoven 1968). These secondary or "key" compounds, include the plant glycosides, essential oils, alkaloids, etc. It has been hypothesized that these chemical substances were originally synthesized by the plant as a defensive mechanism against herbivory, but later, came to be utilized in the host selection process by those insects that were able to adapt to the chemical "flavors" of their hosts (Dethier 1947, Finch 1978). Thus, for insects attracted to these "flavors", selection is dependent upon a behavioral response towards this chemical stimulus (Hawkes 1974, Thibout et. al. 1982).

As early as 1910, Verschaffelt reported that host selection was largely influenced by the presence of certain chemical compounds. Lending support to these early findings, similar types of behavioral responses have been noted in more recent studies. Gupta and Thorsteinson (1960) determined that isothiocyanates stimulated egg-laying by

Plutella maculipennis. Isothiocyanates have also been shown to induce host-finding and ovipositional behaviors by Delia brassicae, the adult cabbage maggot (Nair et. al. 1976, Finch 1978). Manduca sexta and Leptinotarsa decemlineata are known to be stimulated by the steroidal glycoalkaloids associated with the Solanaceous plant family (Yamamoto and Fraenkel 1960a and 1960b, Mitchell and Harrison 1985).

Nutritional Factors: Since the nutrition of the host-plant can affect the nutrition of the insect (House 1969, Fox and MacCauley 1976, Hough and Pimentel 1978, Hare 1983), it is reasonable to hypothesize that insects are equipped with a mechanism that enables them to distinquish, in part, the kinds and quantities of nutrients that a host contains (Thorsteinson 1958). Thorsteinson (1960) was one of the first to argue that although secondary chemicals might be important in host recognition, nutrients also stimulate positive behavioral responses by phytophagous insects. Plant sugars, water and nitrogen are nutritional components known to influence insect performance.

Sugars exert a direct chemotactic influence on phytophagous insects (Dethier 1947). Fermenting sugar baits have been used for collecting nocturnal moths (Utrio 1983) and for monitoring populations of tortricids (Green et. al. 1960). In addition, these compounds are also known to interact as stimuli with several other groups of substances (Thorsteinson 1960, Utrio 1983).

The positive affect of plant water on behavioral responses in herbivorous insects is widely documented (Dethier and Chadwick 1948, Painter 1953, Shorey 1964, Myers 1985). Wolfson (1980) determined that the number of eggs deposited by *Pieris rapae* was positively correlated with leaf water content. Sparks (1973) demonstrated that free moisture and water vapor induced ovipositional behaviors in *Manduca spp*.

Insects are well known to respond to several different nitrogenous compounds (see Thorsteinson 1960). *Rhagoletis polmonella* is attracted to traps containing a bait composed of powdered egg albumin (Howitt and Connor 1965). More recently, Myers and Post (1981) found a positive correlation between host plant quality (measured as percent nitrogen) and the number of eggs oviposited by the cinnabar moth per gram of plant material.

It is now generally recognized that both secondary chemicals and nutritional compounds influence host searching and acceptance (Kennedy 1965, Schoonhoven 1968, Wolfson 1978, Miller and Strickler 1984). Kennedy (1965) proposed the dual-discrimination theory whereby both secondary chemicals and nutritional substances elicit ovipositional responses by insects. It is likely that, at a given time a particular chemical may serve as an allelochemic or as a nutrient depending on its concentration in the plant and the physiological state of the insect (Scriber and Slansky 1981). A current hypothesis suggests that secondary chemicals may be utilized to discriminate among particular plant families or species while nutritional factors are mostly used to discriminate among individual plants within a particular host species (Wolfson 1978).

Nitrogen and Herbivory

An herbivore must find and ingest food that is "capable of being assimilated and converted into energy and structural substances" as well as meeting the nutritional requirements of that insect (Beck and Reese 1976). Nitrogen is a key mineral that is associated with a number of these metabolic processes (Mattson 1980). Not only does this substance influence the physiology of an insect, but it also affects the general behavior of the animal (Beck 1974).

Direct Effects on Insect Nutrition

Acquiring a balanced supply of amino acids and proteins from a particular food source may present a major obstacle for many herbivores (McNeill and Southwood 1978). First, plant nitrogen concentrations vary throughout the host plant and throughout the growing season (Beck 1972, Mattson 1980, White 1984). Secondly, a large portion of the total nitrogen found in plant tissue is not readily available to an herbivore (McNeill and Southwood 1978, Reese 1978, Mattson 1980). Finally, the nutritional value of proteins injested by the herbivore is dependent upon the ratio, pattern and quantity of amino acids that make up those proteins (Rock 1972, House 1969).

Increased levels of plant nitrogen are often beneficial and attractive to an insect. Growth rate, longevity, fecundity, and/or survivorship are known to increase (Shaw and Little 1972, van Emden and Way 1973, Auerbach and Strong 1981, McNeill and Prestidge 1982, Al-Zubaidi and Capinera 1984). Plants with high nitrogen concentrations often have higher infestations of herbivores (Kennedy 1958). White (1979) proposed that increased levels of plant nitrogen cause a subsequent increase in the numbers of insects found on that plant. House (1966a) reported that resistant plant varieties are those having the lowest concentrations of amino acids, implying that susceptible varieties are those having the higher levels. Regan et. al. (1978) and Semtner et. al. (1980) found that nitrogen fertilization increased populations of Manduca sexta.

For other insect species though, plant nitrogen content appears to have little influence on overall performance. Often those individuals developing on a nutritionally inadequate diet can regulate consumption (House 1965, Slansky and Feeny 1977). Scriber and Slansky (1981) determined that a reduction in plant nitrogen had little or no effect on growth due to an increase in the rate of consumption and/or an increase in the amount of time spent feeding, digesting or developing.

Indirect Effects on Insect Nutrition

It is obvious that the availability of nitrogen affects the pattern and quality of amino acids and proteins in the plant and hence, directly influences herbivore nutrition. However, because of its influence on other metabolic processes, plant nitrogen may also indirectly affect insect fitness.

Nitrogen, Plant Water and Herbivory: Plant water content is one aspect of metabolism affected by nitrogen availability. The association between plant nitrogen content and leaf water is well documented (Scriber 1977, Slansky and Feeny 1977, Mattson 1980, Mattson and Haack 1987). Positive correlations between concentrations of plant nitrogen and levels of plant water have been demonstrated by a number of researchers (Benebal and Hall 1967, Slansky and Feeny 1977, Manuwoto and Scriber 1985). Similarly, White (1978) and Mattson and Haack (1987) reported that drought increased the levels of nitrogenous compounds found in plant tissue.

The effect of plant water on ovipositional responses by insects has already been established (see discussion under host selection). Additionally, however, concentrations of plant water can also influence insect fitness. Although once overlooked as a nutrient, it is now well documented that growth and development of an herbivorous insect is dependent upon the water content of its diet

(Pratt et. al. 1972, Beck and Reese 1976, Feeny 1976, Reese 1983, Hagen et. al. 1984). Scriber (1978) determined that various insect growth parameters are positively correlated with percent leaf water. Larvae consumed, grew and utilized their food faster and more efficiently when fed diets containing higher concentration of leaf water (see also Scriber 1977, Scriber and Slansky 1981, Reynolds et. al. 1985).

Nitrogen, Alkaloids and Herbivory: Approximately, one-seventh of the total number of flowering plants or approximately 100 families (McKee 1962, Robinson 1979) contain alkaloids. Many of these alkaloids are derived from amino acids and can form through simple metabolic processes. Other alkaloids are more closely related to steroidal precurssors--steroidal glycoalkaloids--(McKee 1962, Geissman and Crout 1969) and are thought to arise through the degradation of cholesterol (Geissman and Crout 1969). Generally, alkaloids accumulate in young, actively growing tissues. However, these compounds have also been documented in vascular sheaths, latex vessels and epidermal and hypodermal tissues (Robinson 1979).

Within a given plant species, the concentration of an alkaloid varies depending on the particular tissue examined and the supply of minerals, soil moisture, temperature and light that the plant receives (Waller and Nowacki 1978, Robinson 1979, Mattson and Haack 1987). Since alkaloids are compounds containing nitrogen, it is reasonable to assume that the availability of this element will affect alkaloid metabolism in the plant (Waller and Nowacki 1978). This response has, in fact, been demonstrated in several cases. Waller and Nowacki (1978) cite several studies which show that the addition of nitrogen affects the production and subsequent level of alkaloids in a plant. A review of the literature by Mattson (1980) found that "optimal growth conditions and high soil nitrogen levels favored maximal alkaloid synthesis". Geissman and Crout (1969) determined that increased plant nitrogen was paralleled by an increase in alkaloid production. However, high levels of plant nitrogen do not necessarily mean that alkaloid concentrations will increase. For the steriodal glycoalkaloids, commonly found in many species of Solanum and Lycopersicon, biosynthesis is dependent upon the ratio of carbon to nitrogen; therefore, unless sufficient stores of carbon are present, an increase in the availability of nitrogen will not increase total alkaloid levels (Nowacki et. al. 1976, Waller and Nowacki 1978).

Many of the alkaloids are noted for their physiological effects on animals and are known to prevent normal metabolic activities from occurring by disrupting membrane transport, enzyme inhibition and activation, DNA replication, RNA transcription and protein synthesis in the animal (McKee 1962, Robinson 1979). In insects, alkaloids may inhibit growth and development (Harley and Thorsteinson 1967, Nowacki et. al. 1976) or act as a repellent. Leptine I, an alkaloid found in potatoes and tomatoes, deters feeding by the Colorado potato beetle (Tingey 1984, Sinden et. al. 1986).

It has been hypothesized that insects adapted to particular plant families utilize the secondary chemicals associated with those families as host searching cues. Although other secondary chemicals, such as sinigrin and isothiocyanates are known to induce host searching behavior in certain phytophagous insects (Gupta and Thorsteinson 1960, Nair and McEwen 1976, Finch 1978), no studies are known that demonstrate a similar response with plant alkaloids, although, glycosides, a structural component of glycoalkaloids, are thought to induce feeding in _Manduca sexta_ (Yamamoto 1960b).

Literature Review Summary

Manduca spp. are specialist insects that utilize the solanaceous plants (glycoalkaloid containing plants) as hosts. Adult females respond to short range odors and chemical "flavors" of particular plants. The moths also respond to physical attributes associated with their hosts. Both plant moisture and leaf texture influence ovipositional behaviors. When _Manduca_ moths are presented with combinations of host stimuli, a synergistic effect is induced, implying that host selection and oviposition are influenced by an array of factors that, in combination, provides the moth with "unique" information regarding its host species.

In general, host selection by a specialist phytophage may be dependent upon particular "key" plant compounds or certain nutritional components. However, it is likely that combinations of these plant factors interact during different stages of the host selection process to produce a "pattern" of chemical stimuli that elicit a response in the insect.

Nitrogen is one plant component known to directly influence plant physiology and subsequent insect performance. Depleting or increasing the nitrogen pool alters the pattern and concentration of plant substances which incorporate this element as a structural component. These alterations can, in turn, affect host finding, ovipositional response and larval development.

REFERENCES TO LITERATURE REVIEW

Al-Zubaidi PS, Capinera JL (1984) Utilization of food and nitrogen by the beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) in relation to food type and dietary nitrogen levels. Env. Ent. 13:1604-1698.

Auerbach MJ, Strong DR (1981) Nutritional ecology of *Heloiconia* herbivores: experiments with plant fertilization and alternative hosts. Ecological Monographs. 51:63-83.

Baumhover AH (1985) *Manduca sexta*. In: Handbook of Insect Rearing. Vol. 2. (Pritam S, Moore RF, eds.). Elsevier Science Publishers B.V. Amsterdam.

Beck SD (1965) Resistance of plants to insects. Ann. Rev. Entomol. 10:207-232.

Beck SD (1972) Nutrition, adaptation and environment. In: Insect and Mite Nutrition. North-Holland Publishing Co. Amsterdam.

Beck SD (1974) Theoretical aspects of host plant specificity in insects. In: Proc. Summer Institute on Biological Control of Plant Insects and Diseases. (Maxwell FG, Harris FA, eds.). Univ. Press of Miss. Jackson, Miss.

Beck SD, Reese JC (1976) Insect-plant interactions:nutrition and metabolism. In: Biochemical Interactions Between Plants and Insects. (Wallace JW, Mansell RL, eds.). Rec. Adv. Phyto Chem. 10:41-92.

Benebal PS and Hall CV (1967) Biochemical composition of host plants of *Brassica oleracea* var. capitata (L) as related to resistant to *Trichoplusai ni* Hubner and *Pieris rapae* Linne. Proc. Am. Soc. Hort. Sci. 91:325-330.

Bernays EA (1982) The insect on the plant-a closer look. Proc. Fifth Int. Symp. Insect-Plant Relationships. PUDOC, Wageningen.

Courtney SP (1983) Models of hostplant location by butterflies: the effect of search images and search efficiency. Oecologia. 59:317-321.

Dethier VG (1947) Chemical Insect Attractants and Repellents. Blakiston Co. Philadelphia.

Dethier VG, Chadwick LE (1948) Chemoreception in insects. Physiol. Rev. 28:220-54.

Feeny P (1976) Plant apparency and chemical defense. Rec. Adv. in Phytochem. 10:1-40.

Finch S (1978) Volatile plant chemicals and their effect on host plant finding by the cabbage root fly (Delia brassicae). Ent. exp. appl. 24:350-359.

Fox LR, MacCauley BJ (1976) Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. Oecologia. 29:145-162.

Fraenkel G, Nyar J, Nalbandov O, Yamamoto RT (1962) Further investigations into the chemical basis of the insect-hostplant relationship. XI Int. Congr. Entomol. Vienna, Verhandlungen, Bd. III.

Geissman TA, Crout DHG (1969) Organic Chemistry of Secondary Plant Metabolism. Freeman, Cooper and Co. San Fransico, California.

Gilmore JU (1938) Observations on the hornworms attacking tobacco in Tennessee and Kentucky. J. Econ. Ent. 31:706-712.

Green N, Geroza M, Hall SA (1960) Recent developments in chemical attractants for insects. Advances in Pest Control Research 3:129-179.

Gupta PD, Thorsteinson AJ (1960) Food plant relationships of the diamond-back moth (Plutella maculipennis (Curt.)). II. Sensory regulation of oviposition of the adult female. Ent. exp. appl. 3:305-314.

Hagen KS, Dadd RH, Reese J (1984) The food of insects. In: Ecological Entomology. (Huffaker CB, Rabb RL, eds.). John Wiley and Sons, Inc.

Hare JD (1983) Seasonal variation in plant-insect associations:utilization of Solanum dulcamara by Leptinotarsa decimlineata. Ecology. 64:345-361.

Harley KLS, Thorsteinson AJ (1967) The influence of plant chemicals on the feeding behavior, development and survival of the two-stripped grasshopper, Melanoptus bivittatus (Say), Acrididae: Orthoptera. Can. J. Zool. 45:305-319.

Hawkes C (1974) Dispersal of adult cabbage root fly (Erioschia brassicae (Bouche)) in relation to a brassica crop. J. appl. ecol. 11:83-93.

Hough JA, Pimentel D (1978) Influence of host foliage on development, survival and fecundity of the gypsy moth. Env. Ent. 7:97-102.

House HL (1965) Effects of low levels of the nutrient content of a food and of nutrient imbalance on the feeding and the nutrition of a phytophagous larva, Celerio euphobiae (Linnaeus) (Lepidoptera: Sphingidae). Can. Ent. 97:62-68.

House HL (1965b) Insect nutrition. In: The Physiology of Insecta. (Rockstein M, ed.). Academic Press. New York. 2:815-858.

House HL (1969) Effects of different proportions of nutrients on insects. Ent. exp. & appl. 12:651-669.

Howitt AJ, Connor LJ (1965) The response of Rhagoletis pomonella (Walsh) adults and other insects to trap boards baited with protein hydrolysate bait. Proc. Entomol. Soc Ontario. 95:134-136.

Ilse D (1956) Behavior of butterflies before ovipositing. J. Bombay Nat. Hist. Soc. 53:486-488: cited by Miller and Strickler, 1984.

Kennedy JS (1958) Physiological conditions of the hostplant and susceptibility to aphid attack. Ent. exp. & appl. 1:50-65.

Kennedy JS (1965) Mechanisms of host plant selection. Ann. Appl. Bio. 56:317-322.

Kennedy JS, Moorehouse JE (1969) Laboratory observations on locust responses to wind-borne grass odour. Ent. exp. & appl. 12:487-503.

Madden AH, Chamberlin FS (1945) Biology of the tobacco hornworm in the southern cigar-tobacco district. US Dept. of Agric. Tech. Bull. No. 986.

Manuwoto S, Scriber JM (1985) Consumption and utilization of experimentally altered corn by southern armyworm: iron, nitrogen and cyclic hydroxamates. Journal of Chemical Ecology. 11:1469-1483.

Mattson WJ, (1980) Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11:119-161.

Mattson WJ, Haack RA (1987) The role of drought in outbreaks of plant-eating insects. BioScience. 37:110-118.

McFadden MW (1968) Observations on feeding and movement of tobacco hornworm larvae. J. Eco. Entmol. 61:352-356.

McKee HS (1962) Nitrogen Metabolism in Plants. Clarendon Press. Oxford.

McNeill S, Prestidge RA (1982) Plant nutritional strategies and insect herbivore community dynamics. Proc. Fifth Int. Symp. Insect-Plant Relationships. PUDOC, Wageningen. pp. 225-235.

McNeill S, Southwood TRE (1978) The role of nitrogen in the development of insect/plant relationships. In: Biochemical aspects of plant and animal coevolution. (Harborne JB, ed.). Academic Press. London.

Miller JR, Harris MO (1985) Viewing behavior-modifying chemicals in the context of behavior: lessons from the onion fly. In: Chemical Ecology of Insects. (Bell WJ, Carde RJ, eds.,). Sinauer Associates. Sunderland, MA.

Miller JR, Strickler KL (1984) Finding and accepting host plants. In: Chemical Ecology of Insects. (Bell WJ, Carde RT, eds.). Sinauer Associates, Sunderland, MA.

Mitchell BK, Harrison GD (1985) Effects of Solanum glycoalkaloids on chemosensilla in the Colorado potato beetle. A mechanism of feeding deterrence? Journal of Chemical Ecology. 11:73-83.

Myers JH, Post BJ (1981) Plant nitrogen and fluctuations of insect populations: a test with the cinnabar moth-tansy ragwort system. Oecologia. 48:151-156.

Myers JH (1985) Effect of physiological condition of the host plant on the ovipositional choice of the cabbage white butterfly, Pieris rapae. Jour. of Animal Eco. 54:193-204.

Nair KSS, McEwen FL, Snieckus V (1976) The relationship between glucosinolate content of cruciferous plants and ovipostion preferences of Hylema brassica (Diptera: Anthomyiidae) Can. Ent. 108:1031-1036.

Nowacki E, Jurzysta M, Gorski P, Nowacka D, Waller GR (1976). Effect of nitrogen nutrition on alkaloid metabolism in plants. Biochem. Physiol. Pflanzen. 169:231-240.

Painter RH (1953) The role of nutritional factors in host plant selection. Trans. Ninth Int. Congr. Ent. Vol. 2:101-105.

Pratt JJ, House HL, Mansingh A (1972) Insect control strategies based on nutritional principles: a prospectus. In:Insect and Mite Nutrition. (Rodriques JG, ed.). North-Holland Publishing Co. Amsterdam.

Rausher MD (1978) Search image for leaf shape in a butterfly. Anim. Behav. 27:1034-1040.

Rausher MD (1983) The ecology of host selection behavior in phytophagous insects. In: Variable Plants and Herbivores in Natural and Managed Systems. (Denno RF, McClure MS, eds.). Academic Press, New York.

Reese JC (1978) Chronic effects of plant allelochemics on insect nutritional physiology. Ent. exp. & appl. 24:425-431.

Reese JC (1983) Nutrient-allelochemical interactions in host plant resistance. In: Plant Resistance to Insects (Hedin PA, ed.). ACS Symposium Series, No. 208. American Chemical Society. Washington, DC.

Regan TE, Rabb RL, Collins WK (1978) Selected cultural practices as affecting production of tobacco. J. Econ. Entomol. 71:79-82.

Reynolds SE, Nottingham SF, Stephens AE (1985) Food and water economy and its relation to growth in fifth-instar larvae of tobacco hornworm, Manduca sexta. J. Insect Physiol. 31:119-127.

Robinson T (1979) The evolutionary ecology of alkaloids. In: Herbivores Their Interaction with Secondary Plant Metabolites. (Rosenthal GA, Janzen DH, eds.). Academic Press. New York.

Rock GD (1972) Optimal proportions of dietary amino acids. In: Insect and Mite Nutrition. (Rodriquez JG, ed.). North-Holland Publishing Co. Amsterdam.

Schoonhoven LM (1968) Chemosensory bases of host plant selection. Ann. Rev. Ent. 13:115-136.

Scriber JM (1977) Limiting effects of low leaf-water content on the nitrogen utilization, energy budget and larval growth of Hyalophora cecropia (Lepidoptera: Saturniidae). Oecologia. 28:269-287.

Scriber JM (1978) The effects of larval feeding specialization and plant growth form on the consumption and utilization of plant biomass and nitrogen:an ecological consideration. Ent. exp. & appl. 24:494-510.

Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. Ann. Rev. Entomol. 26:183-211.

Semtner PJ, Rasnake M, Terrill TR (1980) Effect of host-plant nutrition on the occurrence of tobacco hornworms and tobacco flea beetles on different types of tobacco. J. Econ. Entomol. 73:221-224.

Shaw GG, Little CHA (1972) Effect of high urea fertilization of balsam fir trees on spruce budworm development. In:Insect and Mite Nutrition. (Rodriquez JG, ed.). North-Holland Publishing Co. Amsterdam.

Shorey HH (1964) The biology of Trichoplusia ni (Lepidoptera: Noctuidae). III. Response to the oviposition substrate. 57:165-170.

Sinden SL, Sanford LL (1986) Leptine glycoalkaloids and resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in Solanum chacoense. Env. Ent. 15: 1057-1062.

Slansky F, Feeny P (1977) Stabilization of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47:209-228.

Sparks MR (1973) Physical and Chemical stimuli affecting oviposition preference of Manduca sexta (Lepidoptera: Sphingidae). Ann. Ent. Soc. Am. 66:571-573.

Stadler E (1984) Contact Chemoreception. In: Chemical Ecology of Insects. (Bell WJ, Carde RJ, eds.). Sinauer and Associates, Sunderland, MA.

Thibout E, Auger J, Lecomte C (1982) Host plant chemicals responsible for attraction and oviposition in Acrolepiopsis assectella. In: Insect-plant relationships. (Visser JH ed.). Minks PUDOC, Wageningen.

Thorsteinson AJ (1958) Acceptability of plants for phytophagous insects. Proc. Tenth Int. Congr. Entomol. Montreal 1956. 2:599-602.

Thorsteinson AJ (1960) Host selection in phytophagous insects. Ann. Rev. Ent. 5:193-218.

Tichenor LH, Seigler DS (1980) Electroantennogram and oviposition responses of Manduca sexta to volatile components of tobacco and tomato. J. Insect Physiol. 26:309-314.

Tingey W (1984) Glycoalkaloids as pest resistance factors. Am. Potato J. 61:157-167.

Utrio P (1983) Sugaring for moths:why are noctuids attracted more than geometrids. Ecological Ent. 3:437-445.

van Emden and Way (1973) Host plants in the population dynamics of insects. Insect/Plant Relationships. Symposia of the Royal Entomological Soc. of London. No. 6. Blackwell. Oxford.

Verschaffelt E (1910) The cause determining the selection of food in some herbivorous insects. Proc. R. Acad. Am. 13:536-42.

Visser H, Thiery D (1985) Behavioral responses of the Colorado potato beetle to stimulation by wind and plant odors. In: Proc. Symp. Colorado potato beetle, Seventeenth Int. Congr. Entomol. (Ferro DN, Voss RH, eds.). Chp. 10. Bull. Mass. Agric. Exp. Stn.

Waldbauer GP, Fraenkel GS (1961) Feeding on normally rejected plants by maxillectomized larvae of the tobacco hornworm, Protoparce sexta (Lepidoptera, Sphingidae). Ann. Ent. Soc. Amer. 54:477-485.

Waldbauer GP (1962) The growth and reproduction of maxillectomized tobacco hornworms feeding on normally rejected nonsolanaceous plants. Ent. exp. & app. 5:147-158.

Waldbauer GP (1964) The consumption, digestion and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco hornworm, Protoparce sexta (Johan.) (Lepidoptera: Sphingidae). Ent. exp. & appl. 7:251-269.

Waller GR, Nowacki EK (1978) Alkaloid Biology and Metabolism in Plants. Pleunum Press, NY and London.

White TCR (1978) The importance of a relative shortage of food in animal ecology. Oecologia. 33:71-86.

White TCR (1979) An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology. 50:905-909.

White TCR (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia. 65:90-105.

Wolfson JL (1978) Environmentally induced variation in *Brassica nigra* (Koch): affects on generalists and specialist herbivores. Ph.D. dissertation. State Univ. of New York. Stony Brook

Wolfson JL (1980) Oviposition response of *Pieris rapae* to environmentally induced variation in *Brassica nigra*. Ent. exp. & appl. 27:223-232.

Yamamoto RT, Jenkins RY, McClusky RK (1969) Factors determining the selection of plants for oviposition by the tobacco hornworm, *Manduca sexta*. Ent. exp. & appl. 12:504-508.

Yamamoto RT, Fraenkel GS (1960a) The specificity of tobacco hornworm, *Protoparce sexta*, to solanaceous plants. Ann. Ent. Soc. Amer. 53:503-507.

Yamamoto RT, Fraenkel GS (1960c) The physiological basis for the selection of plants for egg-laying in the tobacco hornworm, *Protoparce sexta* (Johan.) XI Int. Kongr. Ent. Wien. 3:127-133.

MANUSCRIPT I: The biology and seasonal occurrence of *Manduca quinquemaculata* and *Manduca sexta* (Lepidoptera: Sphingidae) in southwestern Michigan.

ABSTRACT

The seasonal occurrence and development of field populations of *Manduca sexta* and *Manduca quinquemaculata* were monitored at the Kellogg Biological Station in Barry Co., Michigan from late June to early September during 1985 and 1986. Both insect species were equally abundant in field plots during mid-season (end of July to mid August) in both years of the study. In 1985, *M. quinquemaculata* comprised approximately 80% of the hornworm population sampled early in the season, while *M. sexta* was the more prevalent species late in the season, comprising 100% of the hornworm population sampled. In 1986, although *M. sexta* was again the more prevalent species late in the season (73%), both species were relatively common during the beginning of the season. These observations suggest that *M. quinquemaculata* is univoltine in Michigan while *M. sexta* may be multivoltine.

Egg deposition and larval development were found to be closely associated with degree day (DD) accumulation base 50° F. Peak oviposition occurred on DD 1350 (July 11) in 1985 and DD 1320 (July 10) in 1986. Field and laboratory experiments demonstrated that development rates were similar for both species, requiring, on the average, 64 DD per instar.

INTRODUCTION

Studies conducted in the southern tobacco growing regions have documented the biology, distribution and occurrence of field populations of *Manduca sexta* (L.), the tobacco hornworm (Lepidoptera: Sphingidae) on two common host plants, tobacco and tomato (Madden and Chamberlin 1945, Rabb 1966, McFadden 1968, Jones and Thurston 1970, Semtner et. al. 1980, Kennedy et. al. 1984, Borth and Harrison 1984, de Boer and Hanson 1984). However, only one such study has examined this insect in its more northern habitats (Cheng 1977) and only on one host, tobacco. Furthermore, although it is widely known that *M. sexta* shares its resources with a closely related species (Madden and Chamberlin 1938, Gilmore 1938, Borth and Harrison 1984), *M. quinquemaculata* (Haworth), the tomato hornworm, few studies compare the two insects (however, see Gilmore 1938).

In Michigan, larvae of *M. sexta* and *M. quinquemaculata* readily feed on both tomato and potato foliage. Currently, growers achieve control of these pests through the application of one of a variety of insecticides, i.e., *Bacillus thurengiensis*. Unfortunately, although present management techniques limit feeding damage by these herbivores, the potential for pesticide resistance and therefore, economic damage, always exists. As a result, information which contributes to our overall knowledge and understanding of these two species will prove important for future pest management programs.

Given the lack of cooperative studies of the hornworms on tomato and tobacco in more northern habitats, this study was designed to document the seasonal occurrence of M. sexta and M. quinquemaculata in fields in southwestern Michigan and investigate possible differences in development rate of larvae and adults for both species. Furthermore, the research was designed to examine the effectiveness of accumulated degree days as a predictor of egg deposition and subsequent insect development. We hypothesized that seasonal abundance and insect development would be different for the two species and that moth activity and larval growth would be closely correlated with DD base 50° F.

