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LA THÈSE A ÉTÉ
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If the insects ever take over this world, let us hope they remember all the picnics we shared with them.

Anonymous

This thesis is dedicated to my wife, Ann and my daughter, Sara.

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ABSTRACT

The tobacco hornworm, Manduca sexta, was reared under laboratory conditions. Development on seven different meridic diets, reproductive performance of 5 ratios of females to males, and development of the parental and filial generations after exposure to sublethal doses of fenitrothion were studied.

A wheat germ based diet with dried tobacco leaves added and a high water content best suited the nutritional requirements of the tobacco hornworm.

A ratio of 3 females/3 males displayed the greatest reproductive potential. Virgin females began oviposition later and lived longer than mated females.

Larvae exposed to sublethal concentration of fenitrothion ranging from 0.001 ppm to 1.0 ppm required more time to complete development, had larger body weights, and incurred more mortalities than the control group. The reproductive potential of all treated groups was greater than the control.

Treated F_1 generation larva weighed less than the control insects prior to the pupal stage. Treated larvae also had difficulty moulting, had more mortalities than the control, and had insects entering the pupal stage at body weights less than 5 g. The reproductive potential of the 0.001 ppm and 0.01 ppm groups was less

than the control whereas that of the 0.1 ppm and 1.0 ppm groups was greater. Interference with the endocrine system is implicated.

RESUME

Le sphynx du tabac, Manduca sexta, a été élevé en laboratoire. On a étudié son développement sur huit différentes diètes méridiques, sa performance reproductive femelles:males dans un rapport de 5:1, et le développement de ses générations filiales et paternelles après une exposition à des doses sous-létales de fénitrothion.

Une diète à base de germe de blé additionné de feuille de tabac et avec une teneur en eau plus élevée était la plus adéquate pour remplir les exigences nutritives du sphinx du tabac.

Un rapport de 3:3 de femelles:males a démontré le potentiel de reproduction le plus élevé. Les femelles non-accouplées ont commencé l'oviposition plus tard et on vécu plus longtemps que les femelles accouplées.

Les larves qui ont été exposées à des concentrations sous-létales de fénitrothion (de 0.001 ppm à 1.0 ppm) ont requis plus de temps pour compléter leur développement, ont atteint un poids plus élevé, et ont subi un taux de mortalité plus élevé que le groupe étalon. Le potentiel de reproduction de tous les groupes qui ont été traités était plus élevé que celui du groupe étalon.

Le poids des larves de la génération F_1 était inférieur à celui des insectes étalons avant le stade de pupe. Les larves qui ont été traitées ont aussi éprouvé plus de difficulté à muer, ont subi un

taux de mortalité plus élevé que les insectes étalons, et certains d'entre eux ont commencé le stade de pupe à un poids inférieur à 5 g. Le potentiel de reproduction des groupes traités à 0.001 ppm et à 0.01 ppm était inférieur à celui du groupe étalon tandis que celui des groupes traités à 0.1 ppm et à 1.0 ppm était supérieur. Une interférence avec le système endocrinien est suggérée.

INTRODUCTION

1. *Manduca sexta*:

The tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae), is a multivoltine leaf feeder that grows rapidly up to 10 g in the larval stage and has been studied since the beginning of the tobacco industry (Reinecke *et al*, 1980). It inhabits South and Central America, Mexico, the West Indies, the United States, and Southern Ontario and Quebec (Madden and Chamberlin, 1945). This species and a closely related species, the tomato hornworm, *Manduca quinquemaculata* (L.), are serious pests of commercial tobacco plants in the United States, southern Ontario, and Quebec (Cheng, personal communication; Hoffman *et al*, 1966). The primary food sources of the tobacco hornworm are members of the solanaceous family. Although the tobacco plant (*Nicotiana tabacum*) is its principal host, tobacco hornworm larvae also eat the leaves of a wide range of other solanaceous plants including jimson weed, ground cherry, tomato, eggplant, Jerusalem cherry, potato and pepper (Brinkley *et al*, 1975).

The tobacco hornworm has become a choice experimental animal because of its large size and rapid rate of development (Bell and Joachim, 1976). Many field studies and

physiological and endocrine studies have contributed to the extensive accumulation of knowledge about the tobacco hornworm (Bell et al, 1975; Bollenbacher et al, 1975; Borg and Marks, 1973; Kramer et al, 1976; Madden and Chamberlin, 1945; Peterson, 1912; Reinecke et al, 1978, 1980; Sandburg et al, 1975; Truman, 1972). In addition, there are many descriptions on how to rear the tobacco hornworm under field conditions (Hoffman and Lawson, 1964; Madden and Chamberlin, 1945) and under laboratory conditions (Bell and Joachim, 1976; Baumhover et al, 1977; Hoffman et al, 1966; Waldbauer et al, 1964; Yamamoto, 1968, 1969).

The life cycle of the tobacco hornworm lasts 60-70 days (Bell and Joachim, 1976). Eggs are pale green, about 2 mm in diameter, and are laid on the undersurface of leaves. They hatch three to six days after oviposition.

The larvae undergo four moults (five instars) during their development. Newly emerged first instar larvae weigh about 1.5 mg (Reinecke et al, 1980). Most larvae consume the contents of their egg cases immediately after hatching. First instar larvae have silk glands that produce a strand which they can use to descend to a food source. Second, third, and fourth instar larvae frequently ingest parts of the ecdysed cuticle in order to recover

some of the salts present in the cuticle (Philogène, unpublished). This often occurs shortly after ecdysis and prior to the resumption of feeding on the diet. Bell and Joachim (1976) estimated that tobacco hornworm larvae ingested about 35 g of artificial diet during their development. The bulk of this dietary consumption occurred during the fourth and fifth instars. Fifth instar larvae can weigh up to 10 g (Bell and Joachim, 1976). Midway through the feeding stage, they excrete fecal pellets coated with uric acid which gives it a chalky white appearance (Buckner et al, 1980). Nijhout and Williams (1974) termed the phenomenon "frosted frass".

The larval stage lasts about 30 days. Nijhout (1975) and Nijhout and Williams (1975) concluded that a critical weight of 5 g and a head capsule width of 5.1 mm were necessary to initiate the prepupal stage. Once these critical dimensions were reached, an unidentified process was initiated which required 24 hours to be completed. The brain is then rendered competent to release PTTH (prothoracicotropic hormone). In other words, during the fifth instar, the presence of juvenile hormone (JH) inhibits the secretion of PTTH. Once the larva reaches the weight of 5 g or a head capsule width of 5.1 mm, the corpus allatum ceases to secrete JH. The JH is cleared from the haemolymph in about 24 hours. The brain begins to release PTTH. The actual release of PTTH is gated by the

photoperiod. After the larva has passed the critical weight and head capsule width, the gate opens during the very next photophase (Nijhout, 1975).

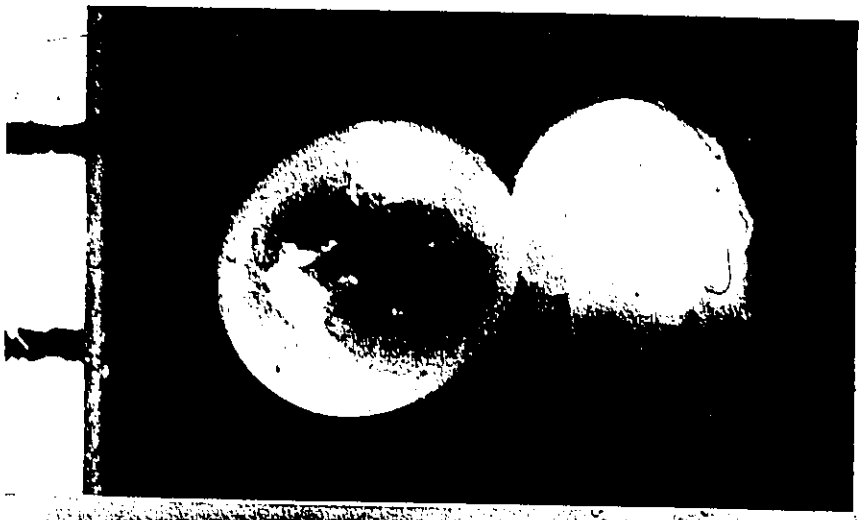
Shortly after the larvae surpass 5.0 g, feeding abruptly stops. This is the first step of the complex larval-pupal transition. Shortly after the cessation of feeding the aorta becomes visible along the dorsal midline near the horn (Brinkley et al, 1975). Body wetting, which follows the exposure of the aorta, was the first reported by Baumhover et al, (1977). This stage is characterized by a sharp turning of the head so that all dorsal and lateral body surfaces are coated with an oral secretion. Immediately after body wetting the wandering stage begins (Truman and Riddiford, 1974) and the prepupae begin to burrow about 12 hours later. The insect will burrow just under the surface for several hours before reaching a final depth of 6-20 cm. A horizontal pupation chamber is formed from frass, food and soil. Once in the pupation chamber, the hornworm purges its gut. The clear, viscous fluid discharge from the anus is responsible for about a 40% loss in body weight (Nijhout and Williams, 1974). The discharge is used to increase the humidity within the pupation chamber (Reinecke et al, 1980). The insect then remains stationary for about two days and larval-pupal ecdysis follows.