MATERIALS AND METHODS

Plot Design

This study was part of a sustainable agriculture project. Investigations into the affects of intercropped systems on the abundance and distribution of insects continues to be an important aspect of this long term project. In accordance with these goals, two separate sites, a tomato monoculture, and a tomato/apple tree intercropping, were included in this particular study. The intercropped site consisted of a determinant cultivar of tomato, var. Sunny, planted between tree rows in a dwarf apple orchard. The tomato monoculture consisted of the same cultivar of tomato planted in an adjacent field. In 1986, the field site was moved from its original location to a similar nearby area. The tomato plots within the orchard site remained at the same location throughout both years of the study.

Both sites were divided into multiple plots of tomato plants. Each plot consisted of double rows of plants 50 m long. Plants in these rows were spaced about .5 m apart. Each plot contained approximately 200 tomato plants. Plots in the field site were spaced 5 m apart while those in the orchard site were spaced 7 m apart and separated by a row of apple trees. There were a total of 8 plots in 1985 and 7 in 1986.

Sampling Procedure

Tomato plants (half plants) were randomly sampled twice weekly during both field seasons. In addition, in 1986 plants were sampled every other day from July 13 to July 21. Actual plants sampled were chosen by utilizing a random numbers table. On each sampling day in 1985, 60 half plants, approximately 15 per plot, were visually scanned for eggs and larvae. In 1986, 96 half plants were sampled each day through August 21. However, beginning on August 25, only 48 half plants were sampled per day (in 1986, plants in the orchard site became heavily infected with *Septoria lycopersici* and in late August, sampling was terminated at this site due to lack of foliage).

Tomato plant stems and flowers and upper and lower leaflets, were searched. The location of all eggs or larvae was recorded and the host plant tagged. For sampled larvae, the growth stage and species were also recorded. Since the two species are readily distinquishable by the third instar (*M. sexta* larvae are characterized by a scarlet horn and a soft, pubescent covering on their body, *M. quinquemaculata* have a black horn and lack pubescence), tagging plants enabled us to return to specific locations to determine the species of previously indistinguishable eggs and early instar larvae. By not sampling tagged plants on successive days, the procedure eliminated the possibility of counting the same eggs or larvae more than once.

Field Cohort Study

Eggs deposited in the field on July 10th and 13th, during 1986, were monitored every other day to determine larval developmental period for both species. Monitoring was continued until a larva could not be located on three subsequent monitoring days. After this time period, monitored larvae were categorized as follows: 1) if the larva had previously been documented as having reached the fifth instar, we assumed it had left the plant as a prepupa, 2) if the larva had last been observed as an earlier instar and was then located on a subsequent sampling day at a later growth stage but falling into the expected sequence of development, the larva was assumed to be the same hornworm originally monitored and was retained in the study, and

3) if the larva had last been observed as an earlier instar and could not be located on any of the three subsequent sampling days, we assumed the larva had "disappeared" and it was dropped from the monitoring study. A second cohort study was conducted later in the season based on eggs deposited on August 8th and 10th using the methodology described above. Larval development time was calculated as the number of days required for a first instar larva to reach the "assumed" prepupal stage.

Lab Cohort Study

Larvae from both hornworm species were reared during May of 1986 from eggs received from the Oxford Tobacco Research Station in North Carolina. Rearing conditions were maintained at ambient temperature (23° - 25° C) and humidity, under a 16:8 light:dark cycle. Eggs from each species were placed into separate plastic petri dishes. To ensure that neonate larvae would immediately have access to food, pieces of tobacco hornworm diet (BioServ, Product No. 59783) were also placed into the dishes in close proximity to the eggs. Upon reaching second instar, 20 larvae were randomly selected from each species, removed from the petri dishes and placed in individual plastic 50 ml containers. As newly molted fifth instars, larvae were transferred to 1000 ml containers to provide ample room for continued growth. All rearing containers were covered with loose weave mesh cheese cloth to ensure air exchange. Frass was removed from the containers and diet was replaced daily. Larvae entering the prepupal or "wandering" stage, were placed into clean containers and allowed to pupate. Sticks, approximately 1 cm wide and 25 cm long, were placed upright in each container to provide a surface for newly emerged moths to crawl up on. Pupae were checked for emergence early each morning. Larval development time was calculated as the amount of time required for a larva to grow from neonate first instar to prepupa. Development to the adult stage was calculated as the amount of time required for a larva to grow from neonate first instar to newly emerged adult.

RESULTS

The number of hornworm eggs and larvae sampled on field grown tomato plants was low during both years of the study (Figure 1). Eggs were first observed on July 2 in 1985 (DD 1150) and July 7 in 1986 (DD 1250). The mean number of eggs/half plant peaked at .62 on DD 1350 (July 11) in 1985 and .5 on DD 1320 (July 10) in 1986. In 1985, egg deposition spanned a period of approximately three weeks. However, in 1986, eggs were almost exclusively laid on July 10 and 13.

Because of the low infestation level of hornworms and the fact that foliage in the orchard site was virtually nonexistent by mid August in 1986 (a result of disease), data were pooled from both the orchard and field sites

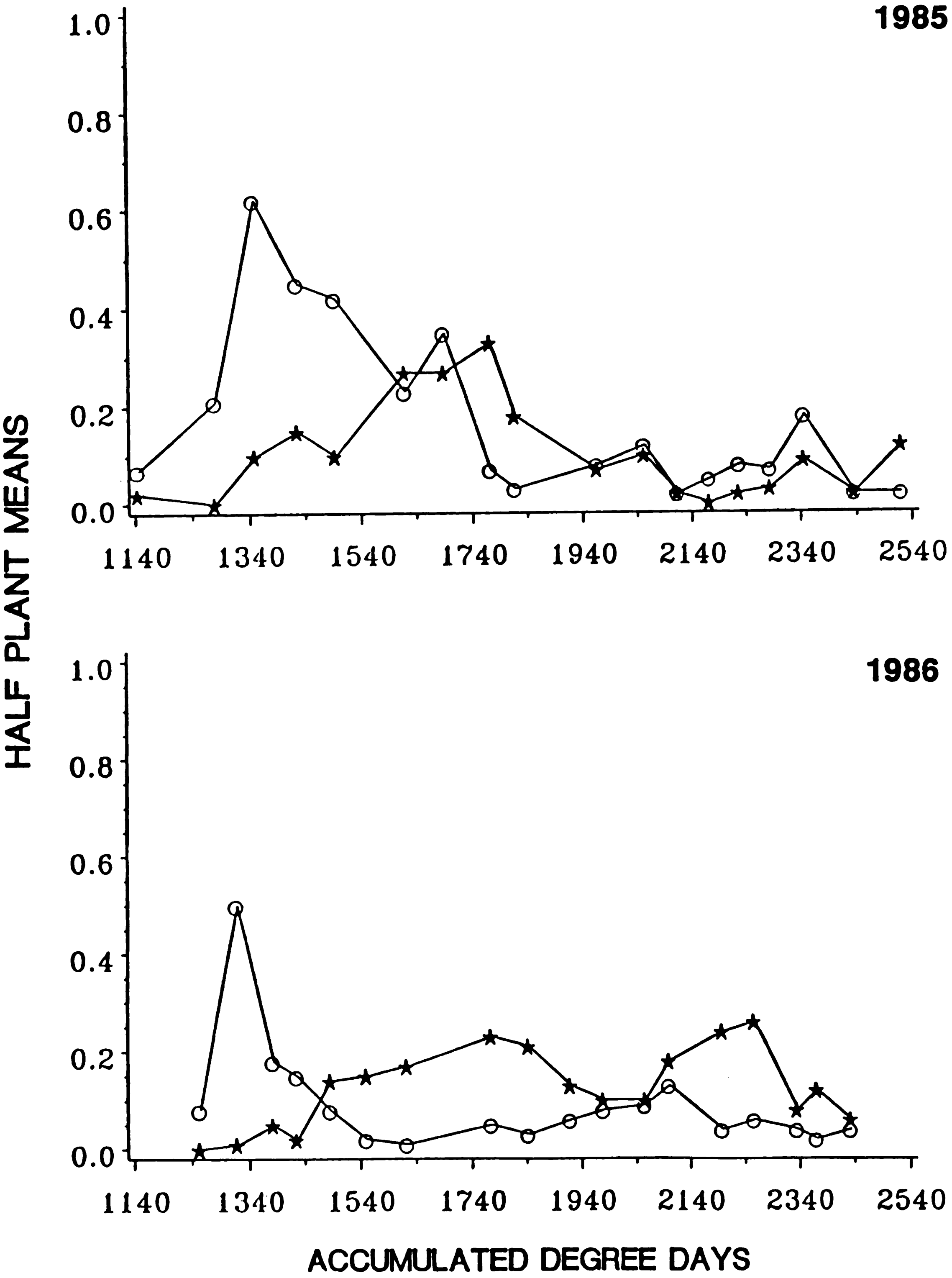


Figure 1. Seasonal trends of *Manduca spp.* (*M. quinquemaculata* and *M. Sexta*) egg and larval means per half plant. Data combined from field and orchard site. Circles = eggs, stars = larvae. In 1985, 60 half plants were sampled per day. In 1986, 96 half plants were sampled per day until August 25, at which time, 48 half plants were sampled.

(Chi-square = 2.176, p > .15 in 1985 and 2.99, p > .10 in 1986) prior to separating eggs and larvae by species.

Figure 2 depicts the seasonal occurrence and relative abundance of eggs from each species (estimated by monitoring egg development through the season and by extrapolating from larvae sampled and identified on a particular date, back to the probable date that oviposition occurred). Moths from both species began depositing eggs early in the season. However, eggs deposition by _M. quinquemaculata_ declined throughout July and by early August (DD 2000), was non-existent. In contrast, _M. sexta_ eggs were observed from the beginning of the season until sampling stopped in early September (DD 2500).

Figure 3 shows the seasonal occurrence and relative abundance of larvae from each species. In both years, _M. quinquemaculata_ larvae were more prevalent during the early part of the season (Table 1). In fact, in 1985, this species was more abundant during the entire first half of the season. Although the same trend existed in 1986, the difference between the proportion of larvae from each species was much smaller (Figure 3). During the middle of the season, the number of individuals from each species was almost equal, especially in 1986 (Table 1). Towards the last 2 1/2 - 3 weeks of the study, _M. sexta_ became the more prevalent species sampled. In 1985, it was the only species observed during this entire sampling period (Table 1 and Figure 3).

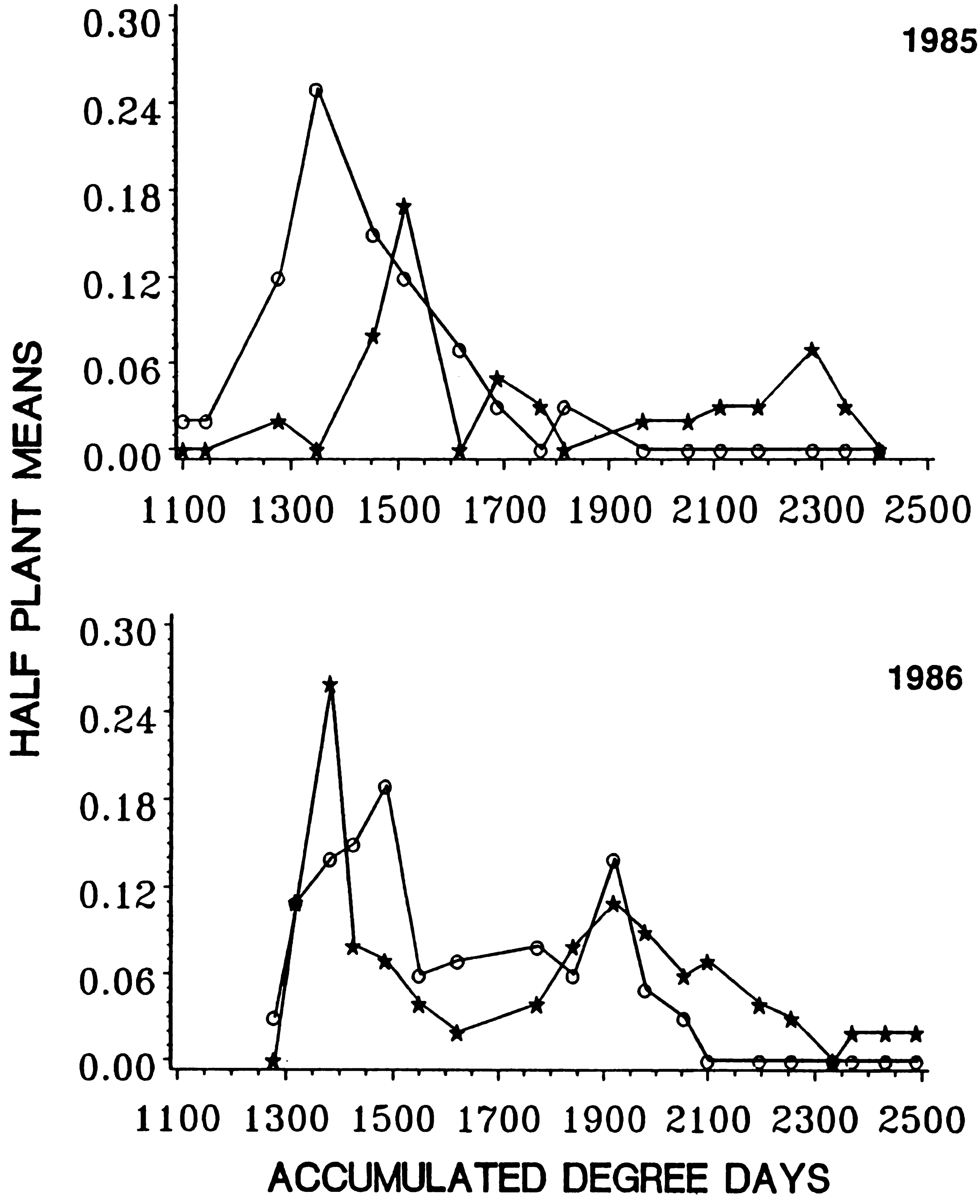


Figure 2. Seasonal egg means of M. quinquemaculata and M. sexta. Circles = M. quinquemaculata, stars = M. sexta. Data combined from field and orchard site. In 1985, 60 half plants were sampled per day. In 1986, 96 half plants were sampled per day until August 25, at which time, 48 half plants were sampled.

Table 1. Relative occurrence of *M. quinquemaculata* and *M. sexta* larvae at different sampling times during each year of the study. Each sampling period consists of approximately 5 separate sampling dates.

YEAR	SAMPLING PERIOD	D Day>50 F	TOTAL NO. LARVAE IDENTIFIED	PERCENT *M. quin.*	PERCENT *M. sexta*
1985	early season				
	8 JUL - 18 JUL	1280-1515	18	89	11
	mid season				
	23 JUL - 2 AUG	1649-1817	45	69	31
	9 AUG - 20 AUG	1967-2184	10	50	50
	late season				
	23 AUG - 6 SEPT	2228-2522	13	0	100
1986	early season				
	7 JUL - 19 JUL	1255-1551	30	77	23
	mid season				
	22 JUL - 4 AUG	1625-1920	64	42	58
	7 AUG - 18 AUG	1980-2199	39	46	54
	late season				
	21 AUG - 2 SEPT	2258-2434	26	27	73

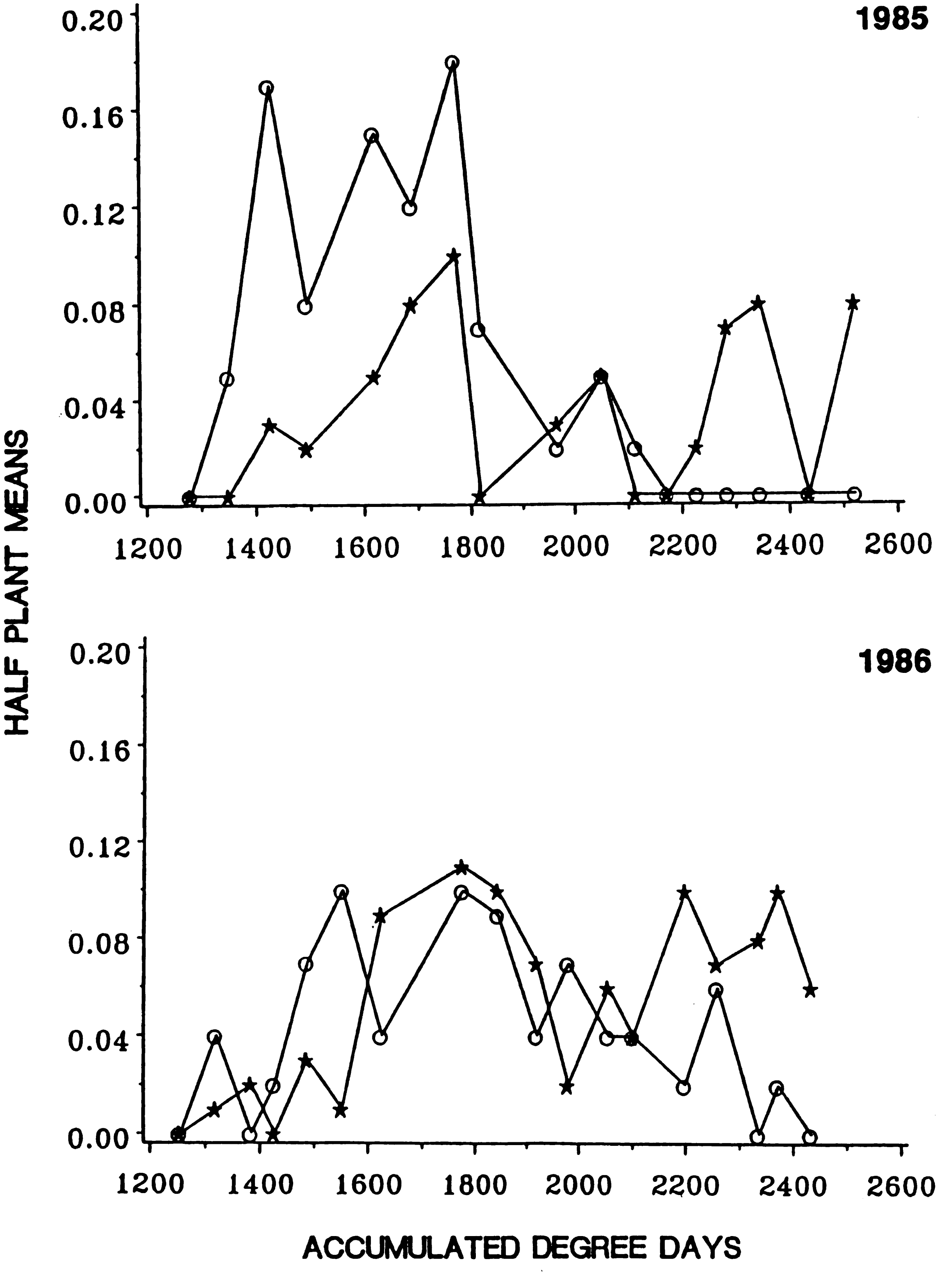


Figure 3. Seasonal larval means of *M. quinquemaculata* and *M. sexta*. Circles = *M. quinquemaculata*, stars = *M. sexta*. Data combined from field and orchard site. In 1985, 60 half plants were sampled per day. In 1986, 96 half plants were sampled per day until August 25, at which time, 48 half plants were sampled.

1986 Cohort Studies

Laboratory studies determined that *M. quinquemaculata* and *M. sexta* larvae require the same amount of time to develop. No significant differences were found between the time required for larvae to reach maturity or for adults to emerge (Table 2).

Results of the first field cohort study demonstrated similar findings. While larval development time was shorter overall for field reared insects compared to laboratory reared insects (a discrepancy which can probably be explained by differences in food quality, i.e. diet vs. foliage, and/or higher temperatures for field reared insects), the period required for larvae to reach the prepupal stage was not significantly different between the two species (Table 2), requiring approximately 2 - 3 days for each of the five instars or 64.30 DD ± 2.60 (N = 29). In fact, plots tracing larval development during the field cohort study, demonstrated that instar peaks occurred on virtually the same degree day for both species (Figure 4).

The second field cohort study produced no results. Of the 17 eggs oviposited on August 8 and 10, only 1 larva could be traced through to the prepupal stage. Of the others, 2 eggs were parasitized, 2 were nonviable, and 13 larvae disappeared during the first instar. However, larval development monitored in the latter part of the season from regular sampling data (monitoring larvae on tagged plants) indicated that larvae required 3 to 4 days to complete an

Table 2. Means and standard errors for development times of field and lab cohorts of *M.quinquemaculata* and *M. sexta* larvae during 1986 study. N=17 for lab cohorts. N=13 and 15 for *M. quinquemaculata* and *M. sexta* field cohorts respectively. Means with same letter not significant at **p** = .05.

SPECIES	LARVAL DEVELOPMENT TIME (DAYS)		TIME TO ADULT EMERGENCE (DAYS)	
	LAB	FIELD	LAB	FIELD
M. sexta	19.82 ± .63a	13.69 ± .62a	48.94 ± 1.44a	N/A
M. quinquemaculata	19.71 ± .24a	13.00 ± .74a	49.65 ± .45a	N/A

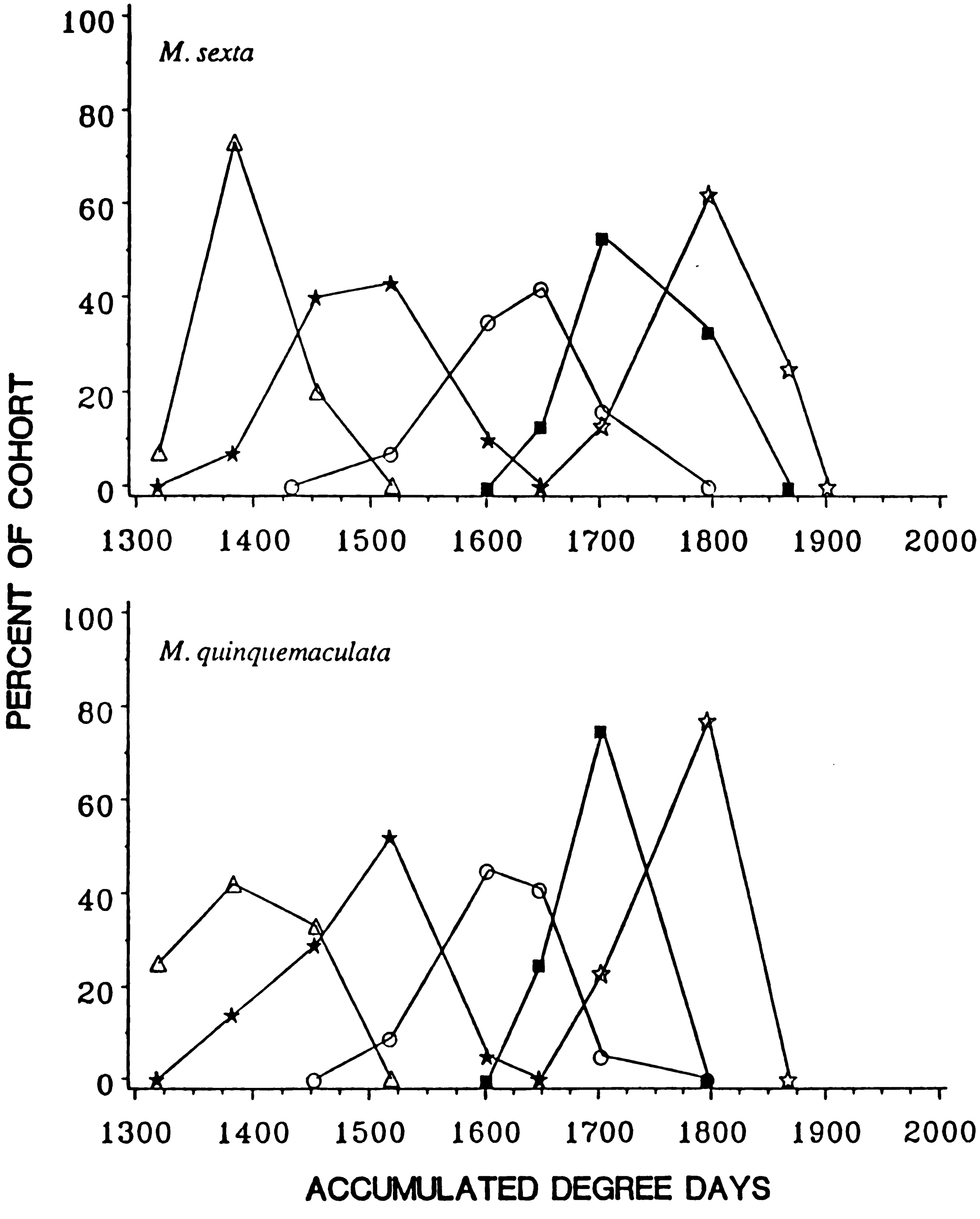


Figure 4. Seasonal development of *M. quinquemaculata* and *M. sexta* field cohorts monitored from egg hatch to prepupa during 1986 (N = 13 and 15 for *M. quinquemaculata* and *M. sexta* respectively). Each data point represents the percent of total larvae for a particular stage of development on each monitoring day. Triangles = eggs, closed stars = 1st and 2nd instars, open circles = 3rd and 4th instars, squares = 5th instars, open stars = prepupa.

instar compared to the 2 to 3 days required for early season larvae; although, the number of DD required was very similar, 67.33 ± 5.95 (N = 10).

DISCUSSION

Since hornworm larvae are ravenous eaters and a single larva can consume an entire tomato plant during development, infestations of less than one larva per plant should be desirable for growth of either *M. sexta* or *M. quinquemaculata*. Thus, it is reasonable to assume that the low population levels documented during this research were conducive to optimum development in these insects; although, other hypotheses are also viable.

Degree day accumulation effectively predicted ovipositional activity and larval development in *M. sexta* and *M. quinquemaculata*. For both species, initial egg lay occurred during the first week of July, a difference of only 100 DD or approximately 4 - 5 calendar days between years. An even closer relationship was demonstrated for degree day accumulation and peak egg deposition. In both years, peak egg lay occurred on virtually the same DD. Larvae developed rapidly and at the same rate in both species. The first larvae of the season reached maturity by the end of July, approximately 12 - 14 days (300 - 350 DD) after egg deposition. Although larvae developing later in the summer required more days to reach maturity, the apparent discrepancy can be explained by the slower rate of heat accumulation late in the season. From mid to late July (DD 1519 - 1903) an average of 24.5 DD were accumulated each calendar day; however, from mid to late August (DD 2081 - 2378), only 18.0 DD were accumulated each calendar day. Hence, larvae still required, on the average, approximately 65 DD per instar to develop.

In the southern states, *M. quinquemaculata* and *M. sexta* may have as many as four generations per year (Madden and Chamberlin 1938, 1945). First generation moths emerge from late May to early June and egg deposition occurs shortly thereafter (Madden and Chamberlin 1938, Madden and Chamberlin 1945). In Southwestern Michigan, egg deposition by *M. quinquemaculata* moths was common throughout July but was virtually non-existent after early August. Larval infestation levels peaked from mid to late July, then decreased thereafter. These trends were especially evident during 1985. From mid August to the end of the summer, not a single *M. quinquemaculata* larva or egg was obtained from 360 samples of tomato plants, suggesting that larvae developing from early season egg deposition completed development, pupated and then entered diapause. While not as apparent, a similar trend was observed in 1986. Although *M. quinquemaculata* larvae were found through August 28, eleven of the thirteen total *M. quinquemaculata* larvae found from August 14 to August 28 (out of 384 plants sampled) were fourth, pre-molt fifth or fifth instar larvae, indicating that egg hatch for these late season larvae occurred in mid July or early August. Based on these observations, it appears that *M. quinquemaculata* has only one generation per year.

In contrast to the seasonal trends of *M. quinquemaculata*, *M. sexta* eggs and early instar larvae were observed from the beginning of the season until sampling stopped in September (Figures 2 and 3), indicating that ovipositon was occurring throughout the season. Unfortunately, it is difficult to determine how many generations of *M. sexta* occur during a year. In 1985, there appeared to be two distinct ovipositional periods (Figure 2 -- note increase in eggs levels late in the season), suggesting that this species is bivoltine, i.e., eggs deposited during the earliest period give rise to a first generation of larvae that complete development, pupate and then emerge as adults in early to mid August. These adults then deposit eggs that develop into a second larval brood.

However, 1986 ovipositional data supports the hypothesis that genetic variability within the species produced individuals that emerged and/or entered diapause at different times during the summer (Tauber and Tauber 1981, Hagen and Lederhouse 1985). Although there were two distinct ovipositional peaks, the period of time between these was not sufficient to permit larval development, pupation and subsequent emergence as an adult. Waldbauer (1973) determined that emergence in *Cecropia* moths was bimodal, i.e., one group of moths emerged in late May and early June while a second group emerged in late June and early July. Rabb (1966) has, in fact, demonstrated bimodal emergence in M. sexta populations in North Carolina. M. sexta adults pupating during one year emerged at two separate times during the following season. In addition, the first "wave" of adults produced larvae that completed development and emerged simultaneously with the later "wave" of adults, producing a field population of larvae consisting of a mixture of first and second generation individuals.