The newly formed pupa is green because the cuticle is initially transparent. The proboscis forms from the tip of the midthorax and is stretched from the head as the insect straightens out from a head down posture to full length (Reinecke et al, 1980). As development progresses the cuticle gradually becomes tanned and sclerotized. This process moves from the posterior to anterior end and deepens in colour as the insect ages (Brinkley et al, 1975). Just before emergence, the cuticle is very dark and soft. The weight of the pupa decreases as it ages. A fully formed pupa weighs about 4 g but, prior to emergence this has decreased to about 2.5 g. Non-diapause pupae require approximately 25 days to complete development whereas diapause pupae require about 100 days (Bell et al, 1975).

Adults emerge in the pupation chamber, defecate, and work their way to the surface. They will climb up onto a vertical surface, begin dorso-lateral revolutions of the wings to a "butterfly" position, and begin to inflate them (Reinecke et al, 1980). Once inflated the wings are folded in the characteristics tent fashion. Adults will use their wings about 3-5 hours after eclosion (Reinecke et al, 1980). These nocturnal sphinx moths (Hoffman et al, 1966) are large animals having a wing span of about 12 cm and weighing approximately 2 g. Almost all activities, i.e. feeding and

Plate 1. Stage of the life cycle of the tobacco hornworm: a: eggs; b: first and second instar larvae; c: fifth instar larvae; d: prepupa; e: pupa; f: adults mating, g: a closeup of the females ovipositor (top) and the males intermitent organ (bottom).

a



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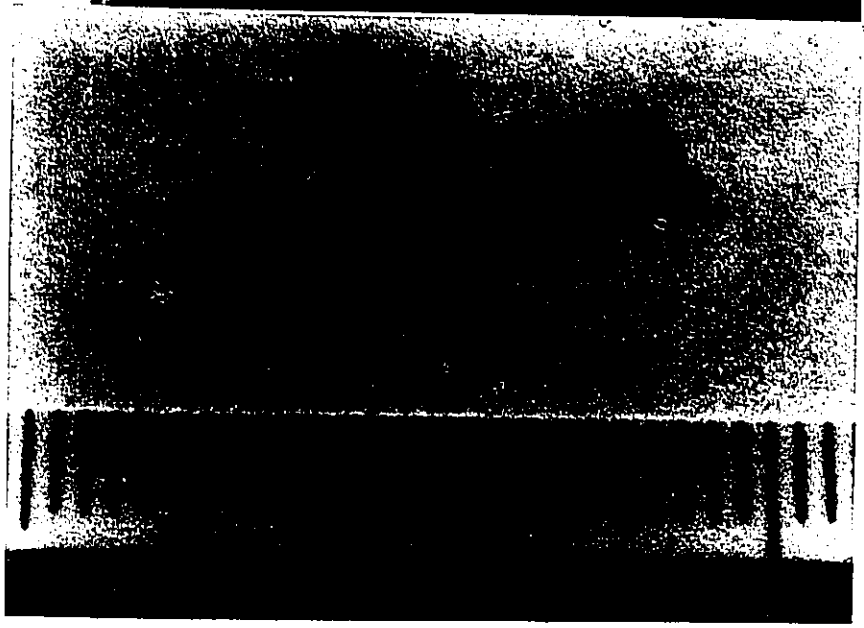

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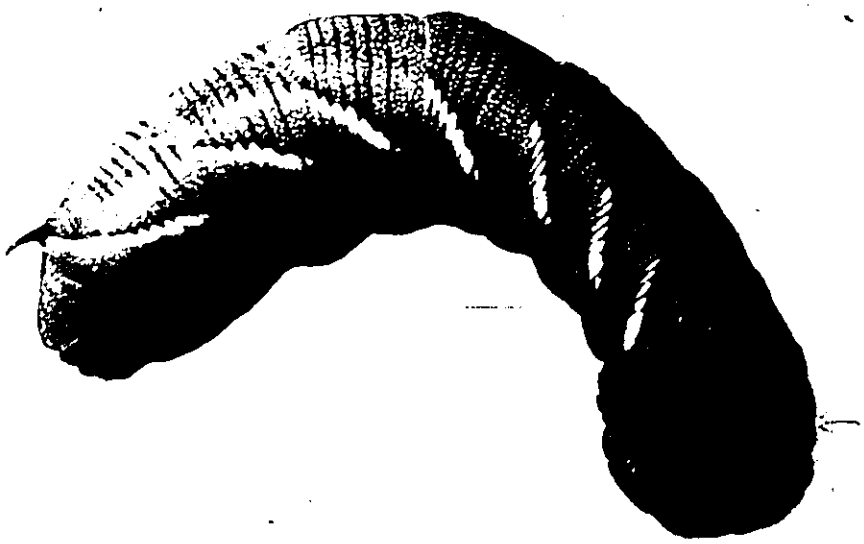
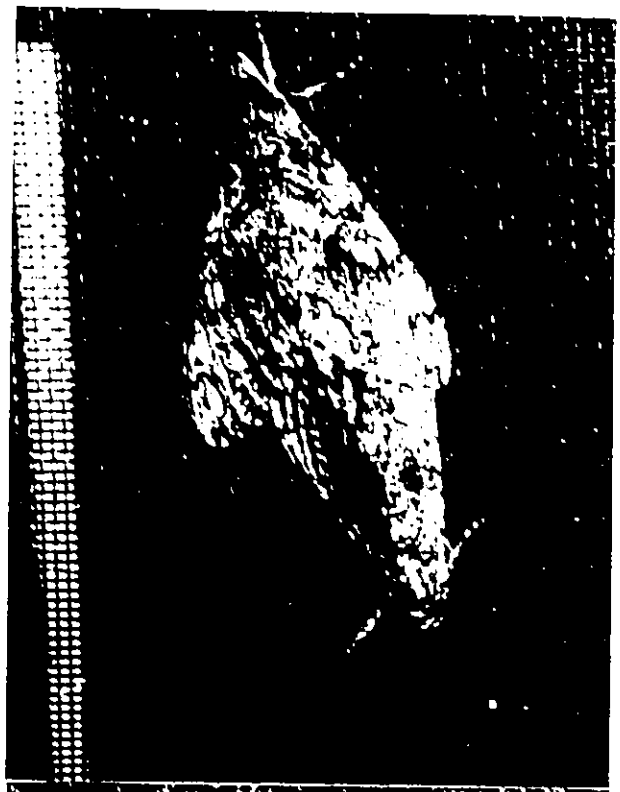


d

MANDUCA SEXTA

e

f



g

oviposition, occur during flight (Hoffman et al, 1966). Feeding, oviposition, and other activities occur in brief flurries during dusk and dawn. Mating usually occurs between 21:00 and 02:00 hours (Allen et al, 1955). Prior to mating the female extends her ovipositor and releases a pheromone which attracts and orients the male. Copulation usually takes place on a vertical surface and lasts from one to three hours (Hoffman et al, 1966). During the period the insects face in opposite directions, their abdomens touching, and the female positioned with her wings overlapping the male. Oviposition usually begins on the third night after emergence and reaches a maximum on the fourth or fifth night (Hoffman et al, 1966). Adults are host specific and, therefore, require a host plant, usually tobacco or tomato as an oviposition site. Females usually lay one egg per leaf.

2. Artificial Diets:

All insects have similar qualitative dietary needs (Dadd, 1973; Fraenkel, 1959). The basic nutrient requirements of insects are: carbohydrates, proteins, sterols, lipogenic factors, polyunsaturated fatty acids, fat soluble vitamins, minerals, B vitamins, ascorbic acid, and water (Dadd, 1973; Hodgson et al, 1977; Navon, 1978; Ouye and Vanderzant, 1964;

Singh, 1977; Vanderzant, 1974).

Excess or inadequacies of nutrients may result in abnormal growth and development (House 1965). A deficiency of ascorbic acid may cause ecdysial failure, larval mortality, reduced adult yield, and deformed insects (Chippendale and Beck, 1965; Dadd, 1960; Navon, 1978; Rock, 1967; Vanderzant et al, 1962). Navon (1978) attributed ecdysial failure to an inhibition of melanization and sclerotization.

The feeding behavior of many phytophagous insects is characterized by the rigid specificity of their diet (Jermy et al, 1968). Many insects, being highly selective in food preference, choose only a single species of plant or family of plants, or feed exclusively on the leaves, fruits or stems (Vanderzant, 1966). Bottger (1942) was the first to successfully rear an insect on an artificial diet. Since then, such diets have been extensively used to rear a variety of insects (House, 1967; Singh, 1977; Smith, 1966; Vanderzant, 1974). A suitable artificial diet should satisfy all the nutritional requirements of the insect and permit ad libitum ingestion of food (Dougherty, 1959; Friend, 1958).

Artificial diets can be classified as holidic, meridic, or oligidic (Vanderzant, 1974). Holidic diets are used

primarily for nutritional studies. The chemical formulae of its constituents are known (Dougherty, 1959). The purity, preparation, and mixing of the holidic diet are usually described in great detail (Gordon, 1959).

Diets containing one or more unrefined substances from plants, animals, or microorganisms such as plant tissue, liver extracts, and yeast products are called meridic diets. Most diets used for rearing laboratory colonies of insects fall into this group. Successful rearing of many colonies of phytophagous insects can be directly attributed to the addition of wheat germ to the diet (Chippendale, 1972; Vanderzant, 1966, 1974).

Oligidic diets consist of those made up of crude plant nutrients. These diets either imitate the insect's natural food or are composed of substances of high nutrient content. They are economical and can be used for mass rearing. Few diets that use unrefined or natural plant products have proven successful (Vanderzant, 1974).

A synthetic diet containing all the necessary ingredients for growth and development is of little value if the insect will not feed on it. Therefore, physical and chemical stimulants must be added to the diet (Vanderzant, 1966). Physical properties such as texture, hardness, palatability, and shape

of the diet affects its ingestion (Vanderzant, 1969; Vanderzant and Davich, 1961; Wardojo, 1967). Cellulose, agar, and other non-nutritive inert materials, which act as carriers for nutrient substances or to thicken the diet, influence the physical properties of artificial diets (Vanderzant, 1966). Chemical stimulants are often provided by the nutrients in the diet. However, some insects require the addition of secondary plant chemicals (Devitt, 1978; Hoffman et al, 1966). Sugars are probably the most important feeding stimulants for phytophagous insects (Beck, 1965, a,b,c).

The ultimate test for a nutritionally adequate diet is to monitor its success in rearing many generations of insects (Vanderzant, 1966). Vanderzant et al (1962) demonstrated that ascorbic acid could be passed from anthonomus grandis adults to the egg. The egg is an important source of trace nutrients which can be carried over from one generation to the next.

There are some problems associated with artificial diets. Insects reared continuously on synthetic diets may demonstrate reduced vigor, fecundity, fertility, longevity, and may lose their ability to survive on

their natural host plant (Lukefar and Martin, 1965; Rathore et al, 1976; Vali et al, 1967). Genetic variation through inbreeding may cause these changes rather than the inadequacy of the diet (Hinks and Byers, 1976; McDonald, 1976).

3. Adult Sex Ratios and Reproductive Performance:

The reproductive potential of adult insects is dependent on the ratio of females to males (sex ratio). The dependency is related to the reproductive physiology and the behavior of the species (Jones et al, 1979). Knowledge of the optimum sex ratio is important in order to maintain an efficient laboratory colony. This knowledge furthers our understanding of the basic biology of the organism and can be incorporated into mass rearing and sterile male release programs (Prostavko and Boreydo, 1971).

All species have the genetic capacity to produce a 1/1 ratio of adults. Sex ratios can be altered by environmental factors, nutrition, and the age of the adults (Brunson, 1934; and Prostovko, 1971). For instance, in nature, the sex ratio of the carpenterworm moth (Prionoxystus robiniae) is 3F/2M (Solomon, 1976).

Sex ratios in laboratory colonies tend to vary (Brinton et al, 1979: Prostavko and Boreyko, 1971).

Overcrowding of the mating cage may physically limit the ability of the insect to mate and oviposit (Bell and Joachim, 1976). Overcrowding may also induce stress related impairment of the normal reproductive behaviour of the insect population (Guerra et al, 1972). Field reared M. sexta females lay a greater number of eggs per individual than do laboratory reared insects (Yamamoto, 1968). The optimum mating space for the corn earworm (Heliolhis zea) and the tobacco budworm (H. virescens) is 1 and 3 pairs of adults per 4 litres respectively (Burton, 1969; Guerra et al, 1972; Jones et al 1975).

High F/M ratios promotes a large egg production with a reduction in spermatophore transfer, and, ultimately, fertility (Brower, 1975; Burton, 1969; Guerra et al, 1972: Jones et al, 1975; Otake and Sakuratami, 1972). Mated females have a greater oviposition rate than do virgin females (Truman and Riddiford, 1974). Unmated females develop the same number of ripe eggs as mated females but only lay about one third of their egg complement (Wigglesworth, 1966).

Wigglesworth postulated that copulation provides the stimulus for egg deposition. Low F/M ratios can result in a high egg production, mating efficiency, and fertility but female longevity is reduced (Jones et al, 1979).

4. Fenitrothion:

Fenitrothion (0,0-dimethyl-0-4-nitro-m-tolyl phosphorthioate) is a broad spectrum organophorphorus insecticide used throughout the world to control agricultural and forest pests, as well as for malaria vector control (NRCC, 1975; Payne et al, 1977). Fenitrothion is registered in Canada for use on apples, woodlands and forests (Agriculture Canada, 1981, unpublished information). The major use of fentrothion in Canada is in large scale forest protection programs for the control of spruce budworm outbreaks in mature or overmature stands of spruce or balsam fir (Prebble, 1975). From 1965 to 1973, New Brunswick, Quebec, Ontario, and Manitoba sprayed 84 million hectares of forest with 3.7 million kg of fenitrothion for spruce budworm control (Prebble, 1975).

Despite its success as an insecticide, fenitrothion does have some adverse environmental effects. Fenitrothion is relatively safe for birds and generally safe for fish, although slight mortality of fish-food organisms has been reported (Tucker and Crabtree, 1970). Yasutomi (1977) and Georghiou (1973) have suggested the development of resistance in Anopheles and Musca species to fenitrothion.

The environmental impact of fenitrothion on target and non-target organisms has been extensively investigated. Areas of studies include the effects of fenitrothion on small mammals (Buckner et al, 1977), birds (Kulczyki, 1977; Moulding, 1976; Pearce and Peakall, 1977), foliage (Moody et al, 1976; Prasad and Moody, 1976; Yule, 1974; Yule and Duffy, 1972), lake ecosystems (Kingsbury, 1977), amphibians (Lyons et al, 1976), fish (Kanazawa, 1977; Peterson, 1975), benthic arthropods (Eidt, 1975), target arthropods (McLeod, 1976; Morris, 1972), non-target arthropods excluding pollinators (Leonhard, 1976) and pollinators (Kevan, 1975; Plowright, 1977; Wood, 1977).

The mode of action of an organophosphous insecticide, like that of a carbamate insecticide, is to inhibit the enzyme acetylcholinesterase (EC 3.1.1.7) which hydrolyzes the synaptic transmittor, acetylcholine, in cholinergic neurons (Corbett, 1974). It should be noted that

neuromuscular junctions of insects unlike those of vertebrates are innervated with neurons which release glutamate rather than acetylcholine (O'Brien, 1978). In insects, cholinergic neurons are restricted to the central nervous system (O'Brien, 1978).

The symptoms of organophosphous pesticide poisoning in insects include restlessness, hyperexcitability, tremors, convulsions, paralysis, and finally death. Although Mengle and Casida (1958) demonstrated that death, in flies, occurred at maximum cholinesterase activity inhibition, the ultimate cause of death in an insect is difficult to prove (Matsumura, 1975).