Regardless of the scenario, or combination thereof, which more appropriately describes the seasonal occurrence of M. sexta in Michigan, the fact that the females deposit eggs very late in the summer would appear to be deleterious to their overall fitness. It seems probable that these newly hatched larvae would not be able to complete development before weather conditions would have an adverse affect on their growth or on that of the plant. In fact, during this study, tomato foliage (determinant variety) was virtually nonexistent by mid to late September. While no explanation is offered for the occurrence of late season emergence and egg deposition, this phenomenon provides an interesting area for future investigations.

CONCLUSIONS

Results of this study demonstrate that egg deposition and larval development can be realistically predicted by degree day accumulation. The study also demonstrates that initial summer emergence of adults and subsequent development of larvae is similar for both species.

The only apparent difference between species appears to be their relative abundance during the season. *M. quinquemaculata* is more prevalent early in the season and becomes relatively rare by mid August, indicating that *M. quinquemaculata* populations are univoltine in Southwestern Michigan. In comparison, *M. sexta* larvae are persistent throughout the season and by mid to late August, are the only species found. The occurrence of eggs and early instar larvae throughout the summer, suggests that *M. sexta* populations are bivoltine, have bimodal emergence or are comprised of some combination between these two scenarios.

REFERENCES

Baumhover AH (1985) Manduca sexta. In: Handbook of Insect Rearing. Vol. 2. (Pritam S, Moore RF, eds.). Elsevier Science Publishers B.V., Amsterdam.

Borth PW, Harrison FP (1984) Within-field distribution patterns of tobacco and tomato hornworm (Lepidoptera: Sphingidae) egg and larval populations in southern Maryland tobacco. Environ. Entomol. 13:859-862.

Cheng HH (1977) Flue-cured tobacco losses caused by the tomato hornworm, Manduca quinquemaculata (Lepidoptera: Sphingidae), at various infestation levels in Ontario. Can. Ent. 109:1091-1095.

de Boer G, Hanson FE (1984) Foodplant selection and induction of feeding preference among host and non-host plants in larvae of the tobacco hornworm Manduca sexta. Ent. Exp. appl. 35:177-193.

Gilmore JU (1938) Observations on the hornworms attacking tobacco in Tennessee and Kentucky. J. Econ. Ent. 31: 706-712.

Hagen RH and Lederhouse RC (1984) Polymodal emergence of the tiger swallowtail, Papilio glaucus (Lepidoptera: Papilionidae): source of a false second generation in central New York State. Ecological Entomology 10:19-28.

Jones GA, Thurston R (1970) Leaf consumption and development of tobacco hornworm larvae feeding on burley and dark tobacco. J. Econ. Ent. 63:1938-1941.

Kennedy GG, Romanow LR, Jenkins SF, Sanders DC (1983) Insects and diseases damaging tomato fruits in the coastal plain of North Carolina. J. Econ. Entomol. 76: 168-173.

Madden AH, Chamberlin FS (1938) Biological studies of the tomatoworm on tobacco in Florida, 1936 and 1936. J. Econ. Entomol. 31:703-706.

Madden AH, Chamberlin FS (1945) Biology of the tobacco hornworm in the southern cigar-tobacco district. US Dept. of Agric. Tech. Bull. No. 986. 51 pp.

McFadden MW (1968) Observations on feeding and movement of tobacco hornworm larvae. J. Econ. Ent. 61:352-356.

Rabb RL (1966) Diapause in Protoparce sexta (Lepidoptera: Sphingidae). Ann. Ent. Soc. Am. 59:160-165.

Semtner PJ, Rasnake M, Terrill TR (1980) Effect of host-plant nutrition on the occurrence of tobacco hornworms and tobacco flea beetles on different types of tobacco. J. Econ. Entomol. 73:221-224.

Tauber CA, Tauber MJ (1981) Insect seasonal cycles: genetics and evolution. Ann. Rev. Ecol. Syst. 12:281-308.

Waldbauer GP, Sternburg JG (1973) Polymorphic termination of diapause by Cecropia: genetic and geographical aspects. Biol. Bull. 145:627-241.

MANUSCRIPT II: Nutritional consequences of variable plant nitrogen: Experiments with plant fertilization and *Manduca quinquemaculata*.

ABSTRACT

Tomato foliage treated at higher rates of nitrogen fertilization and containing higher concentrations of total plant nitrogen and soluble nitrates permitted tomato hornworm larvae to utilize their food more efficiently, grow faster, suffer lower mortality and develop into more fecund adults compared to larvae developing on foliage left untreated or treated with low levels of fertilizer. Larvae reared on untreated foliage were able to partially compensate for lower food conversion efficiencies by consuming food at a higher relative rate; however, larvae reared on foliage treated at low fertilization levels did not demonstrate this compensatory response.

Enhanced larval performance was correlated with both mean percent total plant nitrogen and mean percent soluble nitrates. Plant water content was high for all treatments; hence, probably did not influence larval growth. Other plant components influenced by nitrogen availability and their subsequent affect on herbivores are discussed.

INTRODUCTION

The relatively recent interest in host plant/insect interactions has led to the realization that acquiring a basic understanding of the mechanisms that enable an insect to utilize its available resources, is essential to the development of theory in insect ecology and evolution. This realization has, in turn, produced a widespread interest in the study of nutritional ecology. Much of the recent work has focused on elucidating the biochemical properties of host plants influencing larval development and subsequent adult survival and performance. Results of these studies have indicated that plant nitrogen is a key component in a number of important metabolic pathways (see review by Mattson 1980). Not only does this substance influence the physiology and nutrition of the insect (Shaw and Little 1972, McNeill and Southwood 1978, Scriber and Slansky 1981, Hagen et. al. 1984, Wint 1983), but it also affects the general behavior of the animal (Beck 1974, White 1979, Myers and Post 1981, van der Meijden et. al. 1984).

Unfortunately for the herbivore though, growth and development may be severely limited by inadequate nitrogen reserves. First, plant nitrogen content varies throughout the host plant and throughout the growing season (Beck 1972, Mattson 1980, White 1984). Secondly, a large percentage of plant nitrogen is unavailable to an herbivore (McNeill and Southwood 1978, Reese 1978, Mattson 1980). Finally, nutritional value is dependent upon the ratio, pattern and quantity of amino acids, hence proteins, found in the plant (Rock 1972, House 1969).

The addition of nitrogen fertilizer to agronomic crops is a common cultural practice that, subsequently, alters the physiology of the plant and its value as a food resource for phytophagous insects. Generally, the nutritional value of the foliage increases as the availability of soil nitrogen increases (van Emden and Way 1973, Fox and Macauley 1977, Auerbach and Strong 1981, McNeill and Prestidge 1982, Al-Zubaidi and Capinera 1984, Manuwoto and Scriber 1985). Usually, this increased nutritional value results from increased concentrations of soluble nitrates (Terman et. al. 1976, McNeill and Southwood 1978, Eppendorfer 1978), precurssors of proteins and amino acids (Murata 1969, Hoff et. al. 1974, McNeill and Southwood 1978, Rahier 1978), that provide a ready source of available nitrogen for an insect feeding on the plant. However, increased nitrogen availability also stimulates increased cell elongation and division, generally producing foliage with a high water content, a plant component known to have a large influence on insect fitness (Scriber 1977, 1978, 1979).

Abundant supplies of plant nitrogen do not always enhance insect performance. Increased nitrogen availability may initiate the production of certain metabolic compounds that interfere with food consumption and utilization. Plant alkaloids, chemical compounds found in many plant families, are such substances. Not only do these nitrogen containing compounds generally increase with increased nitrogen availability (Geissman and Crout 1969, Waller and Nowacki 1978), but they also often exert toxic affects on certain insects (Tingey 1984, Sinden et. al. 1986).

This research was designed to examine the interactions between host plant physiology/quality and insect performance. More specifically, we investigated the affect of nitrogen fertilization on plant quality and subsequent larval development and adult survival and fecundity. Field plots of tomato plants (var. Sunny) were treated with four levels of nitrogen fertilizer, thus inducing varying degrees of physiological changes in these plants. *Manduca quinquemaculata* larvae, reared from egg hatch to adult emergence on bouquets of treated foliage, were monitored on a regular basis to determine relative fitness between treatments.

MATERIALS AND METHODS

Fertilization of Field Plot

Nitrogen fertilizer was applied directly to the soil surrounding individual tomato plants at the rate of 0, 30, 120 and 240 lbs./acre. Ammonium nitrate (34% nitrogen) provided the source of nitrogen. To prevent burning the plants receiving high levels of fertilizer, applications were split between July 3 and July 14. The first application of nitrogen occurred approximately 2 1/2 weeks after seedlings were transplanted into the field.

Growth Study

Two growth studies were performed utilizing *Manduca quinquemaculata* larvae reared from eggs received from the Oxford Tobacco Research Station in North Carolina. Although this culture had been maintained for at least five years previous to this study, it was assumed that any inbreeding of the population would not affect relative differences between treatments. The first study examined general larval development and emergence into the adult form. The second study was designed to examine larval growth in detail by measuring different parameters and then calculating several nutritional indices. Originally, both studies were started at the same time. However, after two failed experiments (a result of technical errors), the detailed performance study actually began 24 days later. Altogether, 360 newly hatched, first instar larvae were randomly placed on foliage treated with four levels of nitrogen fertilizer (ninety/treatment). The larvae were initially put into individual 50 ml plastic containers but were later transferred to 1000 ml plastic cups to allow ample room for continued growth.

Rearing facilities were maintained at ambient air temperature and relative humidity to simulate field conditions. Temperature remained fairly constant throughout the study, fluctuating between 24 and 30 degrees C.

General Performance Study

Sixty larvae of the initial ninety/treatment were reared from egg hatch to subsequent emergence as an adult. Each day, fresh tomato foliage was presented to the larvae and uneaten plant material and frass were discarded. Larvae entering the prepupal or "wandering stage" were placed into clean cups and allowed to complete pupation. Six days after pupation, 9 males and 9 females from each nitrogen treatment were randomly selected and oven dryed at 60° C. The remaining pupae were sexed then left to emerge naturally. Small sticks (approximately 7 cm high) were placed upright in each container to provide a surface for newly emerged moths to crawl up on. To be consistent, pupae were checked for emergence every day at 10 pm (the moths always emerged in the late evening). Newly emerged, adult males were recorded and then discarded. Adult females were starved for three days, then dissected so that eggs could be counted.

Detailed Performance Study

The other thirty larvae from each nitrogen treatment were used to calculate performance indices (after Waldbauer 1968). These measurements required that five sets of parameters be determined: a) initial animal weight, b) final animal weight, c) weight of food provided, d) weight of food remaining, and e) weight of feces accumulated. Originally these parameters, measured as grams dry weight, were determined for first instar larvae. However, because of the difficulty of obtaining precise measurements on such low weights (especially dry weight of feces) and high mortality that occurred during the first two failed experiments in which first instars were utilized, the nutritional indices were ultimately calculated for fourth instar larvae.

These indices are defined as follows:

1. Relative Consumption Index, (RCI) = F/(T)(A), calculates the rate of feeding per day relative to the mean weight of the animal.

2. Relative Growth Rate, (RGR) = G/(T)(A), measures the daily rate of growth of the animal relative to its mean body weight.

3. Approximate Digestibility, (AD) = (I - FR)/I x 100, examines the animal's efficiency for converting food consumed into food digested.

4. Efficiency of Conversion of Ingested food, (ECI) = G/I x 100, calculates the overall efficiency of an animal to utilize an ingested food for its own biomass. This index is dependent upon the digestibility and nutritional quality of the food.

5. Efficiency of Conversion of Digested food, G/(I - FR) x 100, measures the overall efficiency of an animal to convert digested material into actual body mass. This index is also dependent upon the digestibility and nutritional quality of the food.

where,

F = dry weight of food eaten,
T = duration of feeding period,
A = mean dry weight of animal during feeding period,
G = dry weight gain of animal during feeding period,
I = dry weight of food ingested, and
FR = weight of frass produced.

Pre-molt fourth and fifth instar larvae were monitored every three hours between 6 am and 12 midnight (pre-molt larvae are easy to distinguish since their head capsule becomes extended from the rest of their body). Newly molted fourth instar larvae were fed pre-weighed foliage collected daily from field grown tomato plants. Frass and uneaten foliage was collected every 24 hours and oven dryed at 60° C. Newly molted fifth instar larvae were immediately removed from the experiment, frozen, then oven dryed.

Initial dry weight of tomato foliage fed to larvae was determined by calculating the mean percent dry weight for a control group of foliage from each treatment each day and then multiplying the fresh weight of foliage actually presented to larvae by this number. This mean was obtained by randomly selecting five freshly picked aliquots of leaves from each treatment from foliage collected each morning and then measuring the fresh and dry weights of these aliquots. The values obtained were also utilized for calculating mean percent water loss for each treatment.

Initial larval weight was determined by randomly selecting a control group of eight newly molted fourth instar larvae from each treatment and obtaining their dry weights. Larval weight during the fourth instar was calculated by multiplying the initial larval weight by the final larval weight, then taking the square root of this number (geometric mean).

All weights utilized in the calculation of the performance indices were determined on an analytic balance. The values obtained were carried to three decimal places. Performance indices for individual larvae were calculated by combining all measurements for each parameter, over the course of the study, into one total measurement, i.e. frass collected each day from each individual larva was combined into one total dry weight of frass produced by that larva.

Foliage Collection and Chemical Analysis

Foliage fed to larvae was collected fresh from the field grown tomato plants early each morning. Similar aliquots of leafs or leaflets were picked. Generally, these aliquots consisted of the first fully expanded leaf from the terminal bud. Foliage from each treatment was placed in a separate plastic bag and stored in a cooler. Foliage utilized in the detailed performance study was immediately weighed and presented to larvae as soon as possible after being collected. Every three to four days, four aliquots of leaves were randomly selected from each bag of leaves and oven dryed at 60° C. These samples were used to determine total nitrogen and soluble nitrates in tomato leaves throughout the course of the study.

Prior to analysis, all foliage was ground using a 40 mesh screen. To determine total nitrogen, 150 mg of dried foliage was digested with Kel-catalyst KC-Mc (3.5 g potassium sulphate + 175 mg mercuric oxide) and 9 ml sulfuric acid for one hour. After cooling for 30 minutes, the solution was brought up to a volume of 75 ml with distilled water. Total ppm nitrogen was read using a Technicon Auto Analyzer II.

To determine soluble nitrates, 20 ml of distilled water was added to 500 mg of dried foliage and then shaken for 30 minutes. Total soluble nitrates were assessed using a nitrate electrode and an expanded scale pH meter.

RESULTS

Foliage Analyses

During both years of the study, plants which were treated with high levels of fertilizer produced foliage that was visibly greener (although not chemically analyzed) than leaves on plants treated with low levels of fertilizer or left untreated.

Chemical analyses conducted on foliage collected during this study demonstrated that mean percent dry weight total plant nitrogen (Table 1) was positively correlated with nitrogen fertilization treatment during both the general and detailed growth studies ($r = .95$, $r = .93$ respectively at $p = .05$). Figure 1 depicts the seasonal trends of this plant component. Even though initial levels of nitrogen were relatively high for all treatments, concentrations declined quickly and then leveled off throughout the remainder of the study.

Mean percent soluble nitrates (Table 1) was found to be positively correlated with fertilizer treatment during the general study ($r = .98$, $p = .01$) but not during the detailed study ($r = .92$, $p = .08$). Each level of fertilization resulted in an initial increase in nitrates that steadily declined during the remainder of the season (Figure 2). For plants treated at the high levels, this decline spanned a period of four to five weeks. However, for plants treated with 30 lbs. nitrogen per acre, decline occurred within 12 days. By the second week of August, nitrate levels for all treatments were at very low levels.

Percent water content analyzed during the Detailed Growth Study was high for all treatments (80 - 88%). Although only slightly higher, foliage treated with 30 lbs. nitrogen per acre had the highest water content and foliage treated with 240 lbs. nitrogen per acre had the lowest. Results of a correlation analysis determined that mean percent water content was negatively correlated with mean percent plant nitrogen ($r = -.95$, $p < .04$). Figure 3

Table 1. Means and standard errors for percent water content, total plant nitrogen and soluble nitrates calculated during each separate aspect of the study. These values were utilized in all correlation analyses requiring data on the concentration of particular plant substances.

		STUDY	
	LBS. Nitrogen Applied/Acre	General Growth (n = 4)	Detailed Growth (n = 4)
Total Plant Nitrogen	0	2.40 (.036)	2.03 (.027)
	30	3.31 (.243)	2.43 (.066)
	120	4.39 (.102)	3.29 (.184)
	240	4.76 (.040)	3.43 (.098)
Soluble Nitrates	0	.047 (.005)	.050 (.004)
	30	.049 (.010)	.042 (.001)
	120	.129 (.031)	.053 (.008)
	240	.194 (.041)	.059 (.003)
Water Content	0		85.04 (.344)
	30	N/A	85.45 (.345)
	120		84.12 (.409)
	240		83.51 (.457)

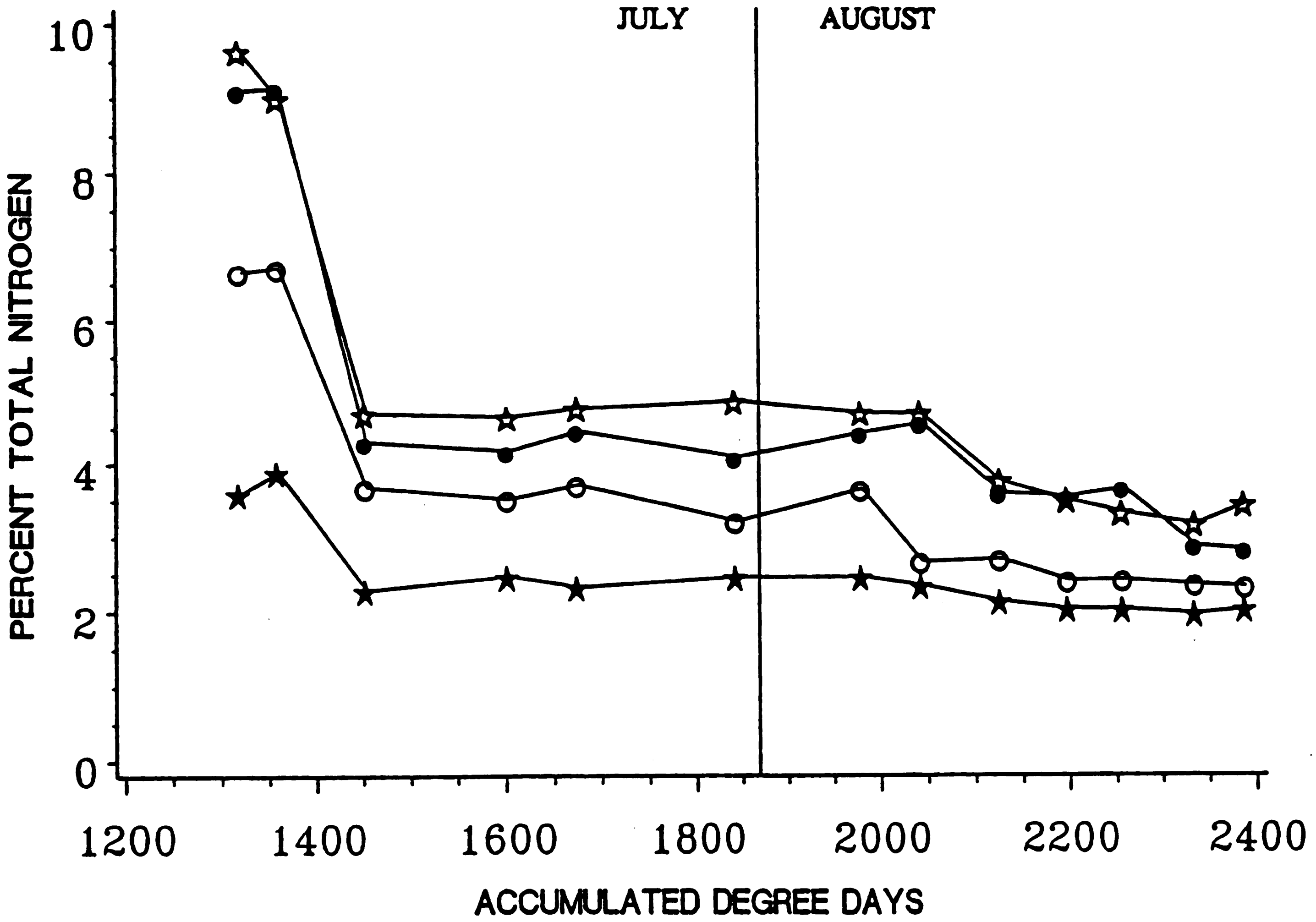


Figure 1. Seasonal trends of percent dry weight nitrogen. Foliage collected during 1986. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

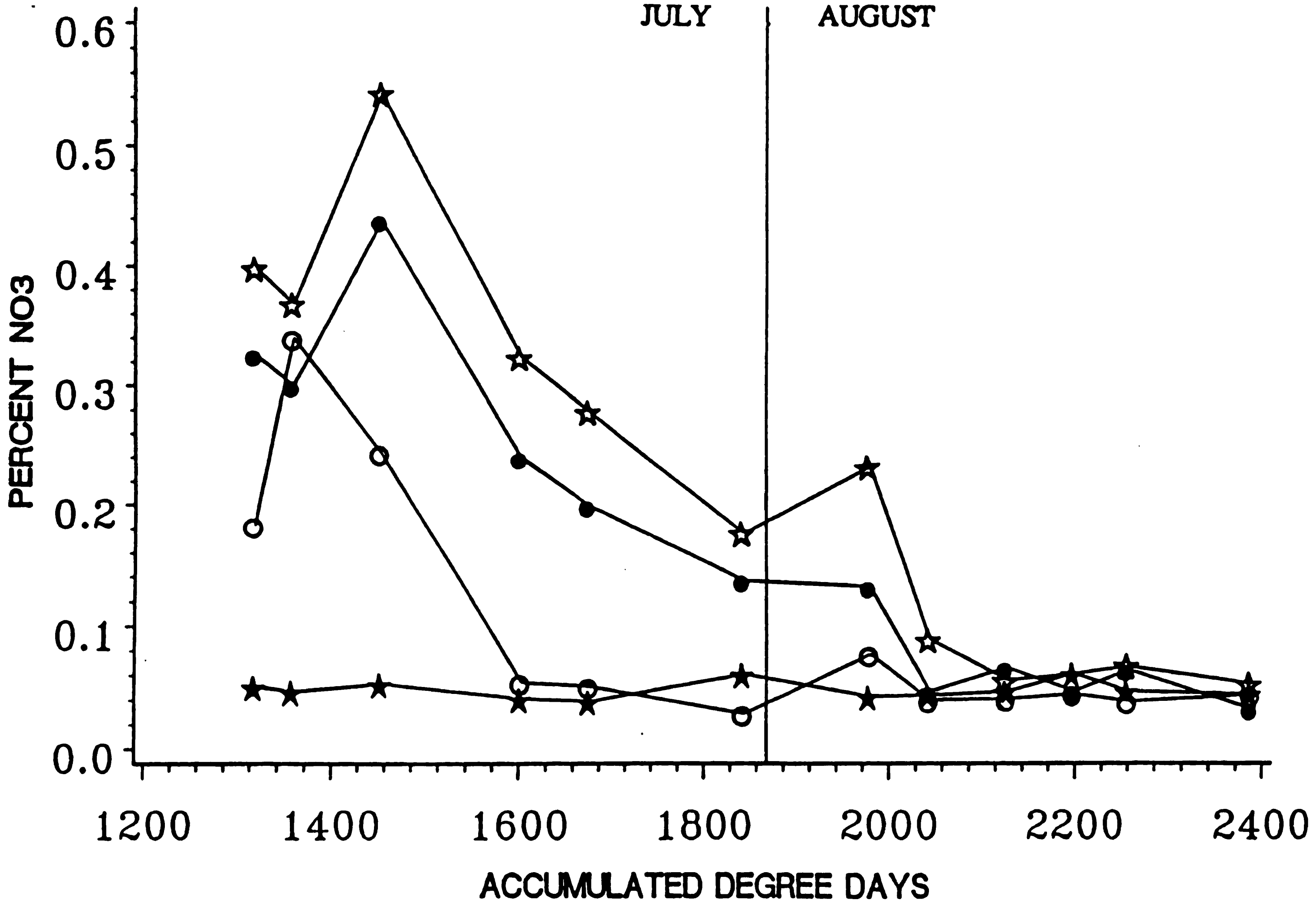


Figure 2. Seasonal trends of percent soluble nitrates. Foliage collected during 1986. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

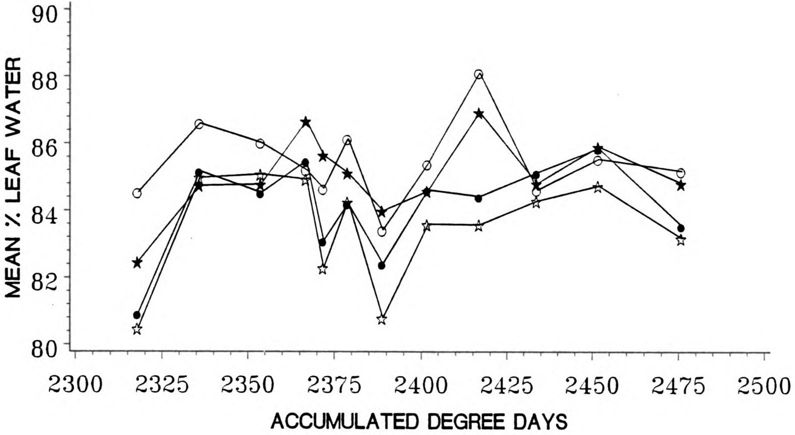


Figure 3. Mean percent plant water at each fertilization level. Foliage collected during Detailed Performance Study. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

depicts the levels of this component during the Detailed Growth Study.

General Performance Study

Larval survival during this study was approximately the same for all treatments (70, 67, 62 and 67% for 0, 30, 120 and 240 lbs. nitrogen applied/acre respectively). Most mortality occurred during the first and second instar and probably resulted from daily handling of larvae. A G-Test for independence determined that percent survival was not associated with fertilization treatment ($G = .47$, $p > .92$).

A linear regression on individual observations of male (Figure 4) and female (Figure 5) pupal dry weights was highly significant ($p < .0002$ and .0001 respectively) although, only a small amount of variability within a treatment was explained by nitrogen fertilization. In both cases, the smallest pupae developed from larvae reared on foliage treated with 30 lbs. nitrogen/acre and the heaviest from foliage treated with 240 lbs. nitrogen/acre. Overall, pupal weights were heavier for females than for males.

A linear regression on percent successful emergence of adult males from the pupal stage did not produce a slope significantly different from zero ($p = .46$). In fact, emergence of males reared on untreated foliage and foliage treated with 120 and 240 lbs. nitrogen/acre was very similar (Figure 6). However, foliar nitrogen, or lack thereof, appeared to have an affect on percent emergence of males

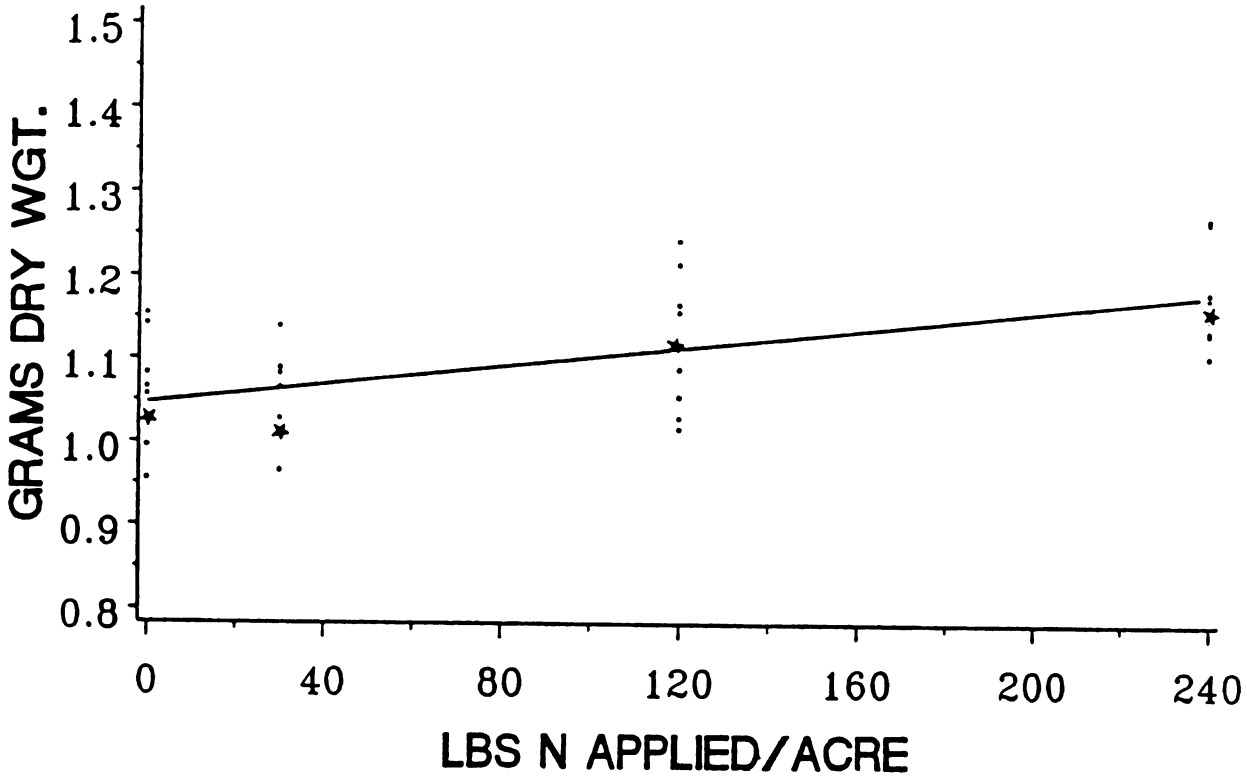


Figure 4. Linear regression of male pupal dry weights, $p < .0001$, $R^2 = .40$, $N = 9$ at each nitrogen level. Mean values depicted by stars. See text for explanation of statistics.

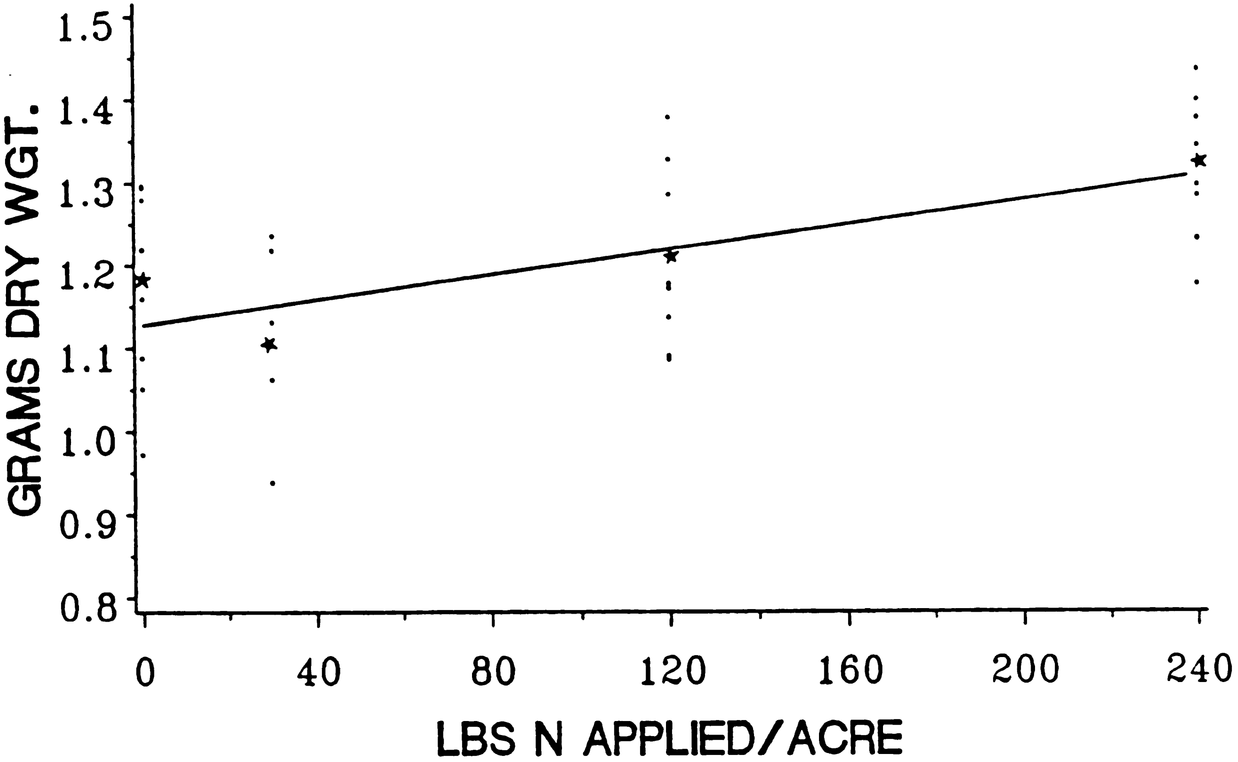


Figure 5. Linear regression of female pupal dry weights, $p < .002$, $R^2 = .34$, $N = 9$ at each nitrogen level. Mean values depicted by stars. See text for explanation of statistics.

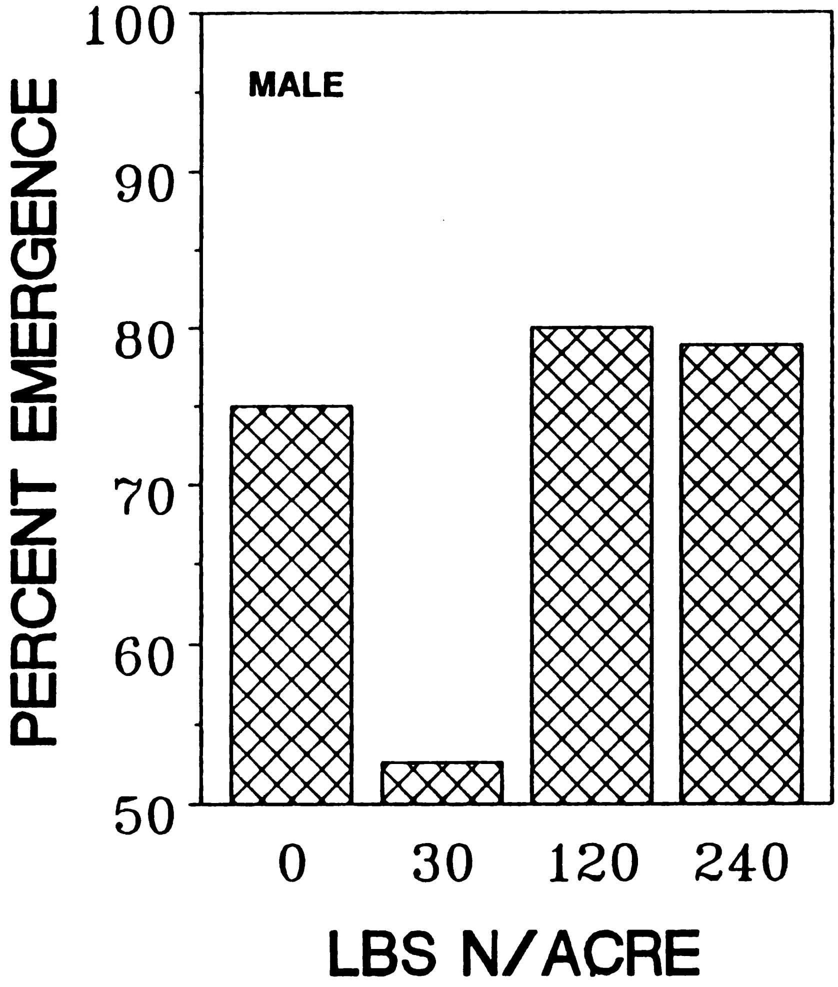


Figure 6. Percent successful emergence of adult males from larvae reared on tomato foliage treated at four different nitrogen levels. A linear regression of arcsine transformed data produced a slope that was not significantly different from zero (R^2 = .46, p < .29). See text for explanation of statistics.

reared on foliage treated with 30 lbs. nitrogen/acre. In contrast, percent successful emergence of adult females from the pupal stage demonstrated an obvious relationship with level of fertilization, suggesting that female emergence was highly dependent upon nitrogen content of foliage fed to larvae (Figure 7).

Female fecundity was significantly influenced by rate of fertilization (Figure 8). A linear regression on egg totals from individual females produced a slope that was highly significant ($p < .009$). However, there was a great deal of variability within a treatment, especially for females reared on foliage treated with 30 lbs. nitrogen/ acre, and nitrogen treatment only accounted for 17% of the variability.

Most General Growth parameters significantly influenced by nitrogen fertilization level were also correlated with mean percent dry weight plant nitrogen and mean percent soluble nitrates (Table 2--mean percent plant nitrogen and soluble nitrates calculated from foliage collected while each separate larval cohort was developing--see Table 1).

Detailed Performance Study

Of the initial thirty larvae per treatment started in this experiment, survival was highest for larvae developing on foliage treated with 120 and 240 lbs. nitrogen/acre (97 and 100% respectively--Figure 9). In fact, only 26 of the

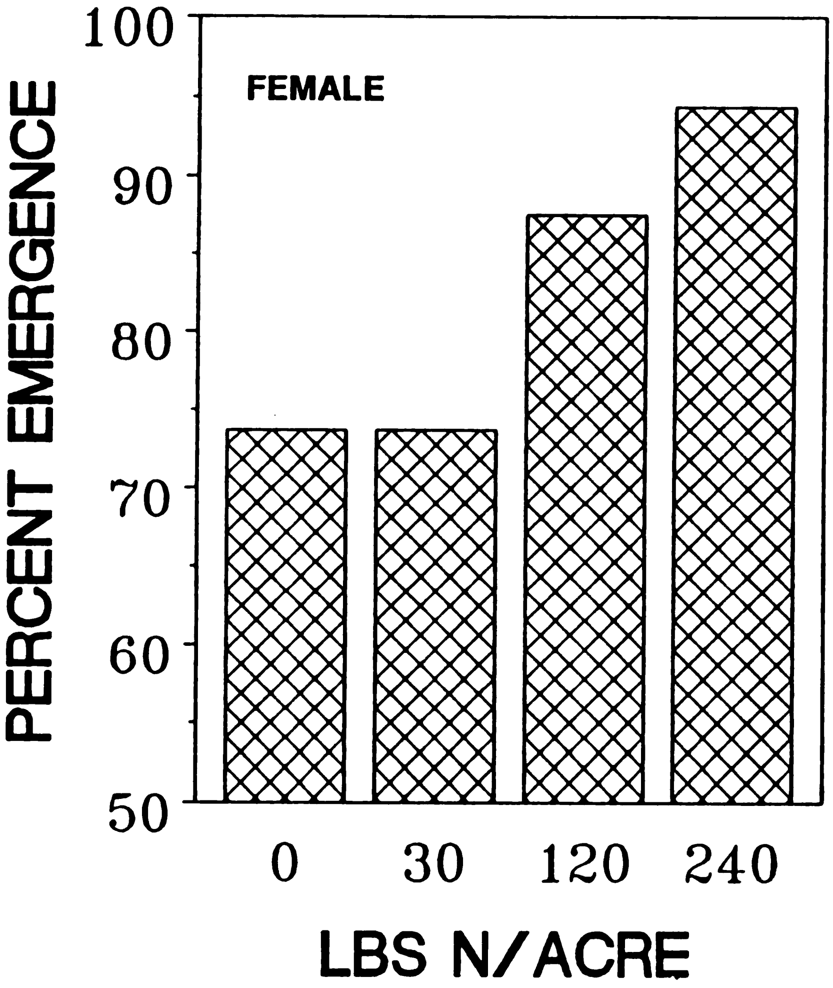


Figure 7. Percent successful emergence of adult females from larvae reared on tomato foliage treated at four different nitrogen levels. A linear regression of arcsine transformed data produced a slope significantly different from zero ($R^2 = .97$, $p < .02$). See text for explanation of statistics.

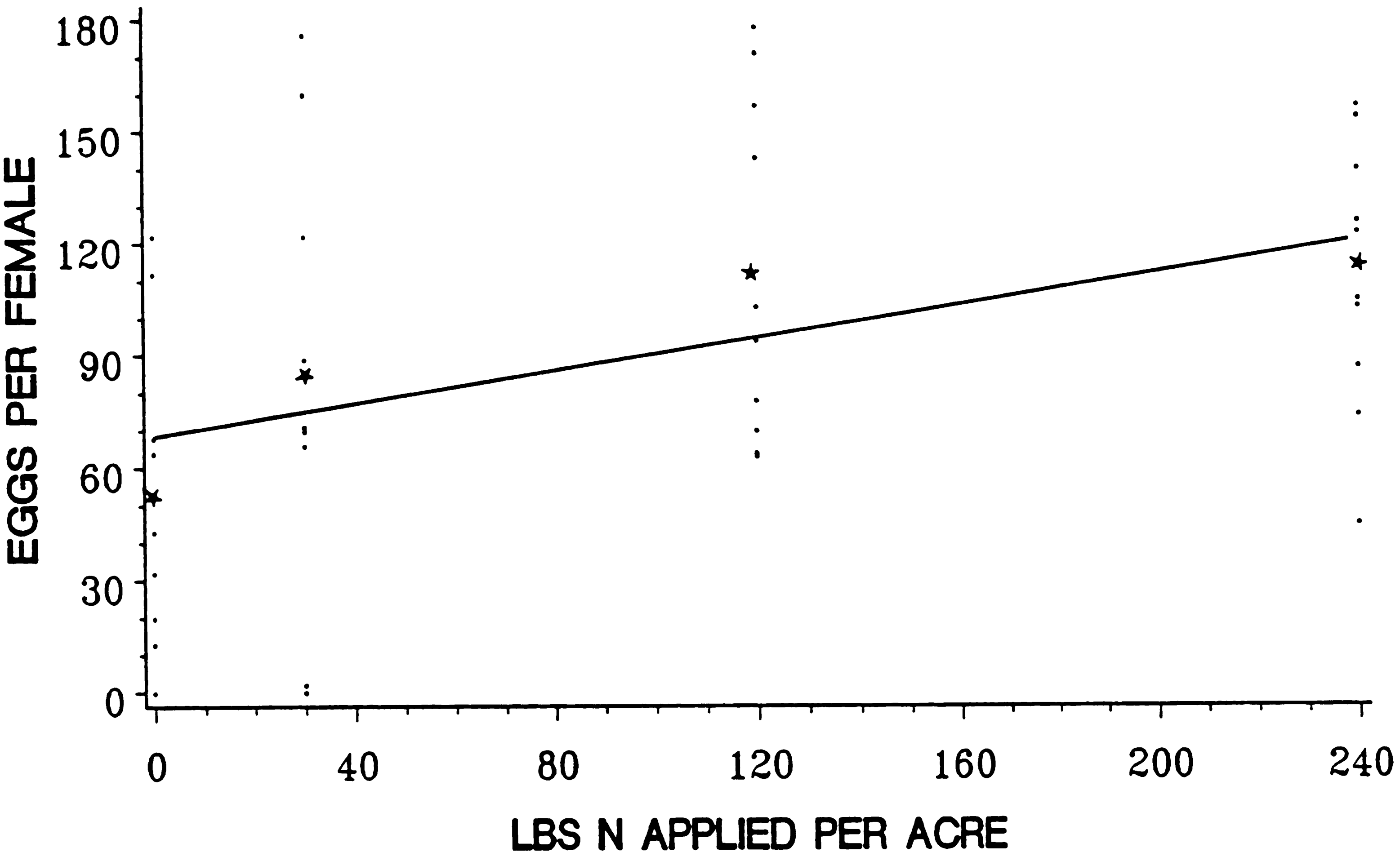


Figure 8. Linear regression of female fecundity. $R^2 = .17$, $p = .009$. $N = 10$ at each nitrogen level. Mean values depicted by stars.

Table 2. Correlation of mean percent dry weight plant nitrogen and soluble nitrates with larval performance parameters calculated from the General Growth Study. Top row of numbers show Pearson's coefficient of correlation. Numbers in parentheses demonstrate level of significance.

	% Successful Emergence		Pupal Dry Weight*		Female Fecundity*	% Larval Survival
	Male	Female	Male	Female		
% Dry Weight Plant Nitrogen	.49 (.51)	.96 (.04)	.57 (.0003)	.47 (.004)	.96 (.03)	-.68 (.32)
% Soluble Nitrates	.64 (.36)	.99 (.001)	.99 (.008)	.95 (.05)	.85 (.15)	-.45 (.53)

*Correlation conducted on individual observations

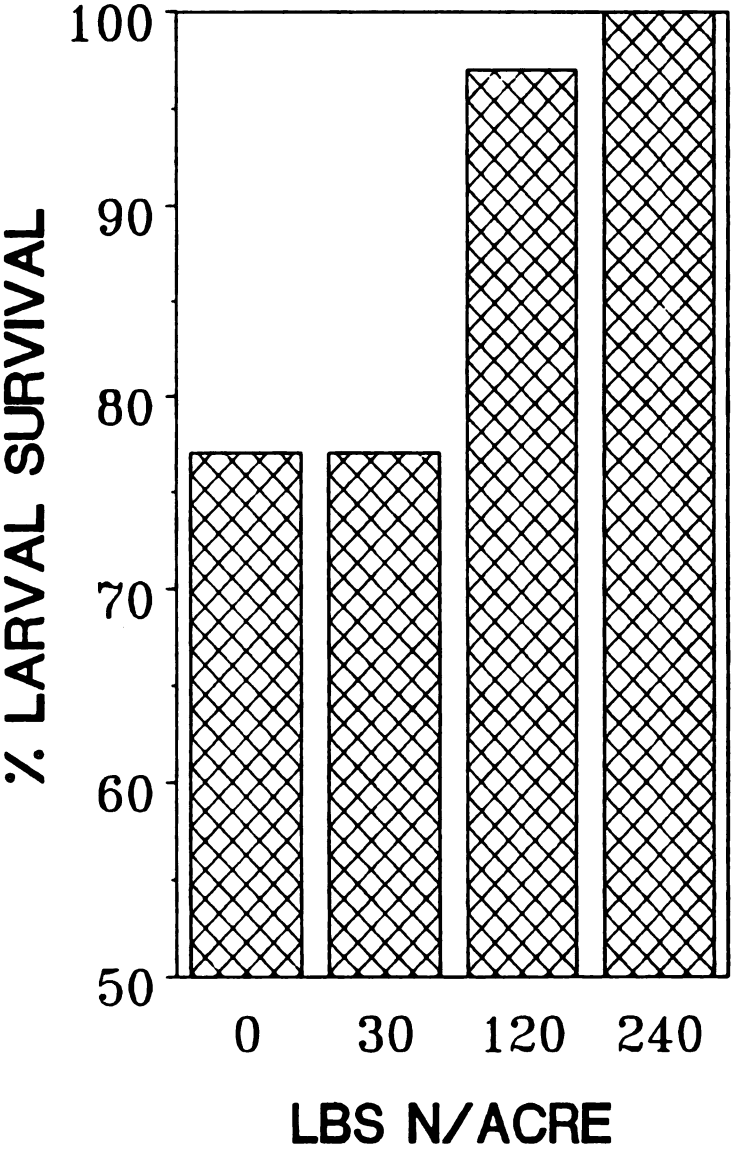


Figure 9. Percent survival of larvae reared from egg hatch to premolt fifth instar on foliage treated at four rates of nitrogen fertilization. A linear regression of arcsine transformed data determined that survival was associated with fertilization level ($R^2 = .95$, $p = .02$). N = 30 larvae per treatment.

30 larvae reared on untreated foliage and only 25 reared on foliage treated with 30 lbs. nitrogen per acre even reached fourth instar. A linear regression of arcsine transformed data determined that larval survival from egg hatch through fourth instar was highly associated with fertilization treatment.

The number of days required for larvae to reach newly molted fourth instar (Figure 10) was slightly shorter for larvae reared on foliage treated at the highest fertilization rate (7.33 ± .10); approximately a half day quicker than individuals reared on foliage treated with 120 lbs. N/acre and untreated foliage (7.90 ± .15 and 7.76 ± .15 for 0 and 120 lbs. nitrogen/acre respectively). Most interesting though, larvae reared on foliage treated with 30 lbs. N/acre required approximately a day longer to reach this stage (8.89 ± .27).

An analysis of variance, utilizing the general linear method procedure (SAS, 1985), was performed on all nutritional indices. Since AD, ECD and ECI calculate relative percentages, these particular indices were converted to their arcsine transformations prior to analysis. In addition, AD, ECD and ECI were analyzed using a weighted regression (Steel and Torrie 1980) to correct for heterogeneity of variance.

Results (Table 3) demonstrated significant differences between treatments for many of the nutritional indices. Only final larval weight and relative foliage consumption

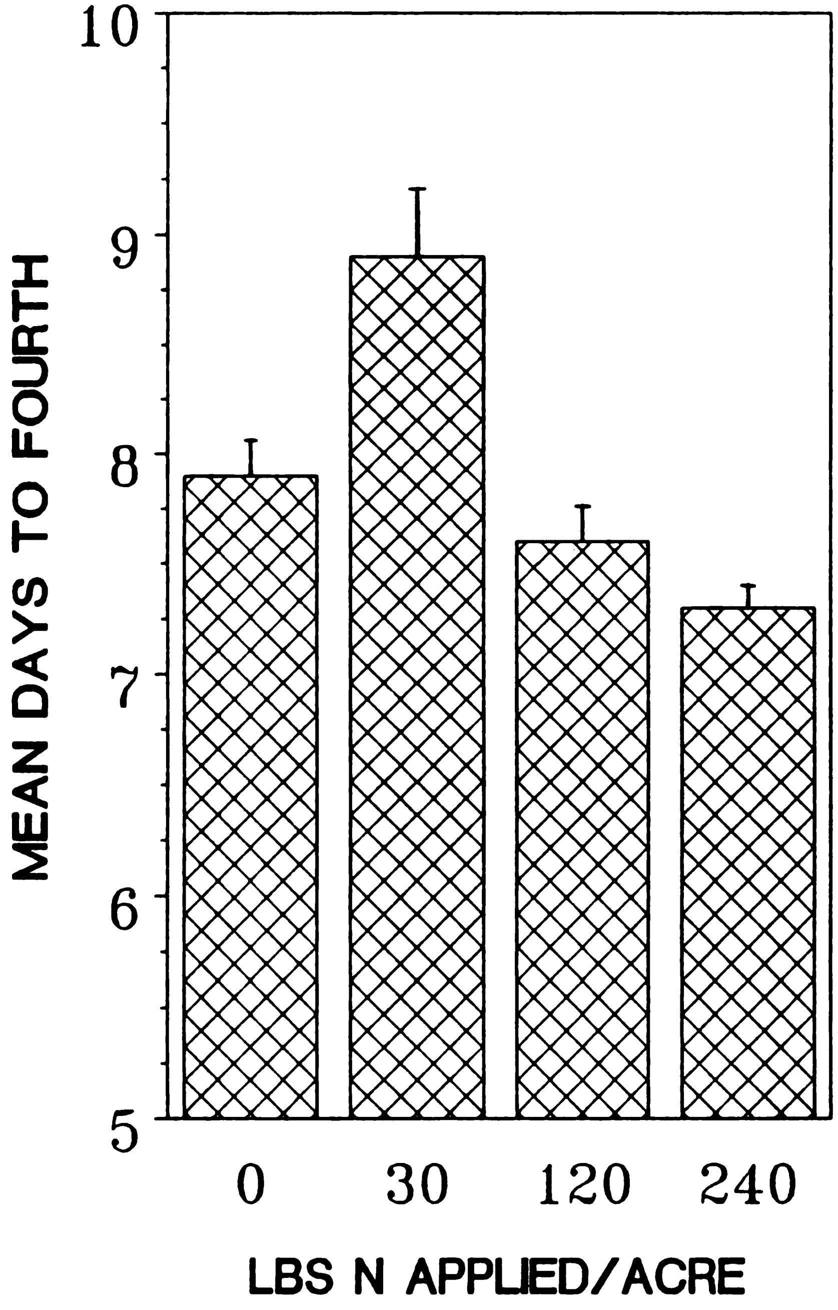


Figure 10. Means and standard errors for days to premolt fourth instar. F = 14.04, p < .0001. N = 26, 25, 30 and 30 for 0, 30, 120 and 240 lbs. nitrogen applied/acre respectively.

Table 3. Analysis of variance of larval performance indices. Larvae reared on foliage treated with four levels of nitrogen fertilizer. Significance of F-statistics (n.s. = not significant at $p = .05$). See materials and methods for explanation of statistics and definition of indices.

Performance Indice	F-Value	P
AD*	3.83	$p < .01$
ECD*	7.87	$p < .0001$
ECI*	11.90	$p < .0001$
RGR	6.17	$p < .0008$
RCI	2.37	n.s. ($p < .07$)
TIME**	3.37	$p < .02$
FINAL WGT	1.57	n.s. ($p < .2$)

*GLM performed on arcsine transformed data. Weighted sums of squares used to correct for heterogenity of variance.

**Time = Duration of fourth instar in days.

were not significantly different at p = .05, although differences in relative consumption were significant at p = .07.

Student-Newman-Keuls mean separation of these results (Table 4) showed that larvae reared on foliage treated with 120 and 240 lbs. nitrogen/acre converted both digested and ingested food more efficiently (ECD and ECI) than larvae reared on the other two foliar treatments. Larvae developing on these other treatments utilized digested plant material with the same approximate efficiency; however, larvae developing on untreated foliage were more efficient at converting ingested food than larvae developing on foliage treated with 30 lbs. nitrogen/acre. Tomato plants with lower fertilization levels produced larvae that had the highest digestion efficiencies (AD). Overall, larvae that were more efficient at converting digested plant material into actual body weight demonstrated lower digestion efficiencies (Figure 11).

Larvae developing on foliage treated at the highest rate of fertilization grew at a faster relative rate (RGR) (Table 4) and reached premolt fifth instar in fewer days than all other larvae (Figure 12). Larvae reared on foliage treated with 30 lbs. nitrogen/acre grew at the slowest relative rate and required the longest amount of time to reach premolt fifth instar. The difference in mean development time between larvae on these two treatments was equal to one day. In contrast, larvae reared on untreated foliage

Table 4. Means and standard errors of larval performance indices. Larvae reared on foliage treated with four levels of nitrogen fertilizer. Means with the same letter are not significantly different at p = .05 (SNK). See Materials and Methods for definitions of indices and explanation of statistics.

LBS Nitrogen Applied/Acre	Duration of Fourth Instar (Days)	RGR	RCR	ECD**	ECI**	AD**
0	3.31 ab (.34)	0.75 a (.05)	5.15 a (.56)	31.11 a (2.78)	15.21 a (.80)	51.98 a (2.62)
30	3.59 b (.25)	0.61 b (.06)	4.51 a (.36)	31.31 a (3.62)	14.44 b (1.41)	52.09 a (3.44)
120	2.97 ab (.16)	0.76 a (.03)	4.01 a (.12)	45.87 b (2.65)	18.89 c (.54)	43.13 b (1.72)
240	2.65 a (.19)	0.87 a (.03)	4.25 a (.19)	44.17 b (2.18)	20.66 d (.60)	48.12 c (1.37)

*N = 15, 18, 22 and 24 for 0, 30, 120 and 240 lbs. nitrogen/acre respectively

**Analysis performed on arcsine transformed data; however, values shown depict actual means and their corresponding standard errors

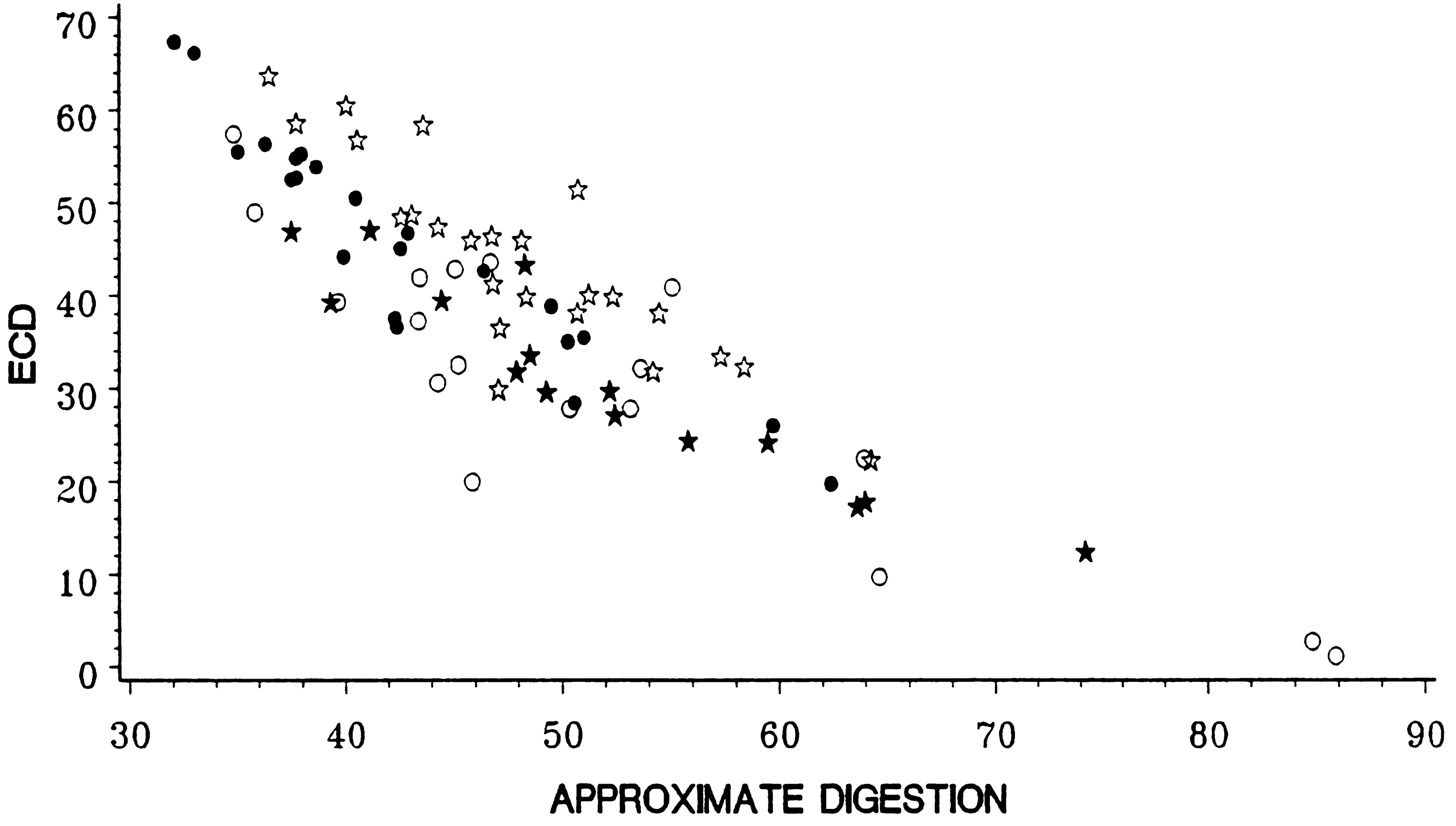


Figure 11. Linear correlation between ECD and Approximate Digestion. Pearson's correlation coefficient = -.90. N = 15, 18, 20 and 22 for 0, 30, 120 and 240 lbs. nitrogen applied/acre. See text for definition of indices. Closed stars 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied/acre.