Most commercially significant pesticides operate by disrupting the passage of nervous impulses in an organism. Since it is the basis of a co-ordination system an animal cannot do without, even for short periods of time, the nervous system of an insect is a suitable target for an insecticide. However, it is unfortunate that the basic molecular events in the nervous systems of all animals, including mammals, tend to be rather similar, so that selective toxicity between animal species is not inherent to the site of action but, rather, must depend on vagaries of uptake, metabolism, and compartmentalization (Corbett, 1974).

5. The Sublethal Effects of Insecticides:

A sublethal dose may be defined as the concentration of a pesticide which does not cause the immediate mortality of an organism but may adversely affect it at some later date.

Insects exposed to sublethal doses of insecticides display a wide range of responses. Generally, growth retardation results when larvae are treated with low doses of insecticides. Pieris brassicae exposed to lindane showed an inhibition of haemolymph carboxyesterase activity, a decrease in the amount of food consumed, but an increase in the digestability of food and the efficiency of converting food into tissue biomass (Truman, 1972, 1975, 1977). Tanton and Khan (1978a) suggested that the growth rate reduction observed in Parapsis atomaria Ol. was due to poor food utilization and an increase in excretion and not the result of food intake inhibition. Poor food utilization could be due to the disintegration of the midgut epithelium proceded by exfoliation of the epithelium from the subadjacent connective membrane. Muscular contraction causes separation of the intestinal epithelium, and the walls of the midgut cells become histologically indistinct

(Pilot, 1935; Tanton and Khan, 1978c; Woke, 1940). Concurrent with the breakdown of the intestinal wall is the dehydration of the insect. Copious amounts of fluids are lost through the mouth and anus of treated insects. This fluid is not replaced by feeding and, consequently, must be drawn from the haemolymphh (Benz, 1963).

Other effects of sublethal doses of pesticides in larvae include a depletion of glycogen, carbohydrate, fat reserves as well as qualitative changes in the protein and liquid content (Brown, 1963; Wongkobrat and Dahlman, 1976). The fat body is involved with pesticide metabolism and reduces the incidence of mortality through detoxification mechanisms (Dawterman and Hodgson, 1978). However, since the fat reserves are consumed after treatment, insects are more susceptible to an additional exposures of pesticides (Bennett and Thomas, 1963; Fast, 1964; Moore et al, 1967; Nakatsugawa and Dahm, 1962). Sublethal doses of organophosphate insecticides also are known to inhibit juvenile hormone esterase activity (Pratt, 1975).

Pupal deformities and mortalities can occur in insects that have been exposed to low concentrations of

insecticides (Bond et al, 1969; Bond and Upitis, 1973; Gough, 1939; Loschiavo, 1960).

Larvae exposed to sublethal doses of insecticides metamorphose into adults that generally show a decrease in fecundity, fertility, and longevity (Boles, 1974; De Coursey and Webster, 1952; Grosch, 1975; Riviere, 1974; Zettler and Le Cato, 1974). Grosch (1975) found a decrease in the number of eggs developing from vitellogenic oocytes. There was also an increase in the resorption of mature ova and the largest oocytes to adjust to the nutritional deprivation resulting from a low pesticide dose. Honek and Novak (1978) reported that Pynhocoris apterus females treated with Intration-50 displayed a faster rate of ovariole maturation and oviposition which was possibly due to a shorter adult life span. Other sublethal effects of pesticides in adults include mating impairment (Zettler and Le Cato, 1974), chemosterilent effects (Abo-elGhar et al, 1972; Flint et al, 1973; Moriarty, 1968; Walker, 1970), adult deformities (Moriarty, 1968; Sherman and Sanchez, 1964), and an increased reproductive potential (Hodjat, 1971; Honek and Novak, 1978).

An increase in the development time of a generation (Hodjat, 1971; Tanton and Khan, 1978b) and behavioural abnormalities in Dysdercus fasciatus (Hodjat, 1971) and in bees (Schricker and Stephen, 1970; Stephen and Schricker, 1970)

have been attributed to sublethal doses of pesticides. The effects of low doses of insecticides on subsequent generations of insects has received limited attention.

It should be recognized that introducting a xenobiotic like a pesticide into the insect body is a form of stress. In addition, there are probably many other stressful agents associated with the day to day maintenance of a laboratory colony of insects. It is often not possible to separate the response to the pesticide stress from the other forms of stress. Excessive stress whether it is physical or chemical can lead to abnormal behaviour and even the death of the insect (Beament, 1958; De Coursey and Webster, 1952; Heslop and Ray, 1959; Roan and Hopkins, 1961; Sternburg, 1963; Sternburg et al, 1959). Hodjat (1971) stated that unrelated environmental stress either insecticidial or non-specific produce analagous effects in insects by the release of pharmocologically active substances which may have profound physiological and pathological consequences when they are present in larger than normal quantities.

6. Objectives:

The objectives of this study were:

(1) To develop a meridic diet which would best promote the growth and development of the tobacco hornworm;

(2) To determine the effects of different adult sex ratios on the reproductive biology of the tobacco hornworm; and

(3) To evaluate the effects of oral, sublethal doses of fenitrothion on the development of parental and filial generations of Manduca sexta.

MATERIALS AND METHODS

1.0 General Rearing Conditions:

Tobacco hornworm eggs were collected every one or two days from the undersurface of the leaves of potted tobacco plants, Nicotiana tabacum, variety Delhi-77. The eggs were placed in plastic Petri dishes (100 mm dia. x 15 mm ht.) which had lids lined with filter paper which were moistened daily to increase the internal relative humidity. Insects, reared under non-diapause conditions, were put in an incubator at 25°C ± 1°C with an 18/6 L/D photoperiod using 20W fluorescent bulbs (Westinghouse Cool White), and at 45% - 55% relative humidity.

Larvae which had emerged within a 24-hour period were placed, in groups of ten, onto pieces of synthetic diet and transferred to glass, foam-stoppered vials (37 mm dia. x 117 mm ht.). The foam plug prevented the larvae from escaping and provided enough ventilation so that the condensation build-up, observed by Bell and Joachim (1976) did not occur. When laid on their sides, the glass vials were large enough to accomodate all larval instars of the hornworm. Larvae were handled with broad, pliable forceps to minimize damage to the insect, especially in the first, second and third instars. Forceps and working area were frequently disinfected with Hinks-Byers Solution (Hinks and Byers, 1976) to reduce the incidence of

microbial contamination. Larvae were transferred to individual containers after 6 - 8 days. It was necessary to isolate the larvae because they can be cannibalistic and display aggressive behaviour when reared in groups. In individual vials, the development of each hornworm can be monitored from the first instar till just prior to adult eclosion. It is difficult to accept Bell and Joachim's (1976) statement that some of the advantages of rearing M. sexta larvae individually were the insurance of uniform rates of development and a decrease in the risk of microbial contamination. It would seem more likely that the uniform rates of development and risk of microbial contamination are more a function of quality and quantity of food available to each insect, and the sanitary conditions employed in maintaining the laboratory colony rather than the isolation of each larvae.

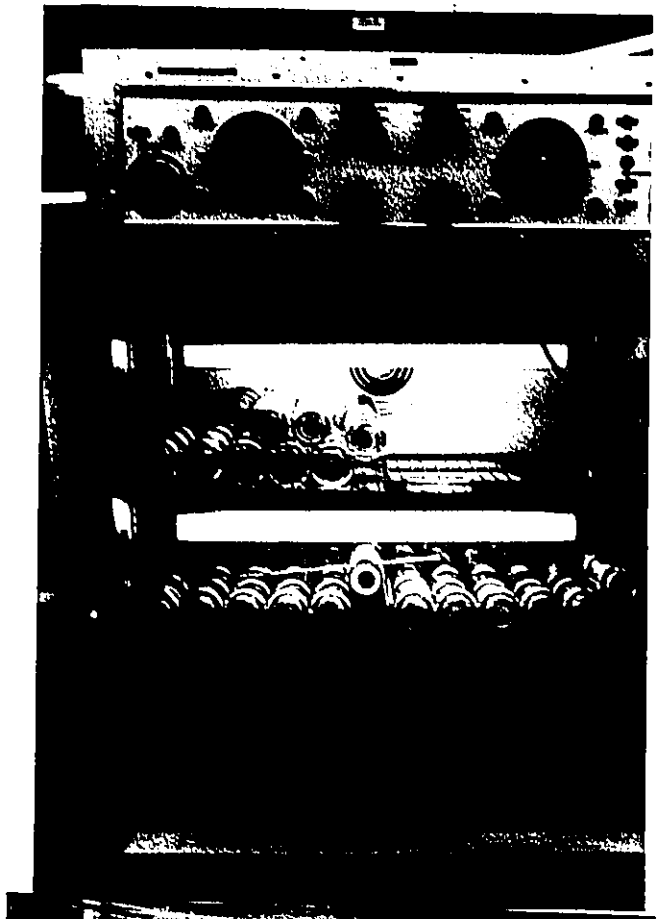
Every two days, unused diet and frass were removed from the vials and the larvae given a fresh piece of diet. The weight of the diet cube ranged from about 0.5 g up to 9.0 g for the last two instars. The diet was cut in to cubes to minimize the amount of water loss. If any mould or other microbial contamination was observed on the unused diet, frass or larva, the insect was transferred to a new container otherwise the larvae were reared in the same container until they reached the prepupal or wandering stage.

Hornworms in the prepupal stage were placed in upright glass vials approximately half filled with vermiculite. The containers were stoppered with foam plugs, placed in groups of 24 in cardboard trays, and kept in the same incubator used to rear the larvae. Since M. sexta prepupae require darkness to complete the pupation process (Bell and Joachim, 1976), the cardboard trays were covered with a black rubber sheet. It was later discovered that laying the vials on their sides decreased the incidence of pupal deformities to a negligible level. The trays were checked at two day intervals for completely tanned and sclerotized pupae. Fully formed pupae were transferred to a 0.357 m^3 (0.63 m l. x 0.63 m wt. x 0.9 m ht.) screened mating cage and allowed to develop to the adult stage.

Adults were maintained under an 18/6 L/D photoperiod, at 23°C, and about 40% - 55% humidity. Day lighting was provided by a 100W incandescent bulb. A dimmed 25W bulb simulated moonlight. The mating cage was equipped with a potted tobacco plant and a feeding station consisting of three milk shake containers taped end to end to form a tower. A scintillation vial with an absorbent cotton wick was filled with a 20% sucrose solution and fastened to the top of the tower. The feeding station which was generally at a greater height than the top of the tobacco plant, was placed in the corner of the cage near the low light source (see Plate 2). Although the

Plate 11. Rearing conditions. a: incubator contaning vials used to rear larvae. Pupae were kept under the black sheet, b: the adult's mating cage. Note the tobacco plant and feeding station on the right.

a



b

internal volume of the cage was sufficient to permit free flight, flying adults would occasionally strike the sides and roof of the cage resulting in slight wing damage.

At two day intervals eggs were collected, the sucrose solution was replaced, and discarded pupal cases and dead adults were removed from the cages. Empty cages were washed with Hinks-Byers solution and allowed to air dry for one day.

1.1 Constitution and Preparation of the Synthetic Diet:

This colony of M. sexta larvae was reared on an artificial diet which was slightly modified from the preparations given by Bell and Joachim (1976) and Yamamoto (1969). The ingredients are listed in Table 1. All of the dry components, except the agar, were mixed in a blender to ensure uniform distribution of nutrients, then stored in a refrigerator (11°C) until needed. It was advantageous to premix four to eight-fold quantities of the dry ingredients at one time. Thus, only one weighing of 131.02 g of dry ingredients was required when the diet was prepared.

About one liter of diet was made from this recipe. The agar was suspended in 400 ml of distilled water and heated to 90°C in a double boiler. An open flame can be used to heat the agar. Although quicker, it was found that the agar tended

Table 1. The constitution of the wheat germ based meridic diet used to rear laboratory colonies of tobacco hornworm larvae.

Ingredients	Quantity
Premix (g)	
Wheat germ	50.00
Casein	22.50
Sucrose	20.00
Torula yeast	15.00
Dried tobacco leaves	10.00
Wesson's salt mixture	7.50
Ascorbic acid	2.00
Sorbic acid	0.75
Methyl-p-hydroxy benzoate	0.50
Cholesterol	0.25
Vitamins (mg)	
Nicotinic acid	5.00
Calcium pantothenate	5.00
Riboflavin	2.50
Thiamine HCI	1.65
Pyridoxine HCI	1.65
Folic acid	0.10
Biotin	0.10
Others	
Agar (g)	10.00
Formalin (ml)	10.00
Raw linseed oil (ml)	2.00
Distilled water (ml)	750.00
Total Weight (g)	900.50

to burn at the bottom of the container and that the Erlenmeyer flask would often crack when exposed to direct heat. Some authors have used an autoclave to melt the agar (Waldbauer et al, 1964). The dry ingredients, linseed oil, formaldehyde and remaining water were poured into the blender and mixed at a low pulsating speed while the agar was heating. Once the agar had reached 90°C, it was added to the other ingredients, thoroughly blended at a high speed for six minutes to a uniform consistency then quickly emptied into plastic containers and allowed to cool to room temperature. The containers were later sealed with cellophane and stored in the refrigerator. The diet was brought to room temperature before being used. Bell and Joachim (1976) argued that chilling and subsequent warming of the diet adversely affected its quality and antimicrobial properties. The quantity of diet made was usually consumed in four to seven days. The antimicrobial capabilities of the diet did not seem to be affected in this time span.

2.0 Diet Experiments:

Fifty newly eclosed larvae were established on one of seven meridic diets in large 125 ml plastic medicine vials (50 mm dia. x 90 mm ht.). The plastic lids were perforated with small pin holes to increase the ventilation. These containers were not as satisfactory as the glass vials.

Condensation from the diet would collect around young larvae causing many to drown or contract microbial infections. Also, the smaller sized container may have confined the larvae decreasing the maximum larval body weight. The ingredients of the meridic diets are listed in Table 2. Survival during the first four days was measured. The thirty healthiest looking larvae were allowed to develop on all diets except group C. In that case, the healthiest 25 larvae were chosen.

Larval body weight, head capsule width, amount of diet consumed per gram insect, development time and mortality were recorded. All measurements were made at two day intervals unless otherwise specified. The larval head capsule width was useful in determining the duration of each stadium, maximum body weight prior to moulting and instar mortality.

The amount of diet consumed per gram insect (ADCPGI) was calculated by taking the difference of the weight of the diet given to the insect and the weight of the diet remaining after two days. This difference was then divided by the weight of the insect on the day the second diet reading was taken. It should be noted that a cube of diet will lose a certain percentage of its weight through water loss even if it is left untouched for two days in the rearing vial. This water loss is dependent on the surface area to volume ratio.

Table 2. The constitution of the seven meridic diets used to rear Manduca sexta larvae. C: wheat germ not ground; F: wheat germ ground to a fine texture; Tf: wheat germ ground to a fine texture, dried tobacco leaves added; Tfi: wheat germ ground to a fine texture, dried tobacco leaves added, a significant increase in water content; Tfiv: wheat germ ground to a fine texture, dried tobacco leaves added, a significant increase in water content, Vanderzant's vitamin mixture added; Bs: BioServe diet; and Bst: BioServe diet, dried tobacco leaves added.