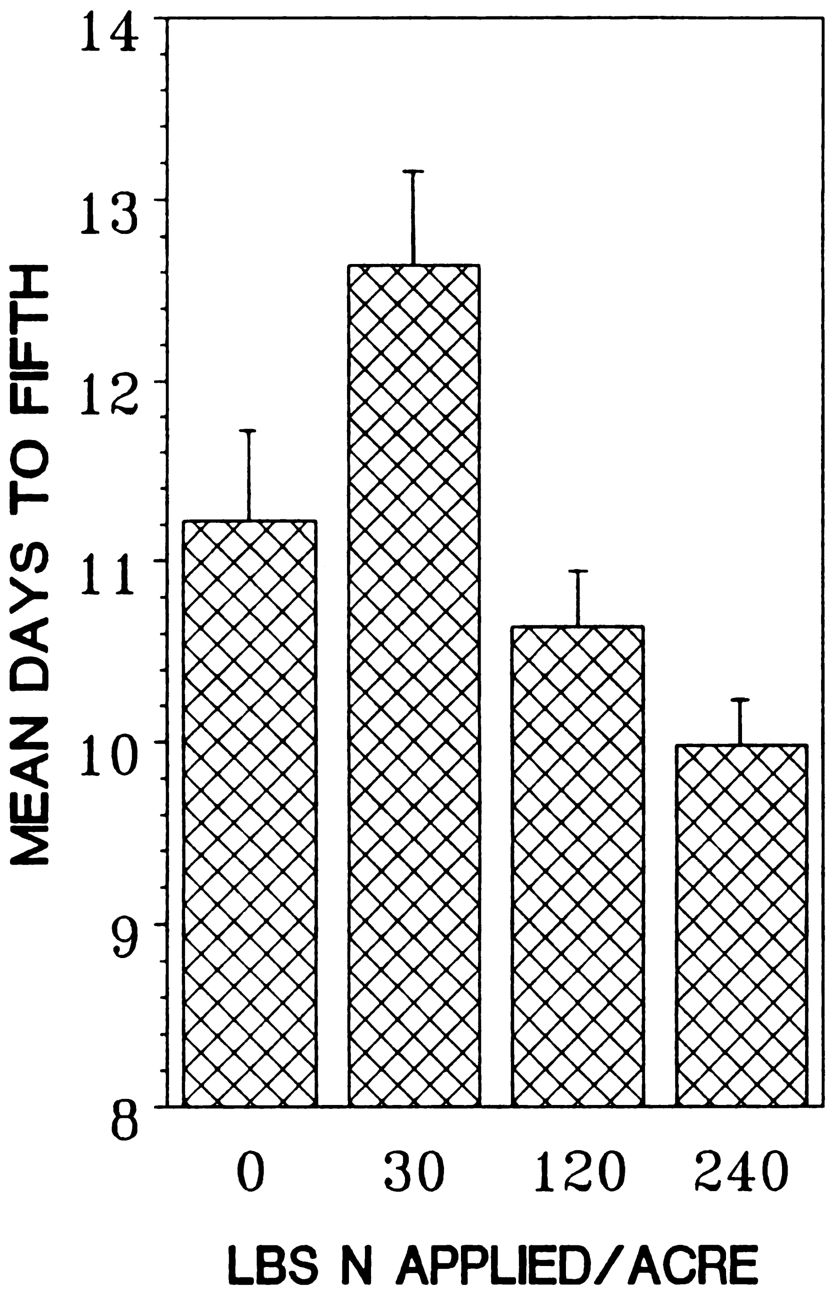


Figure 12. Means and standard errors for days to premolt fifth instar. F = 9.52, p < .0001. N = 15, 18 20 and 22 for 0, 30, 120 and 240 lbs. nitrogen applied/acre respectively.

and foliage treated with 120 lbs. nitrogen acre grew at the same relative rates, although larvae on untreated foliage required approximately one third of a day longer to reach fifth instar.

Even though differences in relative consumption (RCI) were not significant at p = .05, larvae reared on foliage left untreated or treated with a low level of nitrogen fertilizer consumed slightly more plant material relative to their body weight (Table 4). This was especially apparent for those larvae developing on untreated foliage.

Table 5 lists Pearson's correlation coefficients and corresponding level of significance for all growth parameters calculated from data collected during the Detailed Growth Study. Most growth parameters significantly influenced by level of fertilization were also correlated with total plant nitrogen, soluble nitrates or plant water.

DISCUSSION

Results of this study are mostly in agreement with the literature. Increased rates of fertilization were correlated with increased concentrations of total nitrogen and soluble nitrates, which subsequently enhanced larval growth and development and adult fitness. Larvae reared from egg hatch on foliage treated at the two highest rates of fertilization utilized their food more efficiently and developed into heavier pupae and more fecund females. In

Table 5. Correlation of mean percent water content, dry weight nitrogen and soluble nitrates with larval performance parameters calculated from the Detailed Growth Study. Top row of numbers show Pearson's coefficient of correlation. Numbers in parentheses demonstrate level of significance. See text for definition of indices.

	RCR	RGR	ECI	ECD	AD	Duration of Fourth Instar	% Larval Survival
Total Plant Nitrogen	-.91 (.09)	.63 (.38)	.93 (.07)	.97 (.03)	-.89 (.11)	-.91 (.09)	.97 (.02)
Soluble Nitrates	-.38 (.62)	.95 (.05)	.91 (.09)	.80 (.20)	-.56 (.44)	-.38 (.63)	.82 (.17)
Water Content	.62 (.38)	-.91 (.09)	-.99 (.004)	-.95 (.05)	.82 (.18)	.62 (.38)	-.96 (.04)

All correlations calculated from mean values.

comparison, larvae reared on untreated foliage or foliage treated at a very low fertilization rate converted food less efficiently and developed into smaller pupae.

Differences in insect performance were especially apparent for larvae developing on foliage treated with 30 lbs. nitrogen/acre and those developing on foliage treated with 240 lbs. nitrogen/acre. In both the detailed and general growth experiments, larvae reared on foliage treated at the high fertilization rate showed the greatest overall fitness while those reared on foliage treated at the low level were the least fit. Larvae reared on foliage treated with 30 lbs. of nitrogen required, on the average, two additional days to grow from egg hatch to pre-molt fifth instar, a response probably resulting from their low rate of foliage consumption and utilization.

Initially, it was assumed that untreated foliage would contain the lowest levels of total nitrogen and soluble nitrates; hence, produce the slowest developing and least efficient of all larvae. Surprisingly though, larvae developing on foliage treated at 30 lbs. nitrogen/acre, consistently had lower performance values than larvae reared on untreated foliage, even though this foliage contained increased concentrations of total plant nitrogen and soluble nitrates (Table 1; Figures 2 and 3). During the general growth study, calculated values were lower for percent successful emergence of adult males, and pupal dry weights of both males and females (Figures 7, 8 and 9). In addition, performance indices calculated during the detailed growth study, showed that these larvae were less efficient at utilizing ingested food and, as already mentioned, required a longer amount of time to reach both premolt fourth and fifth instar (Figures 10 and 12).

The discrepancy in performance between these two groups of larvae may partially be explained by the higher relative consumption rate of larvae feeding on untreated foliage (Table 4). During each day of the detailed consumption study, these larvae consumed an average of .6 grams of foliage more per day. This observation suggests that, even though larvae reared on untreated foliage did not utilize their food efficiently (compared to larvae developing on foliage treated at the higher rates), they partially compensated for this lack of efficiency by consuming more food per gram of body weight per day. In fact, it appears that this compensatory response enabled larvae developing on untreated foliage to grow at the same relative rate as larvae developing on foliage treated with 120 lbs. nitrogen/acre. Similar types of compensatory behaviors have been demonstrated in other insects (Slansky and Feeny 1977, Slansky 1982). Scriber and Slansky (1981) indicated that a number of larval species adjust their feeding rates in response to decreasing consumption efficiencies, an adaptation apparently demonstrated during this study.

It is uncertain why larvae developing on foliage treated at 30 lbs. nitrogen/acre did not increase consumption to compensate for low food utilization efficiencies. However, this observation, in itself, suggests that foliage treated with 30 lbs. nitrogen/acre probably contained certain repellent compounds or lacked stimulatory ones. One possible explanation for the observed response might be that very slight increases in plant nitrogen produced an imbalance in the chemical composition of the plant by decreasing the substances which elicit feeding behaviors or permit growth.

Nitrogen fertilization directly influences herbivore nutrition by increasing concentrations of amino acids, hence proteins, found in the plant. However, due to its interaction with other plant metabolites, availability of this element can indirectly influence herbivore performance. Two plant factors affected by nitrogen availability and having a large affect on insect nutrition are plant water and plant alkaloids.

Earlier studies have demonstrated a positive correlation between nitrogen concentration and plant water content. However, results of this study do not support these earlier findings. During the two weeks that leaf water content was determined, the level of this plant component was highest in untreated foliage and foliage treated with 30 lbs. nitrogen per acre. However, since percent leaf water content was high for all treatments (80 - 88%), it seems unlikely that water supplies were ever inadequate or at a level that inhibited larval growth. Scriber (1979) indicated that leaf water contents of 75 - 90% would not limit herbivore growth. It is interesting though, that larval fitness, was highest on foliage containing the lowest percentage of plant water. One hypothesis is that lower water levels may have concentrated available nutritional substances (which, due to higher fertilization rates, were probably higher to begin with) and hence, enhanced larval performance. Conversely, higher percentages of plant water in foliage left untreated or treated with low levels of fertilizer may have diluted the nutritional substances in the plant (which, due to lower fertilization rates, were probably lower to begin with) hence, inhibited larval growth (White 1984, Mattson and Haack 1987).

Lycopersicon esculentum, the plant species utilized in this study, contains the steroidal glycoalkaloid α-tomatine. This alkaloid, composed of an aglycone and a glycoside, contains nitrogen as a structural component; thus, will be affected by nitrogen availability. Generally, plant alkaloids increase as plant nitrogen increases. However, because this particular alkaloid has a high C:N ratio, concentrations of this substance may actually decrease with increasing availability of plant nitrogen. When plant nitrogen is abundant, carbohydrates and other carbon containing compounds are utilized to synthesize nitrogen acceptors (Selvendran and Selvendran 1973) thus, depleting the reservoir of carbon utilized to produce glycoalkaloids. Results of experiments conducted on Solanum glycoalkaloids demonstrated that an increase in plant nitrogen resulted in a depletion of the carbohydrate pool and a subsequent decrease in glycoalkaloid synthesis (Nowacki et. al. 1975, 1976).

The affect of glycoalkaloids on M. quinquemaculata are not well known. Yamamoto and Fraenkel (1960b) hypothesized that plant glycosides, commonly found in the host plants of this insect, stimulate feeding; although, this theory remains to be proven. However, even if these compounds initiate feeding behaviors, it is reasonable to assume that at high concentrations they exert a toxic affect on the herbivore. Plant alkaloids are well known for their toxic and repellent effects on certian insects (Harley and Thorsteinson 1967, Nowacki et. al. 1976), even those that are specialists on hosts containing these compounds (Tingey 1984, Sinden et. al. 1986). Based on the preceeding discussion, it seems unlikely that plant glycoalkaloids increased to a level, in any of the treatments, that would have been toxic to M. quinquemaculata larvae. Although not chemically analyzed, it is likely that alkaloid concentrations decreased as plant nitrogen increased. In fact, the responses observed in larvae reared on foliage treated with 30 lbs. of nitrogen per acre may have partially been due to slight increases in the level of alkaloids in these plants, i.e., small increases in nitrogen availability may have been shunted to metabolic pathways producing alkaloids and away from those synthesizing proteins.

The ecological implications of this research are important. Insects with a higher overall fitness will increase their chances of making a genetic contribution to the next generation. Higher survival rates during both the larval and the pupal stage, will increase the number of individuals that emerge as adults and hence, the number available to mate. In addition, heavier pupal weights will produce males and females that have a longer life span and a greater reproductive potential.

The economical considerations are also significant. The application of nitrogen fertilizer is a common cultural practice in agricultural systems. Since higher levels of fertilization can result in insects with faster growth rates and increased fitness, the potential for population outbreaks will also increase. The crop damage caused by these outbreaks could far outweigh the economical advantages of increased yields and may result in considerable losses to the grower.

Conclusions

M. quinquemaculata fitness is significantly enhanced by the application of high levels of nitrogen fertilizer to its host plant. Increased nitrogen availability directly influences both larval and adult performance. Conversion efficiencies, growth rates, pupal weights, fecundity and survival are significantly correlated with rate of fertilization. Nitrogen availability also indirectly affects insect fitness. Since larvae develop faster on foliage treated with high levels of fertilizer, exposure time on the plant is shorter and therefore, the probability of attack by natural enemies will decrease.

REFERENCES

Al-Zubaidi FS, Capinera JL (1984) Utilization of food and nitrogen by the beet armyworm, Spodoptera exigua (Hubner) (Lepidoptera: Noctuidae), in relation to food type and dietary nitrogen levels. Env. Ent. 13:1604-1698.

Auerbach MJ, Strong DR (1981) Nutritional ecology of Heliconia herbivores: experiments with plant fertilization and alternative hosts. Ecological Monographs 51:63-83.

Baumhover AH (1985) Manduca sexta. In: Handbook of Insect Rearing. Vol. 2. (Pritam S, Moore RF, eds.). Elsevier Science Publishers B.V., Amsterdam.

Beck SD (1972) Nutrition, adaptation and environment. In:Insect and Mite Nutrition. North-Holland Publishing Co., Amsterdam.

Beck SD (1974) Theoretical aspects of host plant specificity in insects. In: Proc. Summer Institute on Biological Control of Plant Insects and Diseases. (Maxwell FG, Harris FA, eds.). Univ. Press Miss., Jackson, Miss.

Eppendorfer WH (1978) Effects of N-fertilisation on Amino Acid Composition and Nutritive value of spinach, kale, cauliflower and potatoes. J. Sci. Fd. Agric. 29:305-311.

Fox LR, MacCauley BJ (1976) Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. Oecologia. 29:145-162.

Geissman TA, Crout DHG (1969) Organic Chemistry of Secondary Plant Metabolism. Freeman, Cooper and Co., San Fransico, California.

Gilmore JU (1938) Observations on the hornworms attacking tobacco in Tennessee and Kentucky. J. Econ. Ent. 31:706-712.

Hagen KS, Dadd RH, Reese J (1984) The food of insects. In: Ecological Entomology. (Huffaker CB, Rabb RL, eds.). John Wiley and Sons, Inc.

Harley KLS, Thorsteinson AJ (1967) The influence of plant chemicals on the feeding behavior, development and survival of the two-stripped grasshopper, Melanoptus bivittatus (Say), Acrididae: Orthoptera. Can. J. Zool. 45:305-319.

Hoff JE, Wilcox GE, Jones CM (1974) The effect of nitrate and ammonium nitrogen on the free amino acid composition of tomato plants and tomato fruit. J. Am. Soc. Hort. Sci. 99:27-30.

House HL (1969) Effects of different proportions of nutrients on insects. Ent. exp. & appl. 12:651-669.

Madden AH, Chamberlin FS (1945) Biology of the tobacco hornworm in the southern cigar-tobacco district. US Dept. of Agric. Tech. Bull. No. 986.

Manuwoto S, Scriber JM (1985) Consumption and utilization of experimentally altered corn by southern armyworm:iron, nitrogen and cyclic hydroxamates. Journal of Chemical Ecology. 11:1469-1483.

Mattson WJ (1980) Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11:119-161.

Mattson WJ, Haack RA (1987) The role of drought in outbreaks of plant-eating insects. BioScience. 37:110-118.

McNeill S, Prestidge RA (1982) Plant nutritional strategies and insect herbivore community dynamics. Proc. Fifth Int. Symp. Insect-Plant Relationships. PUDOC, Wageningen.

McNeill S, Southwood TRE (1978) The role of nitrogen in the development of insect/plant relationships. In:Biochemical aspects of plant and animal coevolution. (Harborne JB, ed.). Academic Press. London.

Murata Y (1969) Physiological responses to nitrogen in plants. In: Physiological Aspects of Crop Yield (Eastin JD, ed.) Am. Soc. Agron. Madison, Wis.

Myers JH, Post BJ (1981) Plant nitrogen and fluctuations of insect populations: a test with the cinnabar moth - tansy ragwort system. Oecologia 48:151-156

Nowacki E, Jurzysta M, Gorski P (1975) Effect of availability of nitrogen on alkaloid synthesis in Solanaceae. Bull. Acad. Pol. Sc. Ser. Sc. Biol. 23:219-225.

Nowacki E, Jurzysta M, Gorski P, Nowacka K, Waller GR (1976) effect of nitrogen nutrition on alkaloid metabolism in plants. Biochem. Physiol. Pflanzen. 169:231-240.

Rahier M (1978) Performance of Myzus persicae and production of its host plant, Brassica rapa, related to plant mineral nutrition. Ent. exp. & appl. 24:421-424.

Reese JC (1978) Chronic effects of plant allelochemics on insect nutritional physiology. Ent. exp. & appl. 24:425-431.

Rock GD (1972) Optimal proportions of dietary amino acids. In:Insect and Mite Nutrition. (Rodriquez JG, ed.). North-Holland Publishing Co., Amsterdam.

SAS User's Guide: Statistics (1985) SAS Institute Inc. Cary, NC

Scriber JM (1977) Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth of Hyalophora cecropia (Lepidoptera: Saturniidae). Oecologia. 28:269-287.

Scriber JM (1978) The effects of larval feeding specialization and plant growth form on the consumption and utilization of plant biomass and nitrogen: an ecological consideration. Ent. exp. & appl. 24:494-510.

Scriber JM (1979) Post-ingestive utilization of plant biomass and nitrogen by lepidoptera: legume feeding by the southern armyworm. NY Entomol. Soc. 87:141-53.

Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. Ann. Rev. Entomol. 26:183-211

Selvendran RR, Selvendran S (1973) Chemical changes in young teaplant (Camellia sinensis L.) tissues following application of fertilizer nitrogen. Ann. Bot. 453-461.

Shaw GG, Little CHA (1972) Effect of high urea fertilization of balsam fir trees on spruce budworm development. In: Insect and Mite Nutrition. (Rodriquez JG, ed.). North-Holland Publishing Co., Amsterdam.

Sinden SL, Sanford LL (1986) Leptine glycoalkaloids and resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in Solanum chacoense. Env. Ent. 15: 1057-1062.

Slansky F (1982) Toward a nutritional ecology of insects. Proc. Fifth Int. Symp. Insect-Plant Relationship. PUDOC, Wageningen.

Slansky F, Feeny P (1977) Stabilization of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47:209-228.

Solkal RR, Rohlf FJ (1981) Biometry; The principles and practice of statistics in biological research. Freeman. San Fransico.

Steel RGD, Torrie JH (1980) Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill.

Terman GL, Noggle JC, Hunt CM (1976) Nitrate-N and total N concentration relationships in several plant species Agromony J. 68:556-560.

Tingey W (1984) Glycoalkaloids as pest resistance factors. Am. Potato J. 61:157-167.

van Emden RH, Way (1973) Host plants in the population dynamics of insects. Insect/Plant Relationships. Symposia of the Royal Entomological Soc. of London. No. 6. Blackwell. Oxford.

van der Meijden E, van Bemmelen M, Kooi R, Post BJ (1984) Nutritional quality and chemical defence in the ragwort-cinnabar moth interaction. J. Animal Ecol. 53: 443-453

Waldbauer GP (1962) The growth and reproduction of maxillectomized tobacco hornworms feeding on normally rejected nonsolanaceous plants. Ent. exp. & app. 5:147-158.

Waldbauer GP (1964) The consumption, digestion and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco hornworm, Protoparce sexta (Johan.) (Lepidoptera:Sphingidae). Ent. exp. & appl. 7:251-269.

Waldbauer GP (1968) The consumption and utilization of food by insects. In: Advances in Insect Physiology. 5:229-288.

Waldbauer GP, Fraenkel GS (1961) Feeding on normally rejected plants by maxillectomized larvae of the tobacco hornworm, Protoparce sexta (Lepidoptera, Sphingidae). Ann. Ent. Soc. Amer. 54:477-485.

Waller Nowacki (1978) Alkaloid Biology and Metabolism in Plants. Pleunum Press, NY and London.

White TCR (1979) An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology. 50:905-909.

White TCR (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia. 65:90-105.

Wint GRW (1983) The effect of foliar nutrients upon the growth and feeding of a lepidopteran larva. In:Nitrogen as an Ecological Factor. (Lee JA, McNeill S, Rorison HI, eds.). Sym. British Ecological Soc. Blackwell. London.

Yamamoto RT, Fraenkel G (1960b) Assay of the principal gustatory stimulant for the tobacco hornworm, *Protoparce sexta*, from solanaceous plants.

Yamamoto RT, Jenkins RY, McClusky RK (1969) Factors determining the selection of plants for oviposition by the tobacco hornworm *Manduca sexta*. Ent. exp. & appl. 12:504-508.

MANUSCRIPT III. Conspecific host selection and subsequent larval performance in specialist phytophages: the influence of plant quality on herbivore behavior and physiology (Lepidoptera:Sphingidae).

ABSTRACT

Manduca spp., specialist herbivores, appear to discriminate among individual tomato plants treated at different rates of nitrogen fertilization. Host preference, measured as the percent of total eggs deposited and the percent of plants with eggs, was higher on plants treated at higher fertilization rates and containing higher concentrations of soluble nitrates and total nitrogen.

Plants most preferred by moths also conferred the highest fitness on developing larvae. Larvae reared on foliage from preferred plants grew faster and developed into larger, more fecund adults. Plant components affected by nitrogen availabilty and thought to influence these behavioral responses are discussed.

INTRODUCTION

In many insect species, newly hatched larvae are virtually immobile (Beck 1974, Renwick 1983). For these individuals, survival and growth will be, to a large extent, dependent upon the quality of the plant that the adult female "selects" (Singer 1986, Dethier 1947, Rausher 1979, Mackay 1985). If the female oviposits on an inferior food source, larval performance will be directly affected. Egg deposition on plant species containing toxic substances or lacking minerals and nutrients essential for growth can kill the larva outright (Beck 1965, Rhaodes and Cates 1976, Bernays 1982) or, more commonly, can inhibit the digestion and assimilation of food consumed (Rhoades and Cates 1976, Mitchell and Harrison 1985, Slansky and Feeny 1977, Reese 1978, 1983 Robinson 1979, Barbosa et. al. 1982). Although some species are able to regulate consumption, or otherwise compensate, in response to variations in plant quality (Slansky and Feeny 1977, Slansky 1982), larval development on a nutritionally inadequate food source will generally produce smaller, less fecund, less fit adults (McNeill and Southwood 1978, Scriber and Slansky 1981, Slansky 1982, McNeill and Prestidge 1982, Prestidge and McNeill 1982a, 1982b).

Since host selection by an adult female can affect the success of her offspring and the subsequent success of the adults produced, it is reasonable to assume that host plant quality will influence the evolution of host acceptance behavior and ovipositional response. Through the process of natural selection, insects should have evolved the ability to distinguish between "suitable" and "unsuitable" host plants. Presumably, those individuals that have this capability will have an evolutionary advantage over those insects that lack this mechanism.

Historically, research has focused on elucidating the factors that enable an insect to discriminate among different plant species. These investigations have led to the formulation of hypotheses regarding the ecology of host acceptance behavior. However, there is no reason to assume that discrimination occurs only at the interspecific level. In fact, since host plant quality may influence the evolution of ovipositional response, it seems likely that host searching females will exhibit intra-, as well as, interspecific discriminatory behavior.

Several recent studies have demonstrated that certain insects have the physiological and behavioral capabilities to distinquish among individual plants within a species (Mitchell 1977, Ives 1978, Wolfson 1978, 1980, Myers and Post 1981, Myers 1985, Rausher and Papaj 1983, Papaj and Rausher 1987, McKay 1985). Results of these earlier studies have shown that host searching females cue into plant size (Ives 1978, Karban and Courtney 1987), density (McKay and Singer 1982, McKay 1985), moisture (Shorey 1964, Sparks 1973, Wolfson 1980) and chemistry (Mitchell 1977, Wolfson 1978, 1980, Myers and Post 1981, Myers 1985) when "choosing" among conspecific plants.

The present research was initiated to determine if field populations of a specialist phytophage, in this case _Manduca_ _spp_. (_M._ _sexta_ and _M._ _quinquemaculata_), would discriminate among individual plants of the same host species. In particular, the study focused on the chemical differences among these plants (measured as percent total nitrogen and soluble nitrates). A second objective of the study tested the hypothesis that adult females prefer to oviposit on those plants most suitable for larval growth and survival.

These objectives were investigated by treating small field plots of tomato plants with four different levels of nitrogen fertilizer, thereby inducing physiological changes in these plants. Host preference was determined by documenting the distribution of eggs on treated plants from field populations of _Manduca spp._ Relative fitness was examined by rearing laboratory cohorts of _M. quinquemaculata_ larvae on bouquets of treated foliage and calculating various parameters of fitness.

MATERIALS AND METHODS

Tomato Plot Design

This study was part of a sustainable agriculture project at the Kellogg Biological Station in Hickory Corners, Michigan. Investigations into the affects of intercropped systems on the abundance and distribution of insects continues to be an important aspect of this long term project. In accordance with these goals, two separate sites, a tomato monoculture, and a tomato/apple tree intercropping, were included in this particular study. The intercropped site consisted of a determinant cultivar of tomato, var. Sunny, planted between tree rows in a dwarf apple orchard (designated orchard site). The tomato monoculture consisted of the same cultivar of tomato planted in an adjacent field (designated field site). In 1986, the field site was moved from its original location to a similar nearby area. The orchard site remained at the same location throughout both years of the study (Figure 1).

The study sites were designed such that groups of treated tomato plants occurred in equal proportions and grew in close proximity to one another. Both sites were initially divided into multiple plots of tomato plants. Each plot consisted of double rows of plants approximately 50 m long. Plots in the field site were spaced approximately 5 m apart while those in the orchard site were spaced 7 m apart and interplanted with apple trees (Figure 1). Plots at both sites were subdivided into three blocks. Each block was subsequently divided into four small, rectangular areas containing eight tomato plants. One of four levels of nitrogen fertilizer was randomly applied to plants within a rectangle until all four rectangles within a block had been treated with a fertilizer application (Figure 2). A total of 24 blocks were set up during the first year of the study and 21 during the second year. An area approximately 1 m between rectangles and 2 m between blocks was left untreated (Figure 2). These untreated areas, were designed to prevent or decrease the movement of nitrogen through the soil between adjacent treatments and blocks.