Ingredients	C	F	Tf	Tfi	Tfiv	Bs	Bst
Premix (g)							
Wheat germ, coarse	50.0	-	-	-	-	-	-
Wheat germ, fine	-	50.0	50.0	50.0	50.0	28.1	28.1
Casein	22.5	22.5	22.5	22.5	22.5	25.7	25.7
Sucrose	20.0	20.0	20.0	20.0	20.0	-	-
Fructose	-	-	-	-	-	9.5	9.5
Dextrose	-	-	-	-	-	9.5	9.5
Torula yeast	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Wesson's salt mix	7.5	7.5	7.5	7.5	7.5	10.3	10.3
Sodium propionate	-	-	-	-	-	1.2	1.2
Locust bean gum	-	-	-	-	-	4.2	4.2
Cellulose fibre	-	-	-	-	-	30.7	30.7
Ascorbic acid	2.0	2.0	2.0	2.0	2.6	6.3	6.3
Sorbic acid	0.8	0.8	0.8	0.8	0.8	1.2	1.2
Methyl-p-hydroxy benzoate	0.5	0.5	0.5	0.5	0.5	1.0	1.0
Cholesterol	0.3	0.3	0.3	0.3	0.3	0.7	0.7
Choline dihydrogen citrate	-	-	-	-	-	0.2	0.2
i-Inositol	-	-	-	-	-	0.2	0.2
Vitamins (mg)							
Niacinomide	-	-	-	-	2.0	4.0	4.0
Nictotinic acid	5.0	5.0	5.0	5.0	-	-	-
Calcium pantothenate	5.0	5.0	5.0	5.0	2.0	4.0	4.0
Riboflavin	2.5	2.5	2.5	2.5	1.0	2.0	2.0
Thiamine HCl	1.6	1.6	1.6	1.6	0.5	1.0	1.0
Pyridoxine HCl	1.6	1.6	1.6	1.6	0.5	1.0	1.0
Folic acid	0.1	0.1	0.1	0.1	0.5	1.0	1.0
Biotin	0.1	0.1	0.1	0.1	0.1	0.1	0.1
-Tocopherol	-	-	-	-	15.9	31.7	31.7
Choline chloride	-	-	-	-	49.1	198.1	198.1
i-Inositol	-	-	-	-	39.7	79.3	79.3
B_{12} (0.1%)	-	-	-	-	3.6	7.2	7.2
Others							
Agar	10.0	10.0	10.0	10.0	10.0	19.8n	19.8
Formalin (ml)	10.0	10.0	10.0	10.0	10.0	-	-
Aureomycin (g)	-	-	-	-	-	2.2	2.2
Raw linseed oil (ml)	2.0	2.0	2.0	2.0	2.0	-	-
Coconut oil (g)	-	-	-	-	-	1.1	1.1
Dried tobacco lvs. (g)	-	-	12.5	12.5	12.5	-	12.5
Distilled water (ml)	367.5	367.5	367.5	750.0	750.0	819.0	819.0
Total Weight (g)	507.8	507.8	520.3	902.8	903.5	1001.3	1026.3

Therefore, as the size of the diet cube decreases, the relative amount of water loss through evaporation increases. Weight loss curves for different size diet cubes were constructed and used to help correct the error in the ADCPGI calculation (Fig. 1, Appendix A). The ADCPGI values, particularly in the early stages of larval development, would have been much higher without this correction factor.

The pupal characteristics studied included the percentage of larvae pupating, body weight, number of days till tanned, development time, sex ratio, deformities and mortality. Pupae developing from the prepupal stage till completely tanned are very sensitive. Their body weights had to be measured indirectly by weighing the rearing vial containing the developing pupa and subtracting the weight of the rearing vial. Once the pupa was fully developed, it was placed in an open Petri dish. Thus, each pupa could be observed until the emergence of the adult.

The adult characteristics studied were percentage of pupae surviving to the adult stage, adult development time, sex ratio, the number of eggs laid per female per two days (oviposition rate), the number of eggs produced per female per two days (fecundity rate), proportion of the total egg production retained by females, and the fertility of the eggs laid. The abdomens of females who had died during a two day

period were dissected in Insect Ringer's Solution and the ripe eggs counted. This quantity was combined with the number of eggs laid per female per two days to give an estimate of fecundity. Fifty eggs from the adults of each group were used to determine fertility (% hatch). The difference between the date an insect hatched and the date the resulting adult died gave the total development time.

3.0 Reproduction Experiments:

The effect of the adult sex ratio (females (F) to males (M)) on reproductive performance was determined. Pupae from the colony were sexed and separated. Five groups of six adult F/M combinations ranging from 1F/5M to 5F/1M were put into 0.18m^3 (0.63 m lt. x 0.63 m wt. x 0.45 m ht.) mating cages. The oviposition rate, fecundity rate, the proportion of the total egg production retained by females, and fertility were recorded and used to construct standard curves.

4.0 Toxicology Experiments:

Five samples of 30 newly emerged larvae were reared on diet Tfi under non-diapause conditions. Nine days old larvae were starved for 24-hours then given an oral sublethal dose of 0.001, 0.01, 0.1 and 1.0 ppm fenitrothion. Technical grade fenitrothion was dissolved in 99% ethanol and dispensed

in a volume of 10µl onto a small (0.05 - 0.1 g) piece of diet. Two days after the dose the larvae were given an excess amount of food and allowed to develop to the adult stage. Larval, pupal and adult characteristics recorded for the diet experiments were also noted for this generation and the F_1 generation.

5.0 Statistical Analysis of Results:

Mean values, provided there were more than two samples, were analyzed using a Duncan's Multiple Range Test, $p \leq 0.05$ (Nie et al, 1975).

Numbers presented as proportions were analyzed using confidence intervals for two randomly distributed percentages noted in this text as Difference of Percentages, $p \leq 0.05$ (Larson, 1969; Sokal and Rohlf, 1969).

RESULTS

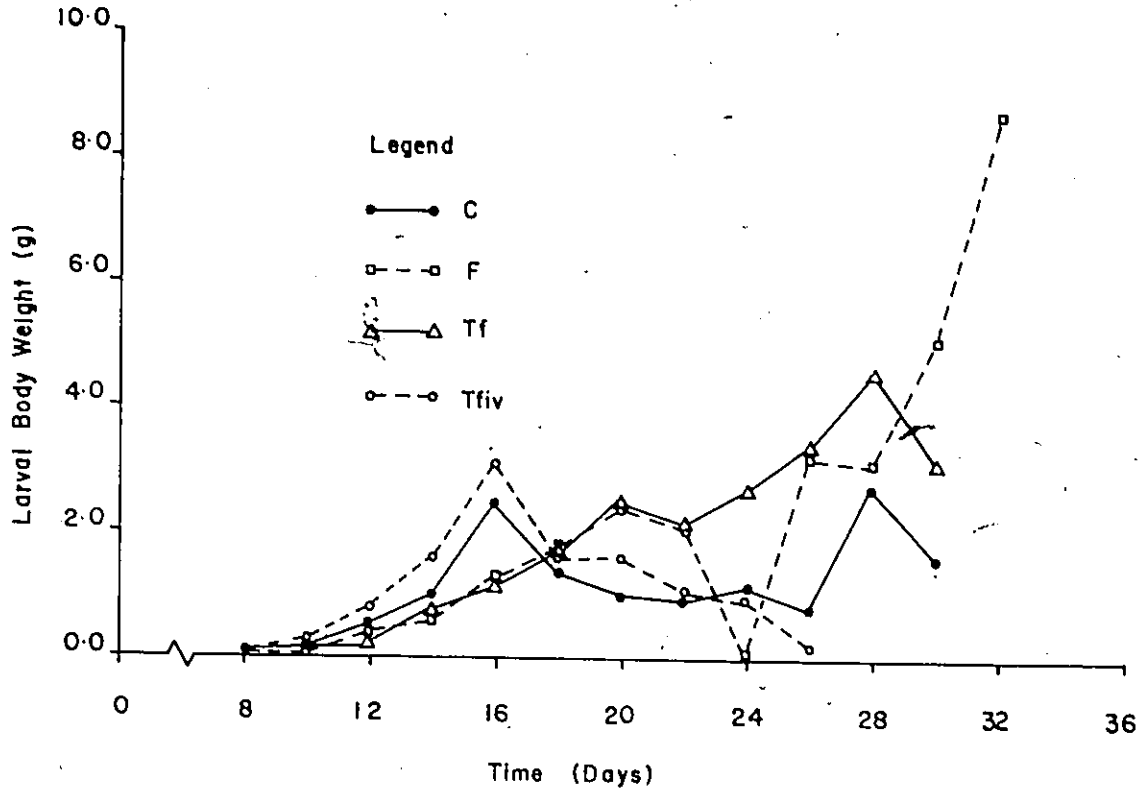
1.0 Diet Experiment:

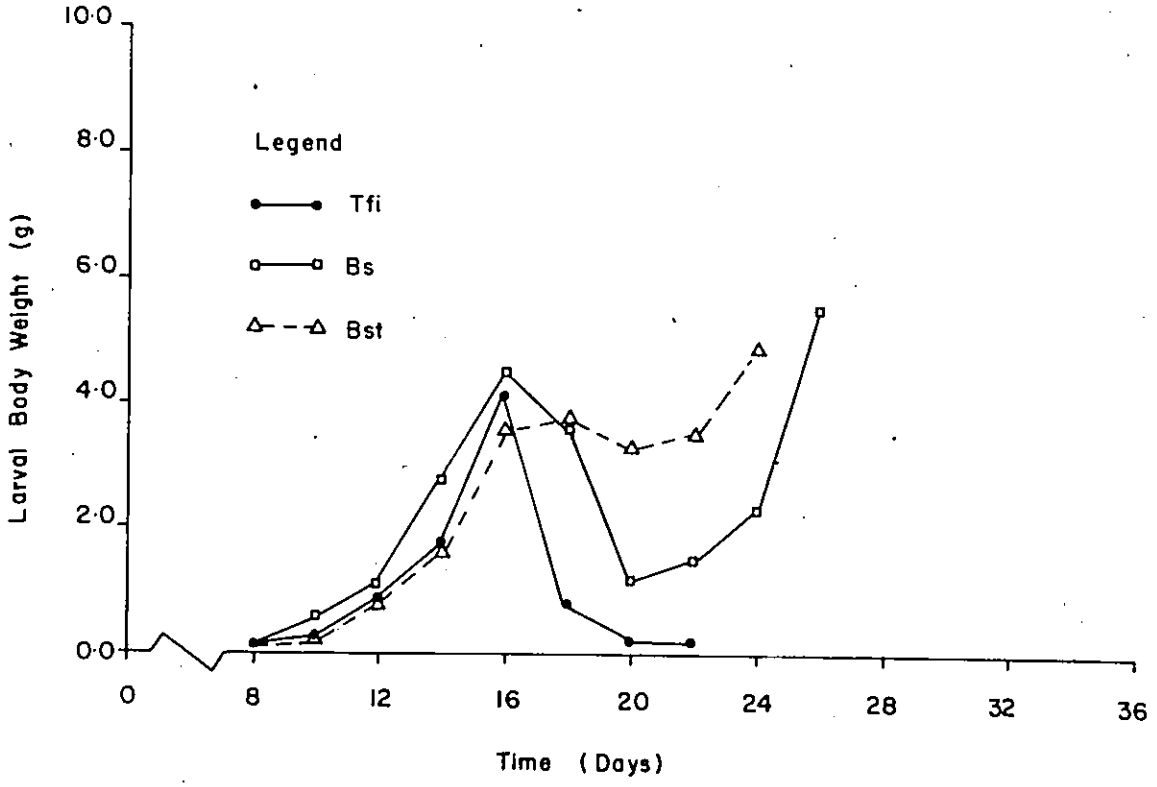
1.1 Larval Development:

The quantity and components of each of the seven meridic diets affected the growth and development of tobacco hornworm larvae. The larval populations of six of the seven diets tested developed asynchronously (Fig. 1). Diets C and F displayed a triphasic response in growth with body weight peaks of 2.448 g, 1.096 g, and 1.622 g and 2.365 g, 3.159 g, and 8.725 g respectively. Biphasic growth rates were observed in groups Tf, Tfiv, Bs, and Bst with peaks of 2.437 g, and 4.618 g, 3.123 g, and 1.615 g, 4.509 g and 5.555 g, and 3.614 g and 4.882 g respectively. Diet group Tfi, the only population to develop synchronously, had a single body weight maximum of 4.107 g. There was a statistical trend for larvae in groups Tfi, Tfiv, Bs, and Bst to have larger body weight means than groups C, F, and Tf from day 4 to day 16 (Table 37 Appendix A). There was no statistical difference amongst any of the diet groups after day 16.

Larval mortality was most prevalent during the first 22 days of development (Fig. 2). Mortality during the first four days of development (initial mortality) was less than 30% for

Fig. 1. Development of tobacco hornworm larvae reared on different meridic diets under non-diapause conditions (25°C /- 1°C, 18/6 L/D photoperiod, and 45% - 55% relative humidity). Mean body weights are presented. C: wheat germ not ground; F: wheat germ ground to a fine texture; Tf: wheat germ ground to a fine texture, dried tobacco leaves added; Tfi: wheat germ ground to a fine texture, dried tobacco leaves added, a significant increase in water content; Tfiv: wheat germ ground to a fine texture, dried tobacco leaves added, a significant increase in water content, Vanderzant's vitamin mixture added; Bs: BioServ diet; and Bst: BioServe diet, dried tobacco leaves added.

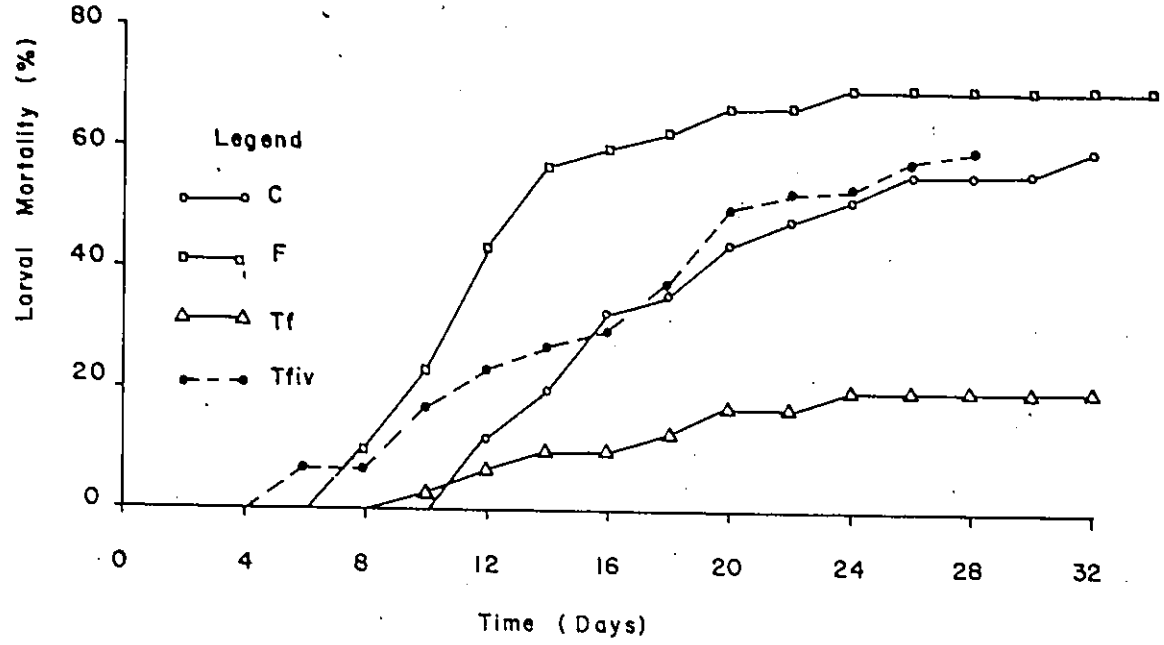

10·0
8·0
6·0
4·0
2·0
0·0
Larval Body Weight (g)
Legend
C
F
Tf
Tfiv
0
8
12
16
20
24
28
32
36
Time (Days)


10·0
8·0
6·0
4·0
2·0
0·0
Larval Body Weight (g)
Legend
Tfi
Bs
Bst
0
8
12
16
20
24
28
32
36
Time (Days)

all groups (Table 3). Samples C and F encountered the greatest mortality (30.3% and 23.8% respectively). Mortality in groups Tfi, Tfiv, Bs, and Bst were approximately 15%. Diet groups Tf recorded the lowest mortality (4.0%). First instar mortality was less than 33.3% for all groups. Second instar mortality was less than 6.7% for all groups except F (20.0%). Third and fourth instar mortalities ranged from 13.3% to 26.7% for all groups except Tf and Bst (less than 4.0%). Samples C and Tfiv incurred the most fifth instar mortalities (28.0% and 26.7% respectively) whereas the other groups recorded mortalities of 10.0% or less. The majority of larval deaths occurred during the third stadium for groups F, Tfi, and Bs (38.1%, 53.3%, and 68.0% respectively). Insects on diets Tf and Bst encountered mortalities of about 20.0% or less during every stadium. Diet groups C and Tfiv lost most of their larvae during the fifth stadium (46.7% and 44.5% respectively). Total larval mortality ranged from 10.0% (Bst) to 70.0% (F). The highest mortalities were recorded by groups C, F, Tfi, and Tfiv (60.0%, 70.0%, 50.0%, and 60.0% respectively).

All groups except Tf spent an average of 4.0 days in the first stadium (Table 4). Time needed for second instar development ranged from 1.93 days (Tf) to 3.58 days (F). The number of days required to complete the third stadium ranged from 3.33 (Bs) to 5.00 (F). The amount of time spent

Fig. 2. Mortality during the course of larval development insects reared on different meridic diets under non-diapause conditions.