Fertilizer Application

Fertilizer was applied directly to the soil surrounding individual plants. In 1985, urea (45% nitrogen) was

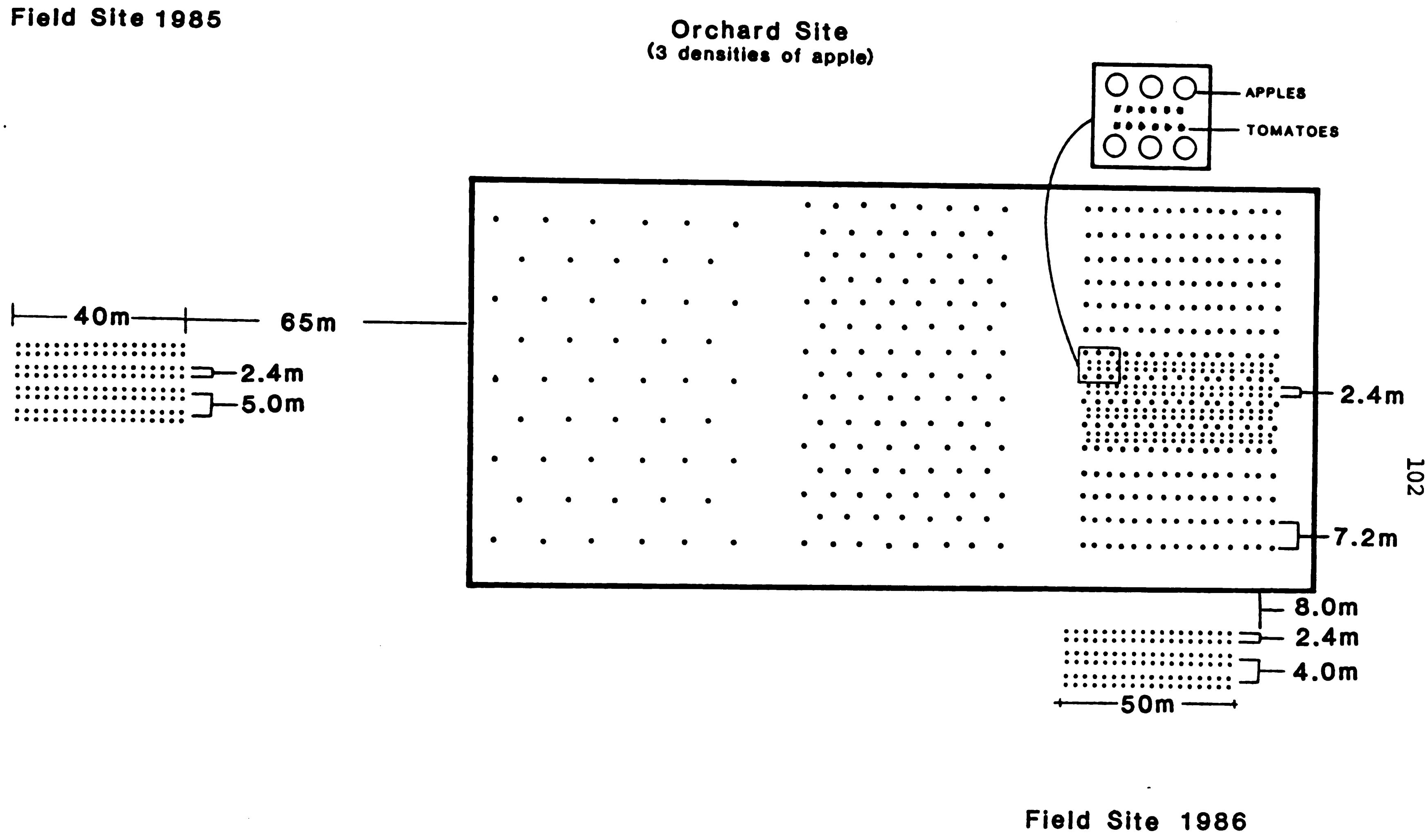
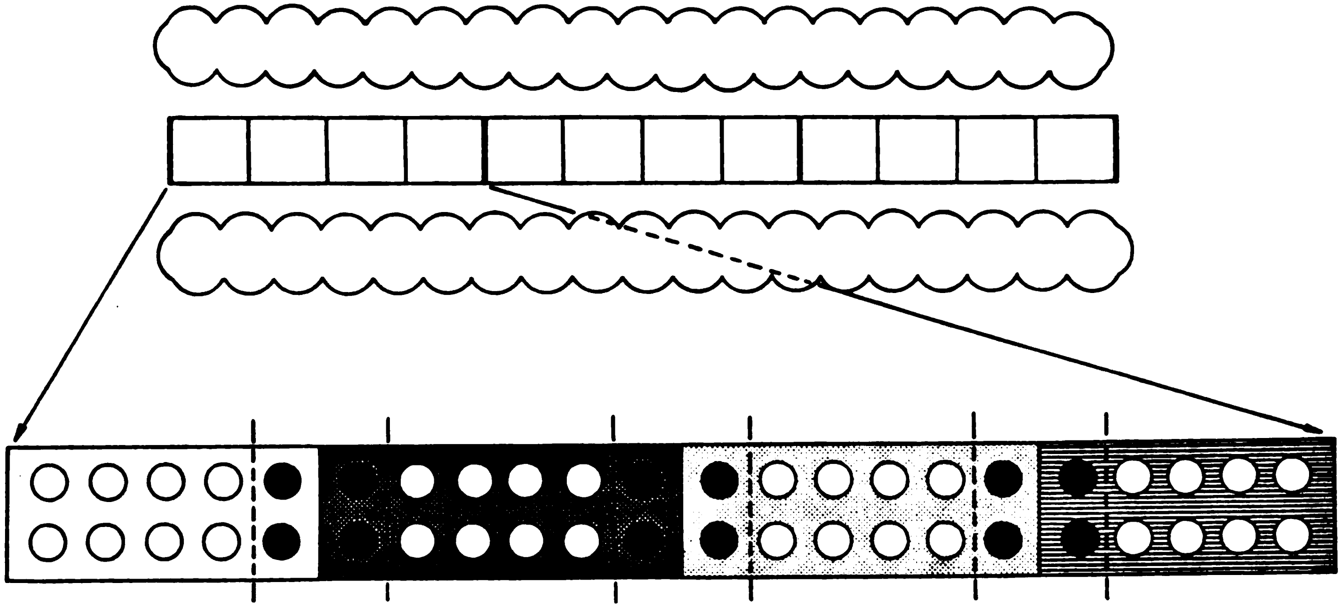


Figure 1. Field design layout for conspecific host preference study. Large dots represent dwarf apple trees, small dots represent tomato plants. See Materials and Methods for further explanation of design.

Layout module of nitrogen treatments.



Single plot subdivided into three blocks.

Single block subdivided into four nitrogen treatments

Figure 2. Plot design depicting fertilization of single block. White circles represent treated tomato plants. Black circles represent untreated tomato plants. See Materials and Methods for further explanation of design.

applied at the rate of 0, 15, 60, and 120 lbs. nitrogen/ acre. Applications were split between June 29 and July 9 to prevent burning the plants receiving high levels of fertilizer. The first fertilizer application occurred approximately 3 and 1/2 weeks after tomato plants were transplanted into the field. Analyses of egg frequencies from the first year of the study revealed that plants treated with 0 and 15 lbs. nitrogen and those treated with 60 and 120 lbs. nitrogen elicited similar responses from the female moths (see Results). Because of this similarity, fertilization rates were changed to 0, 30, 120 and 240 lbs nitrogen/acre for experiments conducted during the second year of the study. These particular levels were chosen for their anticipated effect on ovipositional responses. The highest application, 240 lbs. nitrogen, was hypothesized to be a level at which physiological changes in the plant might actually become deleterious or repellent to the moths. Thirty pounds nitrogen was proposed to be the amount at which differences might first be detected between low and high nitrogen levels (=threshold level). To retain continuity between seasons, two rates of application (0 and 120 lbs. nitrogen applied/acre) were replicated between years. Ammonium nitrate (34% nitrogen) provided the source of nitrogen in 1986. As in 1985, total fertilization levels were divided between two separate dates, July 3 and July 14, with the first application occurring approximately 2 weeks after transplantation of tomato plants into the field.

Sampling Procedure

Since M. quinquemaculata is the more prevalent hornworm species during the early part of the season (see Manuscript I), the period during which moth preference was analyzed, and eggs are virtually identical for both insects, no attempts were made to distinguish between M. sexta and M. quinquemaculata.

Tomato plants (half plants) were randomly sampled twice weekly during both field seasons. In addition, in 1986 plants were sampled every other day from July 13 to July 21. Actual plants sampled were chosen by utilizing a random numbers table. On each sampling day in 1985, 60 half plants, divided among all plots, were visually scanned for eggs. In 1986, 96 half plants (24 per N treatment) were sampled each day until August 21. However, beginning on August 25, only 48 half plants (12 per N treatment) were sampled per day (in 1986, plants in the orchard site became heavily infected with Septoria lycopersici and in late August, sampling was terminated at this site due to lack of foliage.

Tomato plant stems and flowers and upper and lower leaflets, were visually scanned for eggs. When an egg was found, its location was recorded and the host plant was tagged. To eliminate counting the same eggs more than once, tagged plants were not sampled on succeeding days. Documentation of plant height and diameter and general plant quality (vigor, color, etc.) were also recorded for each plant sampled.

Larval Fitness Study

Larval performance was assessed in 1986. For detailed description of Materials and Methods of larval fitness study, see Manuscript II. M. quinquemaculata was chosen as the organism for the laboratory studies because a) it is the most abundant species found in the field during July hence, the majority of eggs sampled during the host preference study will be deposited by M. quinquemaculata moths, and b) few studies examine the biology and ecology of this animal while a large amount of literature has accumulated with respect to the biology and physiology of M. sexta.

Foliage fed to larvae was collected fresh from the field grown tomato plants early each morning. Similar aliquots of leafs were picked from each treatment. Generally, these aliqouts consisted of the first fully expanded leaf from the terminal bud.

Two cohorts of M. quinquemaculata larvae were reared from eggs received from the Oxford Tobacco Research Station in North Carolina. Initially, larval populations were to be generated from field captured moths. However, after attempts to collect moths from natural populations proved futile, it was decided that laboratory populations would be used instead.

The first larval cohort was utilized to determine percent larval survival, pupal dry weights, percent successful adult emergence and female fecundity. Development of this group of larvae coincided with larval development of early field populations. The second larval cohort was utilized to determine percent larval survival and days required to reach newly molted fifth instar. Because of two failed experiments (a result of technical errors), this second larval cohort was begun on foliage three weeks later than the first larval cohort.

To determine overall insect fitness, performance potential (based on Laughlin 1965), was calculated from the following equation utilizing values obtained from each larval cohort experiment:

$$\text{Performance Potential} = \frac{(\%\ \text{Larval Survival})(\%\ \text{Successful Female Emergence})(\text{Mean Pupal Dry Weight})}{\text{Mean Larval Period}}$$

Although this index is not meant to reflect an exact measure of fitness, it does provide a realistic estimate of an individual's potential for conferring genes on to the next generation.

Foliage Analysis

Tomato foliage utilized for chemical analysis was collected in 1986. Every three to four days, four similar aliquots were randomly selected from bags of leaves collected for larval feeding studies. These aliquots were combined into one foliage sample per treatment per each sampling day and then oven dryed at 60° C.

Foliage samples were analyzed for total nitrogen and soluble nitrates. Prior to analysis, all foliage was ground using a 40 mesh screen. To determine total nitrogen, 150 mg of dried foliage was digested with Kel-catalyst KC-M3 (3.5 g potassium sulphate + 175 mg mercuric oxide) and 9 ml sulfuric acid for one hour. After cooling for 30 minutes, the solution was brought up to a volume of 75 ml with distilled water. Total ppm nitrogen was read using a Technicon Auto Analyzer II.

To determine soluble nitrates, 20 mls of distilled water was added to 500 mg of dried foliage an then shaken for 30 minutes. Total soluble nitrates was read using a nitrate electrode and an expanded scale pH meter.

RESULTS

Foliage Analyses

During both years of the preference study, plants treated with high levels of fertilizer produced foliage that was visibly greener (although not chemically analyzed) than leaves on plants treated with low levels of fertilizer or left untreated.

Chemical analyses conducted on foliage collected in 1986 demonstrated that mean percent dry weight total plant nitrogen was positively correlated with nitrogen fertilization treatment ($r = .93$ at $p = .05$), i.e., plants receiving higher levels of nitrogen fertilization contained higher concentrations of total plant nitrogen. Figure 3 depicts the seasonal trends of this plant component. Even though initial concentrations were relatively high for all treatments, decline occurred quickly and concentrations leveled off throughout the remainder of the study. Table 1 lists means and standard errors of percent total plant nitrogen during each separate aspect of the separate study.

Mean percent soluble nitrates (Figure 4) was also found to be positively correlated with fertilizer treatment ($r = .93$ at $p = .05$). Each application of nitrogen resulted in an intial increase in percent soluble nitrates that steadily declined during the remainder of the season. For plants treated at the high fertilization levels, this decline spanned a period of four to five weeks. However, for tomato plants treated with 30 lbs. nitrogen/acre, nitrate decline occurred within 12 days. By the second week of August, nitrate levels for all treatments were very low. Table 1 lists means and standard error of percent soluble nitrates during each separate aspect of the study.

Host Preference

Since host size can influence the number of eggs oviposited on a particular plant (Ives 1978, Karban and Courtney 1985), the amount of foliage available at each nitrogen treatment was determined prior to examining moth preference for a particular treatment. Mean plant volume

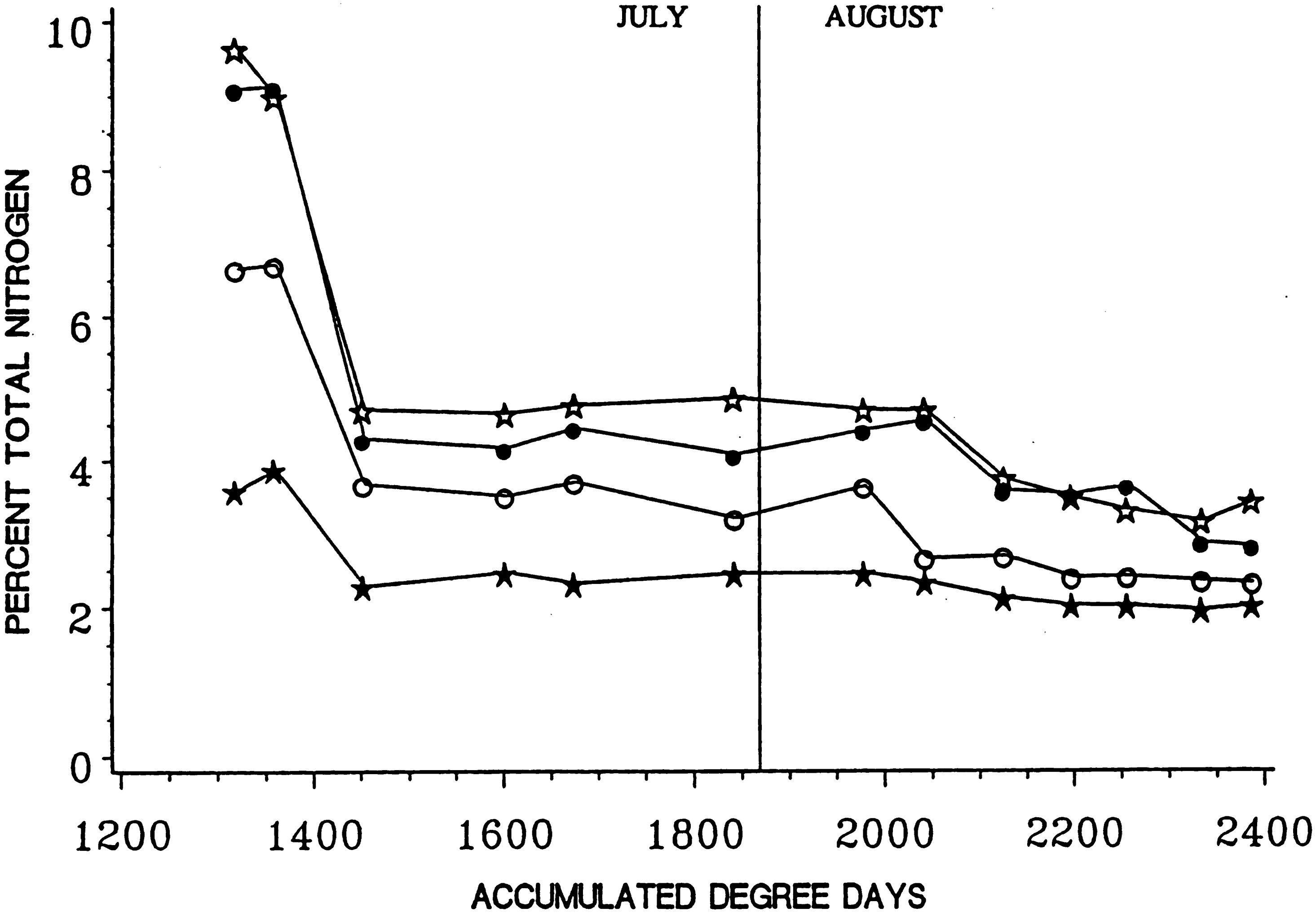


Figure 3. Seasonal trends of percent dry weight nitrogen. Foliage collected during 1986. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Table 1. Means and standard errors for percent water content, total plant nitrogen and soluble nitrates calculated during each separate aspect of the study. These values were utilized in all correlation analyses requiring data on the concentration of particular plant substances.

		STUDY		
	LBS. Nitrogen Applied/Acre	Host Preference (n = 4)	Cohort I (n = 4)	Cohort II (n = 4)
Total Plant Nitrogen	0	3.06 (.400)	2.40 (.036)	2.03 (.027)
	30	5.15 (.893)	3.31 (.243)	2.43 (.066)
	120	6.69 (1.41)	4.39 (.102)	3.29 (.184)
	240	7.01 (1.35)	4.76 (.040)	3.43 (.098)
Soluble Nitrates	0	.048 (.003)	.047 (.005)	.050 (.004)
	30	.205 (.060)	.049 (.010)	.042 (.001)
	120	.326 (.041)	.129 (.031)	.053 (.008)
	240	.408 (.047)	.194 (.041)	.059 (.003)
Water Content	0	N/A	N/A	85.04 (.344)
	30			85.45 (.345)
	120			84.12 (.409)
	240			83.51 (.457)

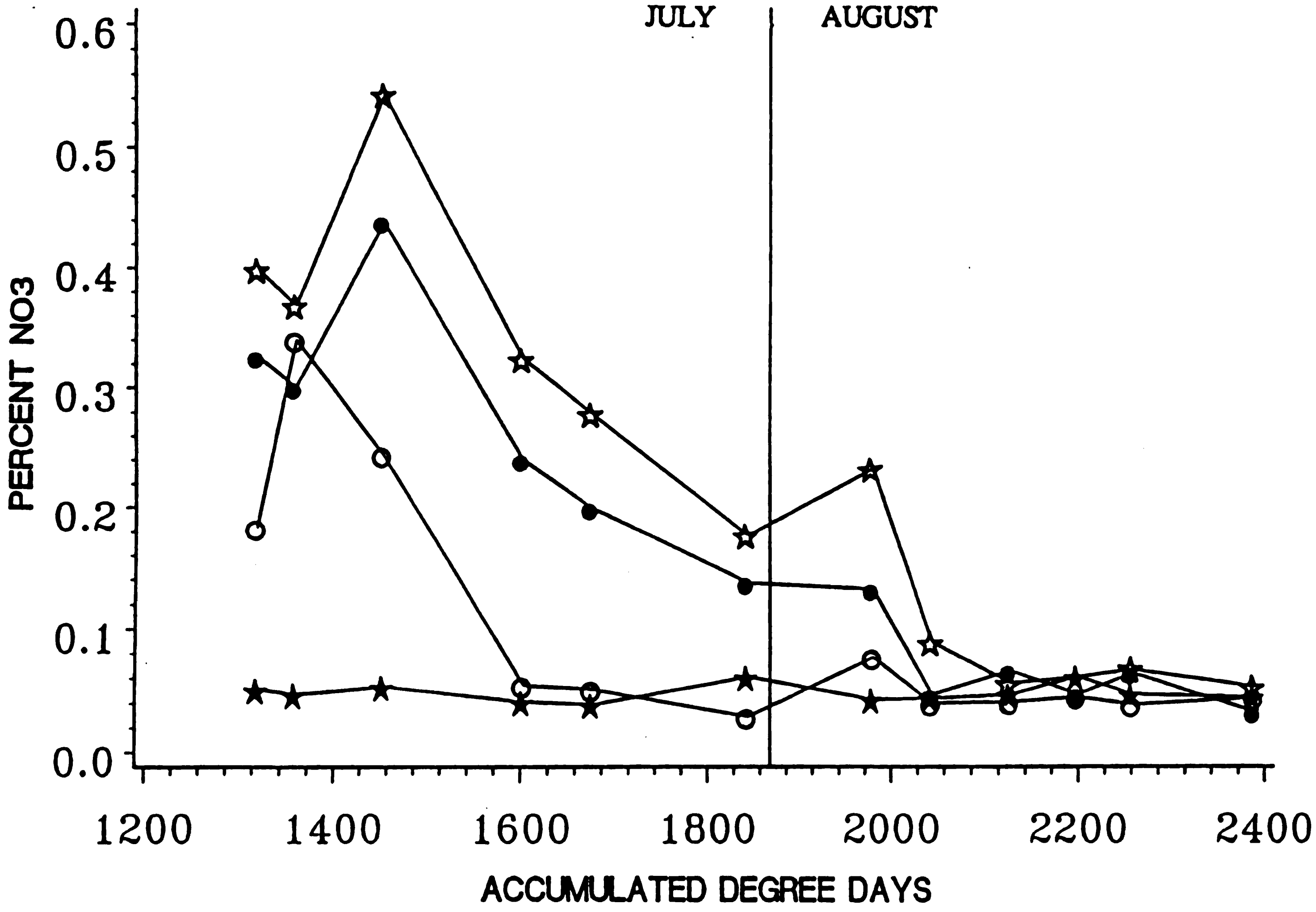
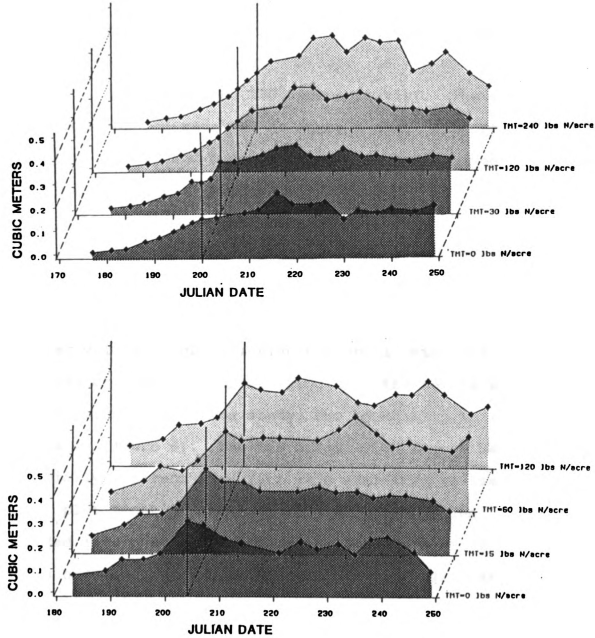


Figure 4. Seasonal trends of percent soluble nitrates. Foliage collected during 1986. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

(plant volume = $\pi r^2 h$ = volume of a cylinder) was calculated from observed plant measurements of height and diameter for each treatment on each day that plants were sampled. The figures obtained provided a close approximation of the amount of plant material available as an ovipositional substrate. Results of an ANOVA performed on mean plant volume for each sampling date determined that there were no significant differences between plant volume and nitrogen application at $p = .05$ from the time that sampling first began each year until July 23 (Julian date 204, DD 1620) in 1985 and July 19 (Julian date 200, DD 1552) in 1986 (Figure 5 represents these analyses-note similarities among areas under the curve for all treatments during a particular year from the time that sampling began at the start of the season up to the point designated by the reference bars). These results suggest that, during this period of time, any differences in numbers of eggs found can be attributed to factors other than plant size (note however, that plant volume does not take into account leaf surface area, a measurement which may be quite different for plants treated with no, or low, amounts of fertilizer compared to those treated with higher levels of fertilizer, although personal observations suggested that this was not the case).

The frequency of hornworm eggs in the field is most appropriately described as a negative binomial distribution (Borth and Harrison 1984), i.e., a distribution heavily skewed towards the larger classes. This study demonstrated



PLANT VOLUME (Y-AXIS) WAS COMPUTED FROM THE FORMULA FOR THE VOLUME OF CYLINDER.

Figure 5. Plot of mean plant volume at each fertilization level. Y-axis represents mean plant volume. X-axis represents Julian date. Z-axis represents nitrogen treatment applied. See Results for further explanation of plots and calculation of plant volume.

a similar frequency distribution. Observed data fit to the negative binomial were not significantly different from expected values (Chi-square = 2.531; $.25 < p < .5$, and 3.68; $.1 < p < .24$ in 1985 and 1986 respectively). The majority of plants sampled received no eggs, a small proportion received one egg and a very low percentage received more than one egg (Table 2).

Moth preference was analyzed from July 2 to 23 (DD 1460-1620) in 1985 and July 7 to 19 (DD 1255-1552) in 1986. In both years, this "window of time" coincided with maximum egg deposition and treated blocks comprised of plants with similar volume (Figure 6; Figure 5--horizontal reference bars depict same day in each figure). Egg levels were low during both years of the study, but especially in 1986 (Table 2; Figure 6). The low infestation levels became especially apparent when data were separated by sampling date, plot location and level of nitrogen applied. When divided into these separate components, only 8% of the total cells had egg frequencies higher than 5 in 1985, and only 6% in 1986. Although negative binomial data are commonly analyzed by a contingency table, a Chi-square test for homogeneity between sites could not be performed since expected cell frequencies were lower than 5 (Little and Hill 1978, Steel and Torrie 1980, Solkal and Rohlf 1981).

To increase cell frequencies, statistical analyses were performed on data that had been combined across all sampling dates even though a more statistically appropriate

Table 2. Distribution of eggs on sampled plants. Data separated by treatment and year. The top row of numbers, within each treatment, represents the frequency of occurrence and the bottom row represents the percent of total.

YEAR	LBS. N APPLIED PER ACRE	NUMBER OF PLANTS SAMPLED BEARING				
		0 EGGS	1 EGG	2 EGGS	3 EGGS	4 EGGS
1985	0	72*	16	3	1	0
		20**	4	<1	<1	0
	15	74	13	2	0	0
		21	4	<1	0	0
	60	65	23	8	0	0
		18	6	2	0	0
	120	52	21	6	0	1
		15	6	2	0	<1
Total Plants Sampled		263	73	19	1	1
Percent Sampled		74%	20%	5%	<1%	<1%
1986	0	125	16	3	0	0
		22	3	<1	0	0
	30	126	16	1	1	0
		22	3	<1	<1	0
	120	118	21	2	2	1
		20	4	<1	<1	<1
	240	118	23	2	1	0
		21	4	<1	<1	0
Total Plants Sampled		487	76	8	4	1
Percent Sampled		84%	13%	1%	<1%	<1%

*Number of plants sampled

**Percent of total plants sampled

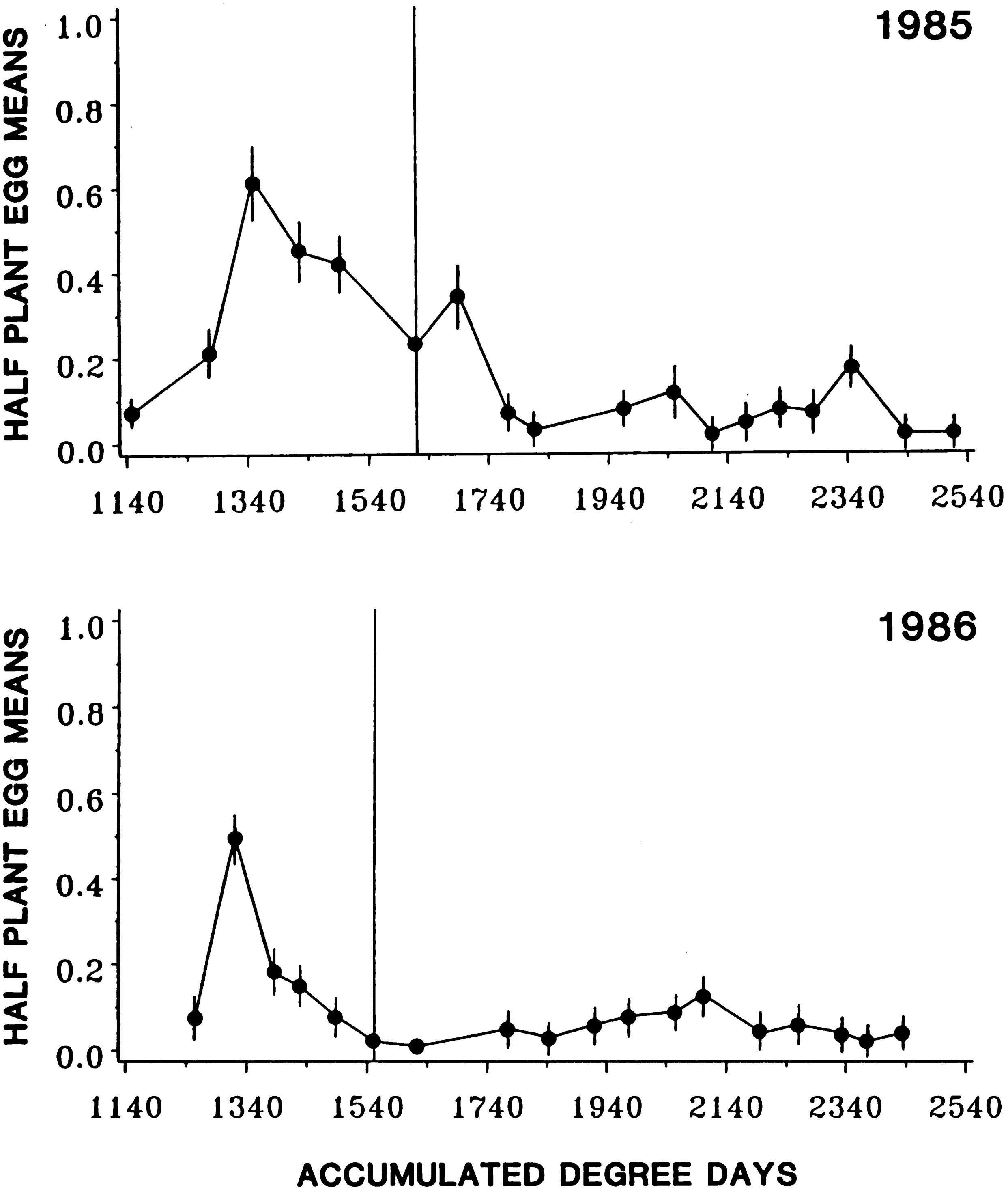


Figure 6. Seasonal hornworm egg densities. Mean eggs calculated per half plant. Reference bars denote period of maximum egg deposition. Data combined from field and orchard site. N = 60 and 96 half plants sampled per day in 1985 and 1986 respectively.

analysis would have been to examine moth preference on a daily basis. Once data had been pooled across dates, a Chi-square test for homogeneity (Solkal and Rohlf 1981) determined that data could be combined across site location, i.e. ovipositional responses were not significantly influenced by site location (Chi-square = 1.50; p > .65, and 3.03; p > .40 in 1985 and 1986 respectively).

Based on the observation that only a small proportion of plants sampled had more than one egg, cells of the table were divided into level of nitrogen applied and plant status, i.e., plants with or without eggs. Data from both years of the study were analyzed separately.

In 1985, a G-test for independence (Solkal and Rohlf 1981) determined that the percent of plants with eggs was associated with the amount of nitrogen applied (Figure 7). However, in 1986 the results of this test did not show a significant association between the number of plants with eggs and fertilization rate (Figure 8) although, results demonstrated that higher nitrogen levels had slightly more plants with eggs.

Since data was collapsed into only two classes (plants with or without eggs) prior to analysis, a test for independence does not take into consideration those plants bearing multiple eggs. This is unfortunate since the majority of plants having more than one egg were also those receiving the higher levels of nitrogen fertilizer, especially during the first year of the study (Table 2). However, because a

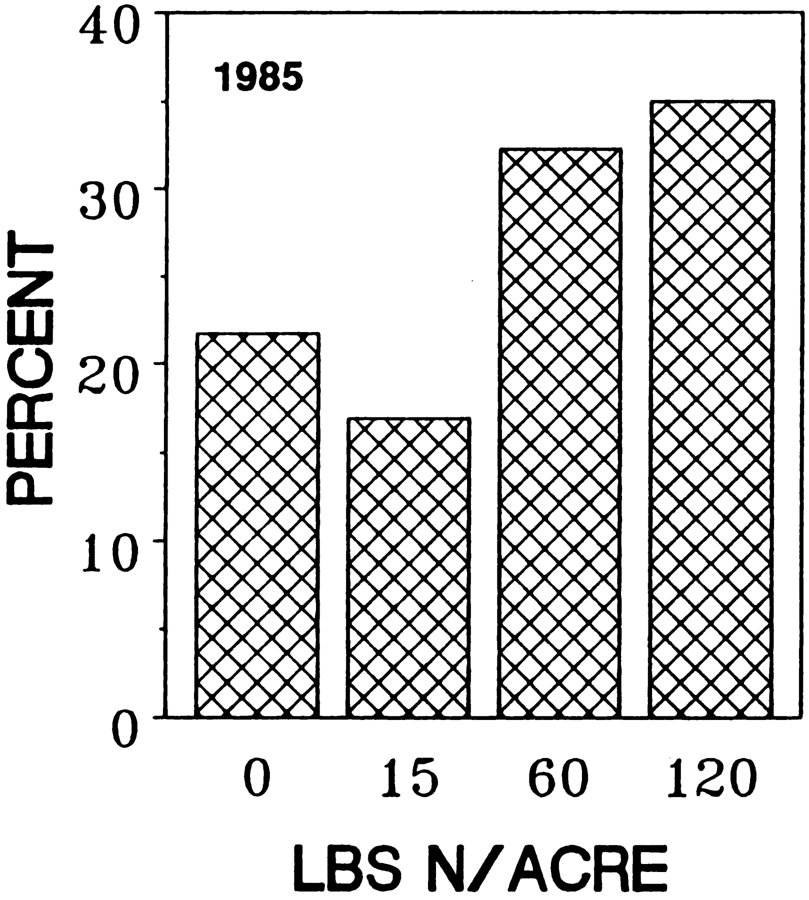


Figure 7. Percent tomato plants with eggs at each nitrogen level. Data combined from field and orchard site--1985 (heterogeneity Chi-square = 1.50, $p > .65$). A G-test analysis for independence determined that the proportion of plants with eggs is associated with the level of nitrogen applied ($G = 10.168$, $p < .025$). See text for explanation of statistics.

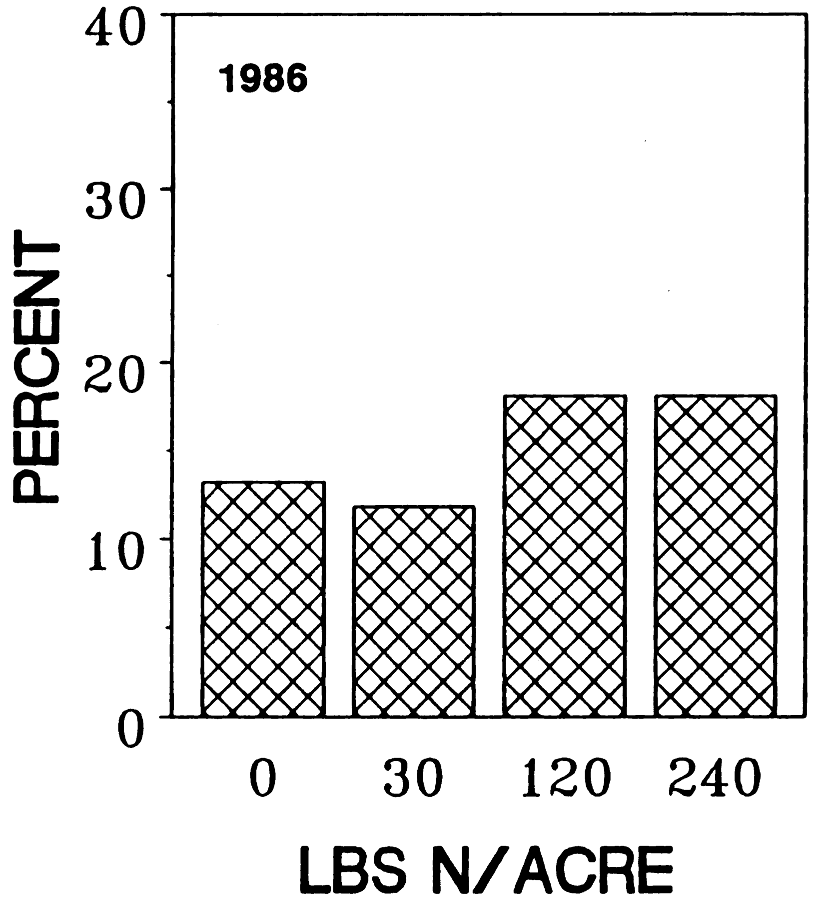


Figure 8. Percent tomato plants with eggs at each nitrogen level. Data combined from field and orchard site--1986 (heterogeneity Chi-square = 3.03, p > .40). A G-test analysis for independence determined that the proportion of plants with eggs is independent of the level of nitrogen applied (G = 5.02, p < .20). See text for explanation of statistics.

large number of total plants was sampled during each year's "window of time" (367 in 1985 and 576 in 1986, see Table 2), it was possible to calculate the percent of total eggs found during both seasons of the study and obtain a very stable approximation of moth response to each nitrogen level (Table 3). To correct for unequal sample size in 1985, the percent of total eggs was calculated by relating the actual number of eggs to a relative sample size of 92 (Table 3). Results of this procedure demonstrated that, in both seasons, approximately 40% of the total eggs were found on plants treated with low levels of nitrogen fertilizer while 60% were found on plants treated with higher levels. Lending support to this analysis is the observation that similar responses were seen during both years of the study for the replicated treatments (0 and 120 lbs. nitrogen applied/ acre). A plot of these percentages and the corresponding quadratic regression line is shown in Figure 9.

In 1986, a correlation analysis utilizing values obtained from foliage analyses and preference data collected from July 7 to July 19, determined that the percent of plants with eggs and percent total eggs were not correlated with mean percent dry weight nitrogen or mean percent soluble nitrates. However, moths did demonstrate higher responses towards plants containing higher percentages of these chemical components (Figures 10 and 11).

Table 3. Total number of hornworm eggs sampled during each year of the preference study. The number of eggs found at each nitrogen level was converted to a percent of the total eggs found during both years. See text for explantion of calculations. N = 357 and 576 total plants sampled in 1985 and 1986 respectively.

YEAR	LBS. N APPLIED PER ACRE	ORIGINAL PLANTS SAMPLED	ORIGINAL NUMBER EGGS	RELATIVE PLANTS SAMPLED	RELATIVE NUMBER EGGS	PERCENT OF TOTAL
1985	0	92	25	92	25.0	20*
	15	89	17	92	17.6	14*
	60	96	39	92	37.4	31*
	120	80	37	92	42.5	35*
TOTAL EGGS 1985			120		122.5	
1986	0	144	22	N/A	N/A	20
	30	144	21			19
	120	144	35			32
	240	144	30			28
TOTAL EGGS 1986			108			

*Computed from Relative Number Eggs.

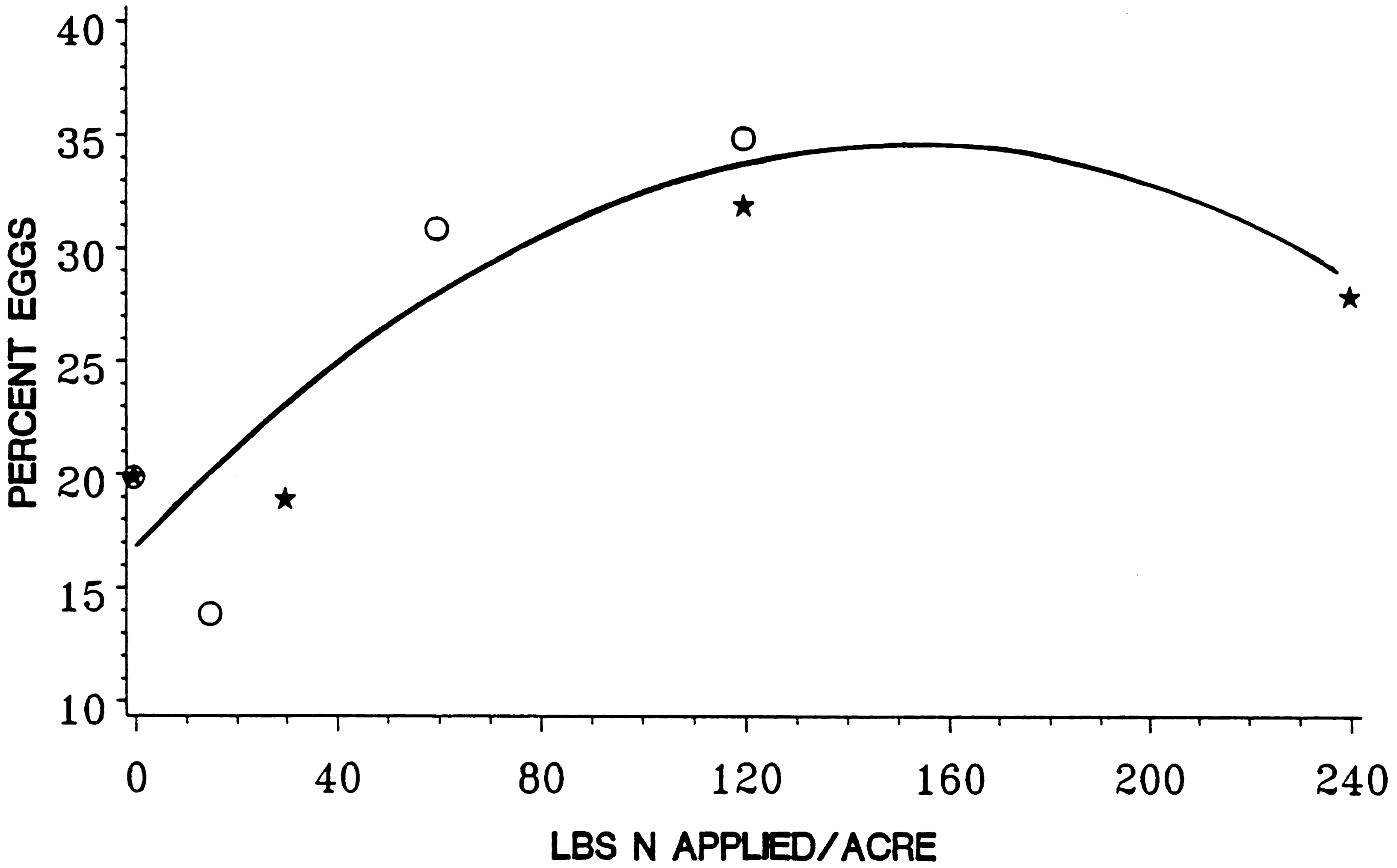


Figure 9. Percent contribution of each nitrogen treatment to total eggs found. Data fit to a quadratic regression, total $R^2 = .78$, $p = .02$. Circles = 1985 data, stars = 1986 data. See text for explanation of statistics.

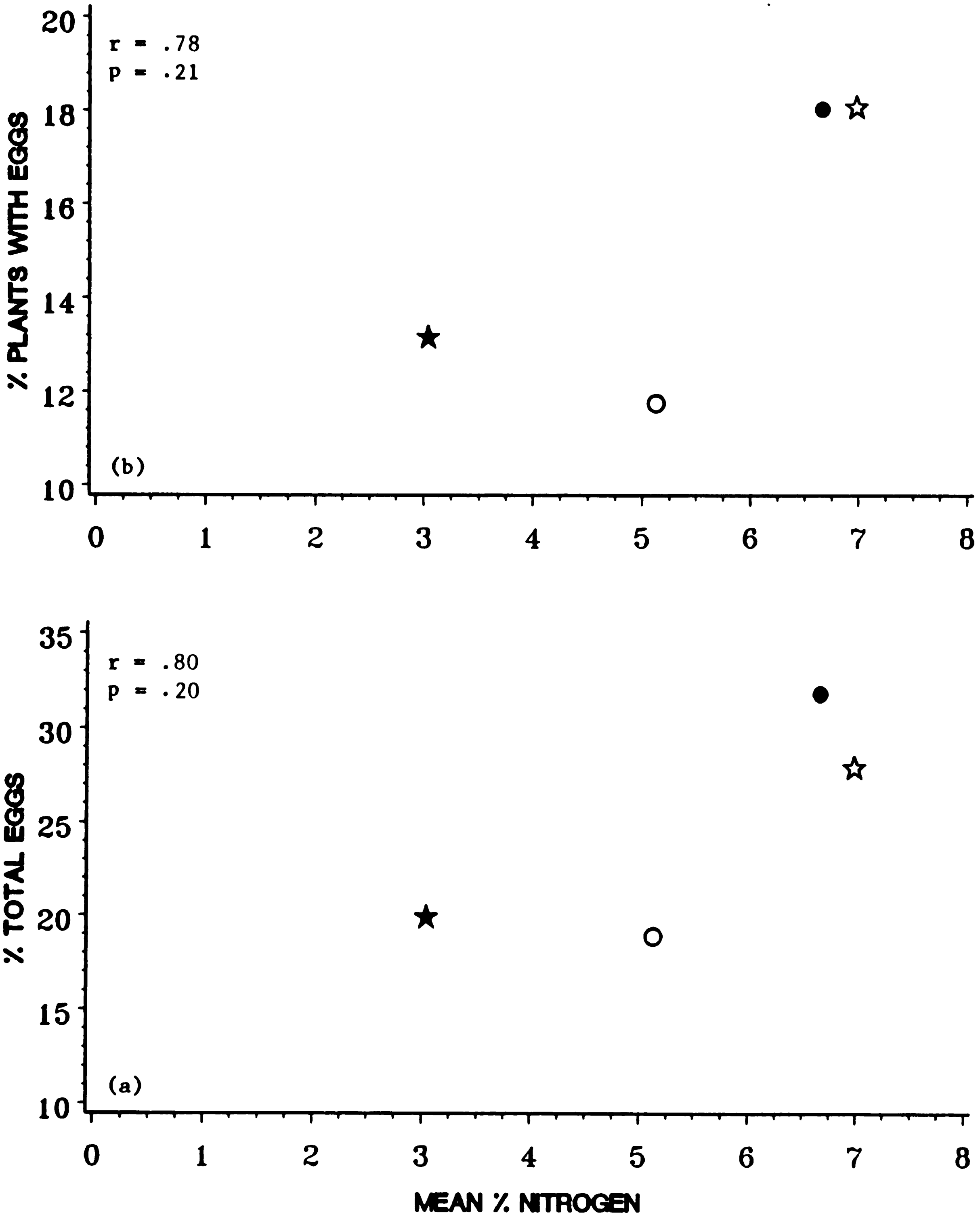


Figure 10. Ovipositional response to treated plants. Correlation of percent nitrogen dry weight with total number of eggs deposited (a) and proportion of plants with eggs (b) at each treatment. Data collected during 1986 only. Total number of eggs deposited = 108. N = 576 total half plants sampled. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

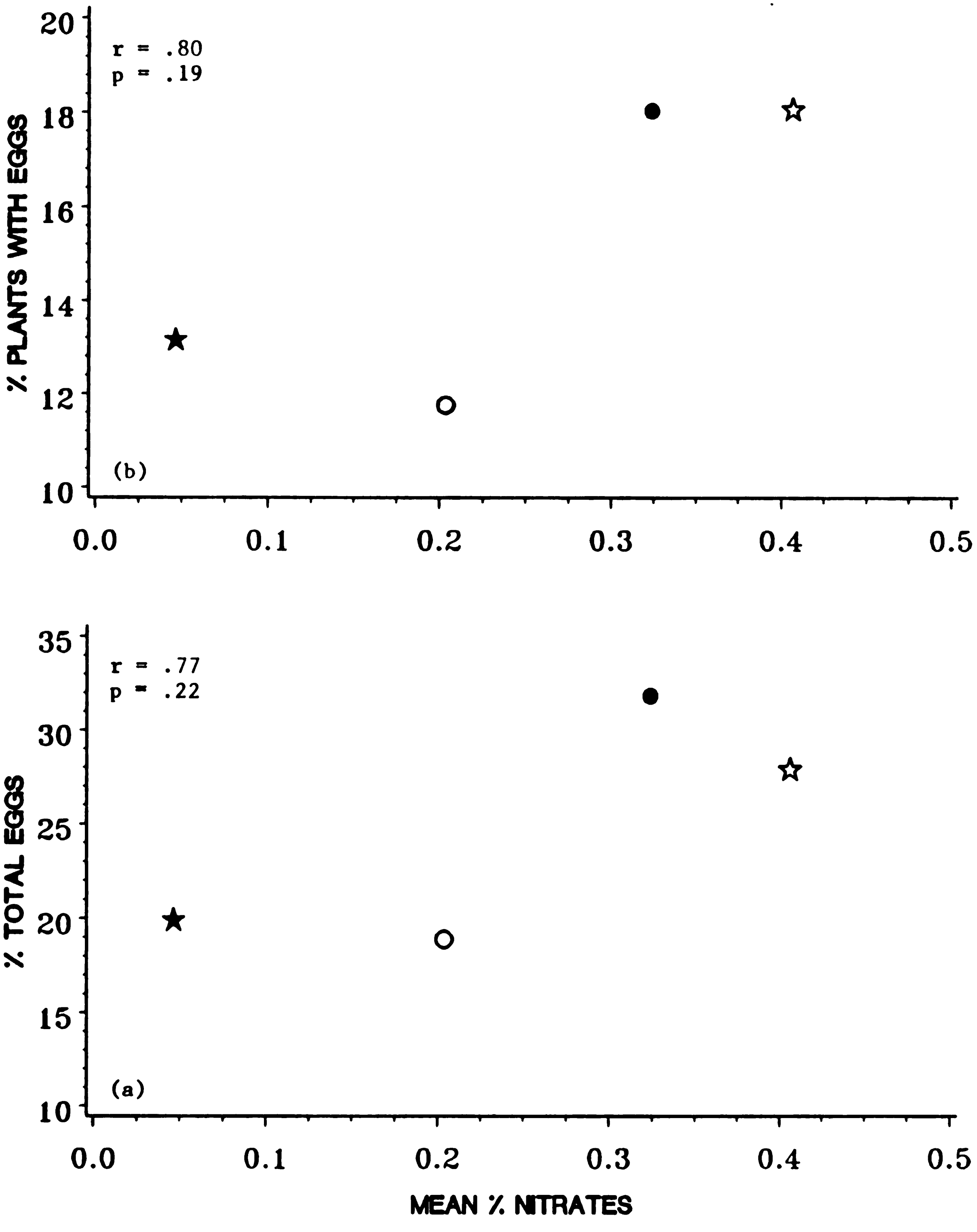


Figure 11. Ovipositional response to treated plants. Correlation of percent soluble nitrates with total number of eggs deposited (a) and proportion of plants with eggs (b) at each treatment. Data collected during 1986 only. Total number of eggs deposited = 108. N = 576 total half plants sampled. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

Larval Fitness

Larval performance was significantly enhanced by nitrogen fertilization. Results of both Cohort I and Cohort II growth studies determined that larvae grew faster and developed into more fit adults when reared on foliage treated at higher rates of fertilization. Table 4 lists the means (where applicable) and standard errors of the means for all measured parameters of fitness for each foliage treatment.

Regression analyses of Cohort I determined that male and female pupal dry weights, successful adult female emergence and female fecundity were significantly influenced by rate of nitrogen fertilization (Table 5). Similar regression analyses of Cohort II demonstrated significant associations between level of fertilization and number of days required for larvae to reach newly molted fifth instar, as well as the percent survival of these larvae (Table 5). Only survival of Cohort I larvae and successful adult male emergence were not linearly correlated with nitrogen treatment, although emergence of males reared on foliage treated with 30 lbs. N per acre was affected by dietary nitrogen--Table 4). Most fitness parameters significantly influenced by nitrogen fertilization were also correlated with mean percent dry weight plant nitrogen and mean percent soluble nitrates (Table 6--mean percent plant nitrogen and soluble nitrates calculated from foliage collected while each separate larval cohort was developing--see Table 1). See

Table 4. Means (± standards errors) for all measured parameters of larval fitness at each level of nitrogen fertilizer applied.

LBS. N Applied	Pupal Dry Weight		% Successful Emergence		Female Fecundity	Days to Fifth Instar	% Larval Survival	
	Male	Female	Male	Female			Cohort I	Cohort II
	(n=9)	(n=9)	(n=19)	(n=19)	(n=10)	(n=23-30)	(n=70)	(n=30)
0	1.06 (.02)	1.17 (.04)	75	74	52.60 (12.75)	11.22 (.48)	70	77
30	1.05 (.02)	1.11 (.03)	53	74	84.00 (20.47)	11.93 (.79)	67	77
120	1.12 .03	1.21 (.03)	80	88	112.10 (14.47)	10.64 (.33)	62	97
240	1.18 (.02)	1.32 (.03)	79	94	111.40 (11.34)	9.98 (.22)	67	100

Table 5. Linear regression statistics of larval fitness study. Numbers depict R^2 value and probability of a greater F-statistic (n.s. = not significant at $p = .05$).

	R^2	p
Pupal Dry Weights:*		
Male	.34	$p < .0002$
Female	.40	$p < .0001$
Adult Emergence:**		
Male	—	n.s.
Female	.97	$p < .02$
Female Fecundity*	.17	$p < .009$
Larval Survival:**		
Cohort I	—	n.s.
Cohort II	.95	$p < .0001$
Days to Fifth*	.18	$p < .0001$

*Regression calculated on individual observations

**Regressions calculated on arcsine transformed percentages

Table 6. Correlation of mean percent dry weight plant nitrogen and soluble nitrates with measured parameters of insect performance. Top row of numbers show Pearson's coefficients of correlation. Numbers in parentheses demonstrate level of significance (n.s. = not significant at p = .05).

	% Successful Emergence		Pupal Dry Weight*		Days to Pre-Fifth*	Female Fecundity**	% Larval Survival	
	Male	Female	Male	Female			Cohort I	Cohort II
% Dry Weight Nitrogen	.49	.96	.57	.47	-.39	.96	-.68	.97
	(n.s.)	(.04)	(.0003)	(.004)	(.0004)	(.03)	(n.s.)	(.03)
% Soluble Nitrates	.64	.99	.99	.95	-.46	.85	-.45	.82
	(n.s.)	(.001)	(.008)	(.05)	(.0001)	(n.s.)	(n.s.)	(n.s.)

*Calculated from individual observations

**Calculated from means

Manuscript II for detailed analysis of the affect of nitrogen fertilization on _M. quinquemaculata_ larval performance.

Host Preference vs. Larval Fitness

Host preference for conspecific plants was significantly correlated with larval performance (Table 7). Pupal dry weights, female fecundity, adult female emergence and percent larval survival of Cohort II were positively associated with moth response, measured as percent of plants with eggs and percent of total eggs deposited at each level of fertilization. Larval periods through pre-fifth instar were negatively associated with moth response. Only percent larval survival of Cohort I was not correlated with moth preference.

Performance Potential (overall larval performance), based on survival values from Cohort I, increased as the percentage of plants with eggs increased, although this relationship was not significantly correlated (Figure 12a). However, Performance Potential based on survival values from Cohort II, did demonstrate a significant correlation with moth response (Figure 12b).

Table 7. Correlation of female preference with larval fitness. Top rows of numbers show Pearson's correlation coefficients. Numbers in parentheses demonstrate level of significance (n.s. = not significant at p = .05.

	% Successful Emergence		Pupal Dry Weight		Female Fecundity	Days to Fifth	% Larval Survival	
	Male	Female	Male	Female			Cohort I	Cohort II
% Total Eggs	.73 (n.s.)	.85 (n.s.)	.51 (.001)	.43 (.009)	.41 (.009)	-.26 (.01)	.81 (n.s.)	.93 (.06)
% Plants With Eggs	.82 (n.s.)	.95 (.05)	.60 (.0001)	.54 (.0006)	.40 (.01)	-.32 (.004)	.62 (n.s.)	.98 (.02)

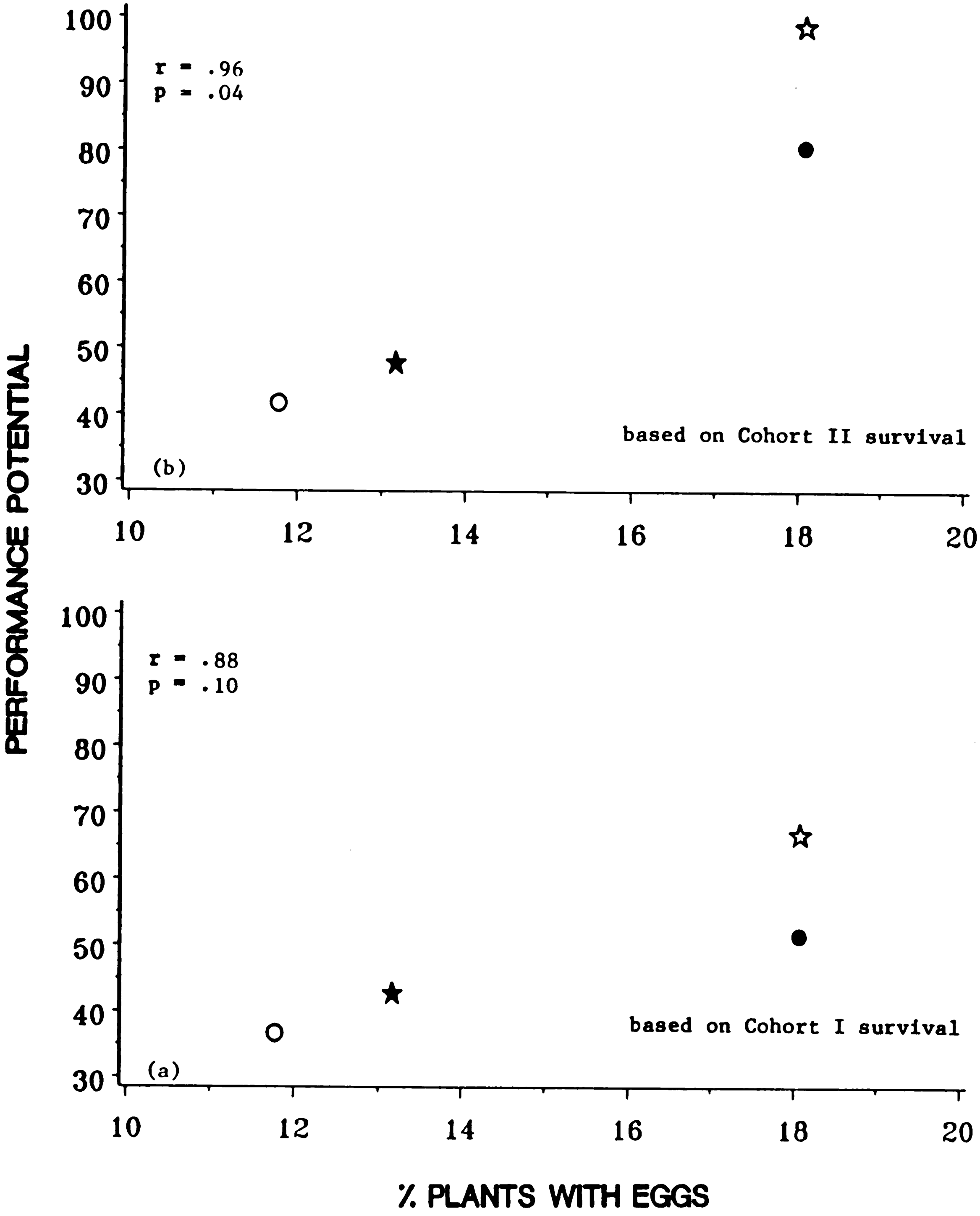


Figure 12. Correlation between percent plants with eggs and larval performance potential. Percent plants with eggs based on 144 plants sampled per treatment. See text for definition of larval performance potential. Closed stars = 0 lbs. N, open circles = 30 lbs. N, closed circles = 120 lbs. N and open stars = 240 lbs. N applied per acre.

DISCUSSION

Host Preference

Results of this research, suggest that Manduca moths show a greater preference for plants that have been treated with higher levels of nitrogen fertilizer. The discriminatory behavior exhibited by the moths was especially apparent in 1985. The proportion of plants bearing at least one egg showed a strong positive association with nitrogen treatment. In addition, sixty-six percent of the total eggs deposited were on plants treated at the two higher fertilization levels. Unfortunately, in 1985 foliage was not collected for chemical analysis. Hence, while we assume that increased rates of fertilization increased concentrations of total nitrogen and soluble nitrates, no conclusions can be drawn concerning the level of moth response to these plant components during this particular year.

A similar response to fertilization treatment was demonstrated in 1986, but of a lower magnitude. Although not significantly correlated, the proportion of plants with eggs and the percent of total eggs were higher on plants treated at higher fertilization levels and on plants containing higher concentrations of total nitrogen and soluble nitrates. Since the percent of total eggs deposited was very similar in both years of the study, i.e., approximately 40% on plants receiving no or low levels of fertilizer versus 60% on plants receiving higher levels (Table 3), it is likely that these responses would have been significant had the total number of eggs deposited, with respect to plants sampled, been as high in 1986 as it was in 1985 (Table 3).

Although fertilization levels were applied in discrete increments, moth response to nitrogen treatment did not appear to increase incrementally. In both years, the percent of total eggs oviposited on plants treated with "low" levels of nitrogen fertilizer was very similar. Likewise, the percent of total eggs oviposited on plants treated with "high" levels of nitrogen fertilizer was very similar (Figure 9; Table 3). Although, this response, at least in 1986, is partially due to the similar concentrations of total plant nitrogen in foliage treated with 120 and 240 lbs. N/acre, these observations also suggest that treated plants only elicited two general levels of response from the moths, i.e., at some "threshold level" physiological changes in the plant, induced by nitrogen fertilization, elicit a higher ovipositional response in the moths; however, below this level, no increased response is observed.

Two results of the study lend additional support to this hypothesis. First, plants treated with 240 lbs. nitrogen/acre and containing the highest concentrations of total nitrogen and soluble nitrates elicited a slightly lower response in moths than plants treated with 120 lbs. nitrogen/acre (Table 3; Figure 9). Although it is impossible to determine if this response is real (since only one data point is available for examination), it seems reasonable to assume that moth response to plant quality will reach some peak and then level off or even decrease thereafter but will not continue to increase incrementally. Secondly, female moths exhibited the lowest response towards plants treated at the low rates of nitrogen fertilization (15 or 30 lbs. nitrogen per acre, depending on the year) not towards untreated plants. This response occurred even though low applications of fertilizer increased levels of both total nitrogen and soluble nitrates in these plants (Figure 3; Figure 4).

Moth preferences demonstrated within this study were generally as predicted although, plants treated with 30 lbs. nitrogen/acre elicited some unexpected responses (discussed in greater detail at a later point). Insect response to nitrogenous compounds has been demonstrated by Howitt and Connor (1965) and Myers and Post (1981) (see also review by Thorsteinson 1960). Unfortunately, since nitrogen interacts with a number of plant components, it is difficult to ascertain which particular plant cues _Manduca_ _spp_. utilize to discriminate among individuals within their host species. It is conceivable that nitrogen fertilization affects other aspects of plant physiology influencing ovipositional responses besides the concentrations of total nitrogen and soluble nitrates.

α-Tomatine, a secondary compound containing nitrogen, is a steroidal glycoalkaloid common to _Lycopersicon_ (tomato). This particular alkaloid is composed of an aglycone and a glycoside (a sugar) and has a high carbon to nitrogen ratio; hence, the availability of either of these minerals will impact synthesis of the alkaloid and subsequently, concentrations found in the plant.

Juvik et. al. (1982) determined that mean α-tomatine concentrations in Lycopersicon esculentum, range from 3.3 mg/g to 16.0 mg/g depending on the particular cultivar analyzed. Although our initial reaction would be to assume that as plant nitrogen content increases, α-tomatine concentrations will also increase, it is probable that increased nitrogen reserves result in decreased glycoalkaloid production. With increased levels of plant nitrogen, carbohydrates and other carbon containing componds are utilized to synthesize nitrogen acceptors, usually aspartic and glutamic acid (Selvendran and Selvendran 1973) thus depeleting the amount of carbon available for glycoalkaloid production. Results of experiments conducted on Solanum glycoalkaloids demonstrated that an increase in plant nitrogen did, in fact, result in a depletion of the carbohydrate pool and a subsequent decrease in glycoalkaloid synthesis (Nowacki et. al. 1975, 1976).

Since glycosidic compounds are thought to stimulate feeding in Manduca larvae (Yamamoto 1960b), concentrations of α-tomatine may also influence host acceptance behavior and egg deposition in the female moths. However, based on the preceeding discussion, it seems reasonable to assume that glycoalkaloid concentrations either did not induce discriminatory behavior in _Manduca_ at the intraspecific level or exerted only a small influence, i.e., the slight decrease in response to plants treated with 240 lbs. nitrogen/acre may have been the result of decreased alkaloid production at this high level of nitrogen.

Leaf water content is another plant component that elicits ovipositional responses in phytophagous insects (Shorey 1964, Sparks 1973), and that is also influenced by nitrogen availablilty. The covariance between this component and plant nitrogen is well documented (Slansky and Feeny 1977, Radin and Parker 1979, Scriber and Slansky 1981, Mattson 1980, Manuwoto and Scriber 1985, Mattson and Haack 1987). Increased levels of plant nitrogen induce lush, succulent foliar growth resulting in plants with a high concentration of leaf water. In contrast, nitrogen deficient plants are chlorotic, stunted, non-succulent and have tough cell walls. Because these two plant components covary, it is difficult to determine which variable actually induces discriminatory behavior in insects. Wolfson (1980) implicated plant water content as the factor controlling ovipositional preferences in _Pieris rapae_. However, in all cases where preferences were significant, nitrogen, as well as water concentrations, were higher for the preferred plants, and in all cases where preferences were not exhibited, nitrogen, as well as water levels, were lower.

Sparks (1973) has already determined that leaf moisture elicits ovipositional responses in M. sexta. Hence, we can hypothesize that higher responses towards plants treated with higher levels of fertilizer may have partially been a result of increased water concentrations and the slight decline in response towards plants treated with 240 lbs. nitrogen/acre a result of the dilution of certain stimulatory compounds caused by increased levels of water. Unfortunately, water content was not analyzed during the host preference study. However, foliage analyzed approximately five weeks later, did not support this hypothesis. Percent water content determined during the larval performance study was negatively correlated with rate of fertilization.

Determining the plant components inducing these behavioral responses is further complicated by the interaction of water and nitrogen on chlorophyll production and subsequent photosynthesis. This interaction may affect host selection in two ways. First, a deficiency of either nitrogen or hydrogen atoms will influence production of chlorophyll, the pigment associated with plant color and hence, plant "greenness". Since recent studies have implicated plant color as a cue utilized by females during host search and acceptance (Miller and Harris 1985, Myers 1985), it is possible that Manduca moths demonstrated higher responses towards plants treated at higher levels of fertilization because of the slightly darker color of these plants.

Secondly, photosynthetic activity influences the production and availability of plant sugars. Generally, decreased photosynthesis, resulting from decreased availability of nitrogen and water, decreases the concentrations of plant sugars (Devlin and Witham 1983). The fact that these compounds are attractive to insects is well documented. With regards to this study, it is reasonable to hypothesize that plants treated at low levels of fertilization or left untreated had inadequate sugar supplies; hence, elicited a lower response from host searching females than plants treated at higher levels of fertilization.

Host Preference vs. Larval Performance

A number of researchers have examined the affect of female host choice on subsequent larval fitness. In some cases, results indicated that certain insect species prefer those plants that produce the most viable offspring. An excellent study by Via (1986) demonstrated a positive genetic covariance between the oviposition preference of *Liriomyzia sativae* and the relative pupal weight of each female's offspring. Studies demonstrating population level associations between host preference and larval performance include those by Chew (1975), Myers and Post (1981), Holdren and Ehrlich (1982), Rausher and Papaj (1983), Williams (1983), Leather (1985), Grossmueller and Lederhouse (1985) and Papaj and Rausher (1987).

Equally well documented though, are results of studies that did not find a correlation between moth response and larval fitness. Courtney (1981) reported that *Anthocharis cardamines* preferred to oviposit on plants that caused the highest larval mortality. Other studies demonstrating these seemingly maladaptive host acceptance behaviors (although, see Singer 1984) include those by Rausher (1979), Renwick and Radke (1982), Mackay (1982, 1985 and Karban and Courtney (1987).

This study indicates that *Manduca* not only exhibits conspecific discriminatory behavior, but that "preferred" plants are also the most suitable for larval growth and development. Ovipositional responses were significantly correlated with most individual measurements of insect fitness (Table 7). In addition, performance potential, an overall measure of insect fitness, was higher on the most preferred plants (Figure 12a,b).

At the beginning of the study, we assumed that untreated plants would be the least preferred and most nutritionally inadequate. However, plants treated with 30 lbs. nitrogen/acre) elicited the lowest response from female moths and provided the least amount of nutritional support for developing larvae. Larvae reared on foliage from these plants required more days to reach fifth instar, had the lowest pupal weights and also the lowest overall fitness. In fact, 50% of the male larvae died during the pupal stage compared to a 20-25% mortality rate for larvae reared on the other three treatments. These results suggest that small increases in nitrogen availability may adversely affect herbivores, probably by creating an imbalance among those plant factors eliciting positive responses and/or providing adequate nutrition and those factors inhibiting oviposition, feeding and/or development. Since the actual behavioral or physiological mechanisms involved in these responses are not known, it would be worthwhile to investigate the chemical differences between untreated foliage and foliage treated with 30 lbs. nitrogen/acre and subsequently, the affect of these differences on insect performance and host selection.

Overall larval performance appeared to be affected by nitrogen fertilization in a manner similar to that demonstrated by ovipositing moths, i.e., tomato plants left untreated or treated at lower fertilization rates induce one level of response while plants treated at higher fertilization rates elicit a greater level of response. Although certain measured parameters of fitness in this study were higher or lower for particular treatments, larval performance appears to show a similar "two level" response. Larvae reared on foliage treated with high levels of fertilizer show a high survival rate and develop into heavier pupae and more fecund females. Conversely, larvae reared on untreated foliage or foliage treated with 30 lbs. nitrogen/ acre show a lower survival rate and develop into smaller pupae and less fecund adults.

Since both host seeking female moths and developing larvae exhibit similar responses to plant nitrogen content, it appears that similar plant components influence both the behavior and the fitness of these phytophages, thus implying a genetic basis for the correlations demonstrated in this study. Females that "select" and then deposit eggs on plants supporting the highest larval fitness will increase their chances of making a genetic contribution to the next generation by increasing the probability that their offspring will complete devlopment and emerge as viable, fecund adults. In addition, the genetic "program" that enabled these females to distinquish between "suitable" and "unsuitable" hosts will be conferred onto their offspring, thus producing a new generation of individuals with similar behavioral mechanisms for host discrimination.

However, a second explanation for the responses documented during this study is equally viable. It is possible that adult females become "conditioned", thru prior experience, to select plants comprised of physical and chemical components similar to those contained in their larval host plants. This phenomenon, commonly reffered to as Hopkins Host Selection Principle (Hopkins 1917) has recently been demonstrated by Jaenike (1983). In fact, the affect of conditioning on <u>Manduca</u> has already been demonstrated. Yamamoto and Fraenkel (1960a) determined that <u>M. sexta</u> moths preferred to oviposit on the host plant species fed on during the larval stage. With regards to this study, an abundance of nitrogen rich host plants, either other tomato plant varieties or other host species, occurring near the study sites may have preadapted <u>Manduca</u> moths to oviposit on those plants containing higher concentrations of nitrogen.

Unfortunately, the responses demonstrated in this study do not provide the information needed to eliminate one or the other of these two hypotheses. While the results are interesting, further experimentation will be required to test these theories. However, regardless of the complexity or simplicity of this system and of the mechanisms involved, it is inherently interesting that Manduca females preferentially oviposit on plants producing individuals with the highest overall fitness. Both the ecological and economical implications of these findings are important. Hopefully, future studies will investigate, in detail, the factors in both the plant and the insect, that elicit these responses and the physiological and behavioral basis of these interactions.

Conclusions

Manduca spp., specialist herbivores, appear to discriminate among conspecific plants. Plants treated at higher rates of nitrogen fertilization and containing higher concentrations of total nitrogen and soluble nitrates elicited the highest responses from female moths. Unfortunately, because of the complexity of interactions that occur among plant minerals, water, secondary compounds and metabolism, it is difficult to delineate the particular factors that induce discriminatory behavior in these moths.

Furthermore, plants most preferred by ovipositing females also produced individuals with the greatest overall fitness. Larvae reared on foliage from "preferred" plants grew faster and developed into larger, more fecund adults.

REFERENCES

Barbosa P, Saunders JA, Waldvogel M (1982) Plant mediated variation in herbivore suitability and parasitoid fitness. Proc. Fifth Int. Symp. Insect-Plant Relationships. PUDOC, Wageningen.

Beck SD (1965) Resistance of plants to insects. Ann. Rev. Entomol. 10:207-232.

Beck SD (1974) Theoretical aspects of host plant specificity in insects. In: Proc. Summer Institute on Biological Control of Plant Insects and Diseases. (Maxwell FG, Harris FA, eds.). Univ. Press Miss., Jackson, Miss.

Bernays EA (1982) The insect on the plant-a closer look. Proc. Fifth Int. Symp. Insect-Plant Relationships, PUDOC, Wageningen.

Borth PW, Harrison FP (1984) Within-field distribution patterns of tobacco and tomato hornworm (Lepidoptera: Sphingidae) egg and larval populations in southern Maryland tobacco. 13:859-862.

Chew FS, Robbins RK (1984) Egg-laying in butterflies. In: The Biology of Butterflies (Vane-Wright RI, Ackery PR, eds.). Academic Press.

Chew FS (1975) Coevolution of Pierid butterflies and their cruciferous foodplants. I. The relative quality of available resources. Oecologia. 20:117-127.

Courtney SP (1981) Coevolution of Pierid butterflies and their cruciferous foodplants. III. Anthocharis cardamines (L.) survival, development and oviposition on different host plants. Oecologia. 51:91-96.

Dethier VG (1947) Chemical Insect Attractants and Repellents. Blakiston. Philadelphia.

Grossmueller DW, Lederhouse RC (1985) Oviposition site selection: an aid to rapid growth and development in the tiger swallowtail butterfly, Papilio glaucus. Oecologia. 66:68-73.

Gulmon SL, Shu CC (1981) The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry mater allocation in the chaparral shrub, Diplacus aurantiacus. Ocologia. 49:207-212.

Gunn A, Gatehouse AG (1985) Effects of the availability of food and water on reproduction in the African armyworm, *Spodoptera exempta*. Physiological Entomology. 10:53-63.

Holdren CE, Ehrlich PR (1982) Ecological determinants of food plant choice in the Checkerspot butterfly *Euphydryas editha* in Colorado. Oecologia. 52:417-423.

Hough JA, Pimentel D (1978) Influence of host foliage on development, survival and fecundity of the Gypsy Moth. Env. Ent. 7:97-102.

House HL (1965) Effects of low levels of the nutrient content of a food and of nutrient imbalance on the feeding and the nutrition of a phytophagous larva, *Celerio euphobiae* (Linnaeus) (Lepidoptera: Sphingidae). Can. Ent. 97:62-68.

Howwitt AJ, Connor LJ (1965) The response of *Rhagoletis pomonella* (Walsh) adults and other insects to trap boards baited with protein hydrolysate bait. Proc. Entomol. Soc. Ontario. 95:134-136.

Ives PM (1978) How discriminating are cabbage butterflies? Australian J. of Ecology. 3:261-276.

Jaenike J (1983) Induction of host preference in *Drosophila melanogaster*. Oecologia. 53:320-325.

Juvik JA, Stevens MA, Rick CM (1982) Survey of the genus *Lycopersicon* for variability in α-tomatine content. Hort. Sci. 17:764-766.

Karban R, Courtney S (1987) Intraspecific host plant choice:lack of consequences for *Streptanthus tortuosus* (Cruciferae) and *Euchloe hyantis* (Lepidoptera:Pieridae). Oikos. 48:243-248.

Laughlin R (1965) Capacity for increase: a useful population statistic. J. Animal Ecology. 34:77-91.

Leather SR (1985) Oviposition preferences in relation to larval growth rates and survival in the pine beauty moth, *Panolis flammea*. Ecol. Ent. 10:218-217.

Lindroth RL, Batzli GO, Avildsen SI (1986) *Lespedeza* phenolics and *Penstemon* alkaloids: Effects on digestion efficiencies and growth of voles. Journal of Chemical Ecology. 12:713-728.

Mackay DA (1985) Conspecific host discrimination by ovipositing *Euphydryas editha* butterflies:its nature and its consequences for offspring survivorship. Res. Popul. Ecol. 27:87-98.

Mackay DA, Singer MC (1982) The basis of an apparent preference for isolated host plants by ovipositing *Euptychia libye* butterflies. Ecol. Ent. 7:299-303.

Manuwoto S, Scriber JM (1985) Consumption and utilization of experimentally altered corn by southern armyworm: iron, nitrogen and cyclic hydroxamates. J. of Chem. Eco. 11:1469-1483.

Mattson WJ (1980) Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11:119-161.

Mattson WJ, Haack RA (1987) The role of drought in outbreaks of plant-eating insects. BioScience. 37:110-118.

McNeill S, Prestidge RA (1982) Plant nutritional strategies and insect herbivore community dynamics. Proc. Fifth Int. Symp. Insect-Plant Relationships. PUDOC, Wageningen.

McNeill S, Southwood TRE (1978) The role of nitrogen in the development of insect/plant relationships. In:Biochemical aspects of plant and animal coevolution. (Harborne JB, ed.). Academic Press. London.

Miller JR, Harris MO (1985) Viewing behavior-modifying chemicals in the context of behavior: lessons form the onion fly. In:Chemical Ecology of Insects. (Bell WJ, Carde RT, eds.). Sinauer Associates, Sunderland, MA.

Mitchell BK, Harrison GD (1985) Effects of *Solanum* glycoalkaloids on chemosensilla inthe colorado potato beetle. A mechanism of feeding deterrence? J. Chem. Eco. 11:73-83.

Mitchell ND (1977) Differential host selection by *Pieris brassicae* (The large white butterfly) on *Brassica oleracea* subsp. *Oleracea* (The wild cabbage). Ent. exp. & appl. 22:208-219.

Myers JH, Post BJ (1981) Plant nitrogen and fluctuations of insect populations:a test with the cinnabarmoth-tansy ragwort system. Oecologia. 48:151-156.

Myers JH (1985) Effect of physiological condition of the host plant on the ovipositional choice of the cabbage white butterfly, Pieris rapae. Jour. of Animal Eco. 54:193-204.

Natr L (1972) Influence of mineral nutrients on photsynthesis of higher plants. Photosynthetica. 6:80-99.

Nowacki M, Jurzysta M, Gorski P (1975) Effect of availability of nitrogen on alkaloid synthesis in Solanaceae. Bull. Acad. Pol. Sc. Ser. Sc. Biol. 23:219-225.

Nowacki E, Jurzqsta M, Gorski P, Nowacka D, Waller GR (1976) Effect of nitrogen nutrition on alkaloid metabolism in plants. Biochem. Physiol. Pflanzen. 169:231-240.

Papaj DR, Rausher MD (1987) Components of conspecific host discrimination behavior in the butterfly Battus philenor. Ecology. 68:245-253.

Prestidge RA, McNeill S (1982) Instar duration, adult consumption, oviposition and nitrogen utilization efficiencies of leafhoppers feeding on different quality food. Ecological Entomol. 7:91-101.

Prestidge RA, McNeill S (1983) The role of nitrogen in the ecology of grassland Auchenorryncha. In: Nitrogen as an Ecological Factor. (Lee JA, McNeill S, Rorrison HI, eds.). Symp. British Ecological Soc. Blackwell Scientific Publications. London.

Radin JW, Parker LL (1979) Water relations of cotton plants under nitrogen deficiency. I. Dependence upon leaf structure. Plant Physiol. 64:495-498.

Rausher MD, Papaj DR (1983) Demographic consequences of discrimination among conspecific host plants by Battus philenor butterflies. Ecology. 64: 1402-1410.

Rausher MD (1979) Larval habitat suitability and oviposition preference in three related butterflies. Ecology. 60:503-511.

Reese JC (1978) Chronic effects of plant allelochemics on insect nutritional physiology. Ent. exp. & appl. 24:425-431.

Reese JC (1983) Nutrient-allelochemical interactions in hostplant resistance. In: Plant Resistance to Insects (Hedin PA, ed.). ACS Symposium Series, No. 208. American Chemical Society. Washington, DC.

Renwick JAA (1983) Non-preference mechanisms: plant characteristics influencing insect behavior. In:Plant Resistance to Insects. (Hedin PA, ed.). ACS Symposium Series, No. 208. American Chemical Society, Washington, DC.

Renwick JAA, Radke CD (1982) Ovipositional choice and larval survival of the Spruce Budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae). Env. Ent. 11:503-505.

Rhoades DF, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. Rec. Adv. Phytochem. 10:168-213.

Robinson T (1979) The evolutionary ecology of alkaloids. In:Herbivores, Their Interactions with Secondary Metabolites. (Rosenthal GA, Janzen DH, Applebaum, SW, eds.). Academic Press. New York.

Selvendran RR, Selvendran S (1973) Chemical changes in young tea plant (Camellia sinensis L.) tissues following application of fertilizer nitrogen. Ann. Bot. 453-461.

Scriber JM (1978) The effects of larval feeding specialization and plant growth form on the consumption and utilization of plant biomass and nitrogen: an ecological consideration. Ent. exp. & appl. 24:494-510.

Scriber JM, Slansky F (1981) The nutritional ecology of immature insects. Ann. Rev. Entomol. 26:183-211.

Shimishi D (1970) The effect of nitrogen supply on some indices of plant-water relations of beans (Phaseolus vulgaris L.) New Phytol. 69:418-424.

Shorey HH (1964) The biology of Trichoplusia ni (Lepidoptera:Noctuidae). III. Response to the oviposition substrate. 57:165-170.

Singer MC (1986) The definition and measurement of oviposition preference in plant-feeding insects. In: Insect-Plant Interactions. (Miller JR and Miller TA, eds.). Springer-Verlag. New York.

Singer MC (1984) Butterfly-hostplant relationships: host quality, adult choice and larval success. In: The Biology of Butterflies. (Vane-Wright RI, Ackery PR, eds.). Academic Press.

Slansky F (1982) Toward a nutritional ecology of insects. Proc. Fifth Int. Symp. Insect-Plant Relationship. PUDOC, Wageningen.

Slansky F, Feeny P (1977) Stabilization of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47:209-228.

Slansky F, Scriber JM (1985) Food consumption and utilization. In: Comprehensive Insect Physiology Biochemistry and Pharmacology (Kerkut GA, Gilbert LI, eds.). Pergamon Press.

Solkal RR, Rohlf FJ (1981) Biometry: The principles and practice of statistics in biological research. Freeman. San Fransico.

Sparks MR (1973) Physical and Chemical stimuli affecting oviposition preference of Manduca sexta (Lepidoptera: Sphingidae). Ann. Ent. Soc. Am. 66:571-73.

Taylor MFJ (1984) The dependence of development and fecundity of Samea multiplicalis on early larval nitrogen intake. Insect Physiol. 30:779-785.

Thorsteinson AJ (1960) Host selection in phytophagous insects. Ann. Rev. Ent. 5:193-218.

Tichenor LH, Seigler DS (1980) Electroantennogram and oviposition responses of Manduca sexta to volatile components of tobacco and tomato. J. Insect Physiol. 26:309-314.

Treshow M (1970) Environment and Plant Response. McGraw-Hill.

Via S (1986) Genetic covariance between oviposition preference and larval performance in an insect herbivore. Evolution. 40:778-785.

Williams KS (1983) The coevolution of Euphydryas chalcedona butterflies and their larval host plants. III. Oviposition behavior and host plant quality. Oecologia. 56:336-340.

Wolfson JL (1978) Environmentally induced variation in Brassica nigra (Koch): affects on generalists and specialist herbivores. Ph.D. dissertation. State Univ. of New York. Stony Brook.

Wolfson JL (1980) Oviposition response of Pieris rapae to environmentally induced variation in Brassica nigra. Ent. exp. & appl. 27:223-232.

Yamamoto RT, Fraenkel GS (1960a) The specificity of tobacco hornworm, Protoparce sexta, to solanaceous plants. Ann. Ent. Soc. Amer. 53:503-507.

Yamamoto RT, Fraenkel GS (1960c) The physiological basis for the selection of plants for egg-laying in the tobacco hornworm, *Protoparce sexta* (Johan.) XI Int. Kongr. ent. Wien. 3:127-133.

POSTCRIPT

As this research project progressed, it became apparent that few investigators had examined conspecific host selection. This is unfortunate. Based on the findings presented in this thesis, it seems obvious that intraspecific discriminatory behaviors are as important and have as many implications as interspecific ones. Results demonstrated that nitrogen fertilization of field grown tomato plants induced physiological changes in the plants which, subsequently, influenced conspecific host discrimination and larval performance in Manduca spp. Plants treated at higher fertilization rates elicited a greater response in gravid females and produced larvae with a greater overall fitness. Results further indicated that plants most preferred by females also conferred the highest fitness on larvae reared on these plants. Future studies should focus on describing these behaviors and elucidating the components involved. This study, alone, generated a number of interesting questions.

The observation that plants treated at low levels of nitrogen fertilizer elicited the lowest response in moths and supported the lowest larval performance is of particular interest. It appears that small amounts of fertilizer induced a biochemical reaction in the plant that repelled the moths and inhibited feeding and digestion in the larva. Unfortunately, because of the number of plant components that are affected by nitrogen availability and the complexity of their interactions, it is impossible to conclude which factors, or combination thereof, elicited these responses.

The finding that female moths prefer to oviposit on those plants conferring the highest fitness on their offspring presents a whole area of research that remains to be described. Observations on individuals within a family, instead of the population level approach utilized in this study, is a more appropriate method for determining the physiological basis of these responses. Experiments with full sibs could provide basic information regarding the genetic components that induce these behaviors. Simple experiments could determine, and possibly rule out, the existence of host plant conditioning fairly quickly. If the behavioral response has a genetic basis, then similar, but more detailed backcrossing studies could be utilized to examine the gene complexes involved.

The ecological implications of these findings are important. Moths selecting plants with a higher nutritional content will produce offspring that complete development earlier, suffer lower mortality and develop into more fecund adults. As a result the female will increase her genetic contribution to the next generation. For ecologists and evolutionary biologists interested in examining these plant/herbivore interactions, this system provides a method for investigating the components of nautral selection and other related processes in phytophagous insects.

The economical considerations are also significant. The development of cultural programs that consider the affect of nitrogen fertilization on insect behavior and performance may enable the grower to balance the economical advantages of increased yield with the potential disadvantages of increased pest populations. Furthermore, researchers investigating plant resistance may be able to develop a program that confers resistance on crop plants by manipulating the nutritional quality of those plants. The results of this study suggest that low levels of nitrotgen fertilizer may actually repel insects. Hence, it would be interesting to determine if other insect populations demonstrate similar responses. At the very least, a system that examines insect abundance in relation to host quality could provide important information on the plant factors that induce resistance in certain crop plants.