Larval Mortality (%)
80
60
40
20
0
Legend
C
F
Tf
Tfiv
0
4
8
12
16
20
24
28
32
Time (Days)

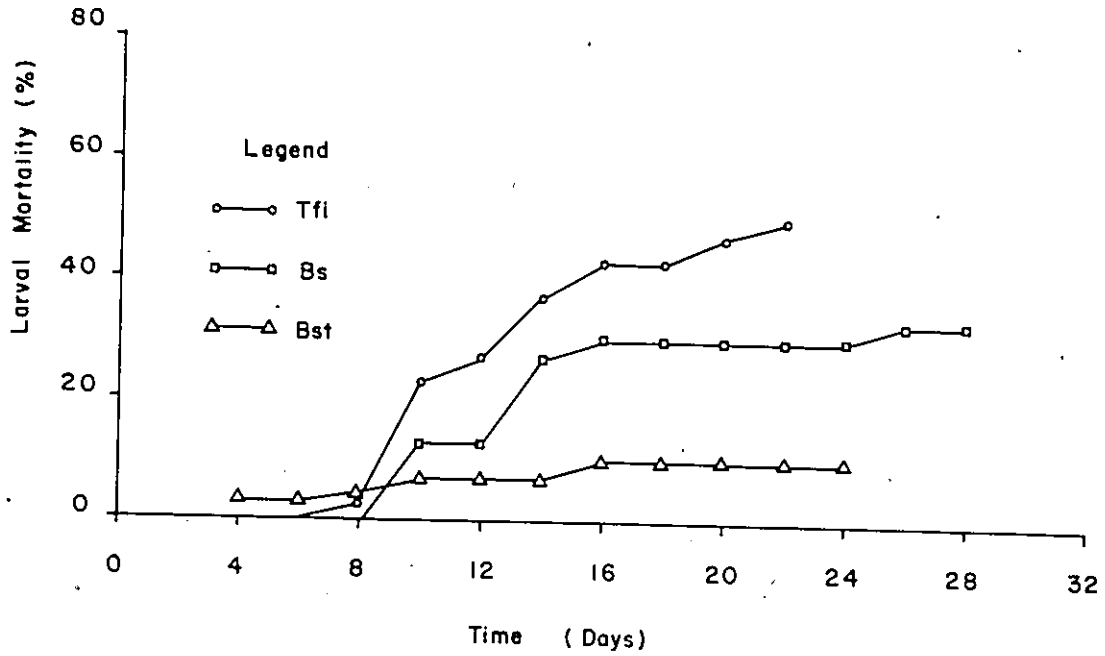

Larval Mortality (%)
80
60
40
20
0
Legend
Tfi
Bs
Bst
0
4
8
12
16
20
24
28
32
Time (Days)

Table 3. Mortality in each instar of M. sexta larvae reared on different diets under non-diapause conditions. Initial mortality is the mortality incurred by larvae from eclosion till four days old. Numbers in brackets are the proportion each instar mortality contributes to the total. Numbers in a column followed by a similar number are statistically similar (differences of percentage).

Diet	Init. Mort. (%)	Instar Mortality I	II	III	IV	V	VI	Total
C n=25	30.3	0.0^{1} (0.0)	0.0^{1} (0.0)	$16.0^{2,3}$ (26.7)	16.0^{2} (26.7)	28.0^{2} (46.7)	-	60.0^{4}
F n=30	23.8	0.0^{1} (0.0)	20.0 (28.6)	36.7^{3} (38.1)	20.0^{2} (28.6)	$3.3^{1,2}$ (4.3)	0.0	70.0^{4}
Tf n=30	4.0	0.0^{1} (0.0)	$3.3^{1,2}$ (16.7)	3.3^{1} (16.7)	3.3^{1} (16.7)	10.0^{2} (50.0)	-	$20.0^{1,2}$
Tfi n=30	10.0	0.0^{1} (0.0)	6.7^{2} (13.3)	26.7^{3} (53.3)	13.3^{2} (26.7)	$3.3^{1,2}$ (13.3)	-	$50.0^{3,4}$
Tfiv n=30	8.8	0.0^{1} (0.0)	6.7^{2} (11.1)	13.3^{2} (22.2)	13.3^{2} (22.2)	26.7^{3} (44.5)	-	60.0^{4}
Bs n=30	18.1	0.0^{1} (0.0)	0.0^{1} (0.0)	$20.0^{2,3}$ (60.0)	13.3^{2} (40.0)	0.0^{1} (0.0)	-	$33.3^{2,3}$
Bst n=30	15.0	3.3^{1} (33.3)	0.0^{1} (0.0)	3.3^{1} (33.3)	3.3^{1} (33.3)	0.0^{1} 0.0	-	10.0^{1}

as a fourth instar larva varied from 4.10 days (C) to 5.20 days (F). Fifth instar development time ranged from 2.73 days (Tfiv) to 4.50 days (Bs). A supernumerary sixth instar was observed for an insect in diet group F. Total larval development time (DAL) varied from 17.64 days (Tfiv) to 21.33 days (F). There was no significant difference in the total larval development time taken by samples within groups (1) C, Tfi, Bs, and Bst, and (2) C, F, and Tf. Groups C, F, and Tf took an average of two or three more days to complete larval development than did group Tfi. Compared to Tfi, group C required more time to complete the second and fifth stadia whereas group F spent more time in the second, fourth, and fifth stadia. Sample Tf took longer to complete first, third, and fifth instar development than did Tfi.

Table 5, illustrates the mean body weights at the end of each instar for each of the diets tested. All groups with the exception of Tf had similar second instar body weights. Body weights at the end of the the third stadium ranged from 0.126 g (Bs) to 0.190 (Tfi). Fourth instar body weights varied from 0.935 g (F) to 1.392 g (Tfi). Fifth instar body weights ranged from 2.817 g (Tf) to 4.890 g (Bs). Maximum larval body weights were least for group Tf (6.614 g) and greatest for group C (7.814 g).

All groups consumed a large quantity of diet relative to

Table 4. Mean number of days spent in each instar and total development time for Manduca sexta larvae. DAL: days as larvae. Numbers in brackets are times needed for the first and last larvae to reach the prepupal stage (range). Numbers in a column followed by the same letter are not significantly different from each other (Duncan's Multiple Range Analysis of Variance).

Mean Number of Days Spent in Each Instar

Diet	I	II	III	IV	V	IV	DAL
C	4.00^{a}	3.36^{c}	4.10^{abc}	4.10^{a}	4.00^{bc}	-	19.60^{ab}
SEM	0.00	0.25	0.45	0.45	0.42	-	1.26
n=25							(16-30)
F	4.07^{a}	3.58^{c}	4.38^{abc}	5.20^{a}	3.78^{abc}	4.00	21.33^{b}
SEM	0.07	0.29	0.33	0.33	0.33	-	1.73
n=30							(16-32)
Tf	6.00	1.93^{a}	5.00^{c}	4.44^{a}	3.58^{abc}	-	21.00^{b}
SEM	0.00	0.05	0.41	0.16	0.40	-	0.81
n=30							(18-32)
Tfi	4.00^{a}	2.71^{b}	4.50^{bc}	5.00^{a}	3.33^{ab}	-	17.89^{a}
SEM	0.00	0.18	0.35	0.22	0.25	-	0.13
n=30							(16-18)
Tfiv	4.00^{a}	2.79^{b}	3.91^{ab}	5.05^{a}	2.73^{b}	-	17.64
SEM	0.00	0.19	0.15	0.28	0.30	-	0.24
n=30							(16-18)
Bs	4.00^{a}	2.40^{ab}	3.33^{a}	4.70^{a}	4.50^{c}	-	18.50^{a}
SEM	0.00	0.22	0.26	0.64	0.56	-	0.79
n=30							(16-28)
Bst	4.07^{a}	2.55^{b}	3.86^{ab}	4.44^{a}	3.78^{abc}	-	18.74^{a}
SEM	0.07	0.17	0.20	0.25	0.25	-	0.44
n=30							(16-26)

Table 5. Weight at the end of each instar for tobacco hornworm larvae reared on seven different synthetic diets. Mean body weights (g) are presented with the standard error of the mean (SEM). Diet group F was the only sample to have insects in the first stadia when body weight measurements were taken. Numbers in a column followed by a similar letter are not statistically different from each other (Duncan's Multirange Analysis of Variance).

Diet	Larval Wt. (g) at the End of each Instar						Max. Larval Wt. (g)
	I	II	III	IV	V	VI	
C	-	0.024^{a}	0.139^{ab}	1.020^{ab}	4.365^{ac}	-	7.814^{b}
SEM	-	0.002	0.009	0.008	0.427	-	0.035
n=25							
F	0.007	0.022^{a}	0.157^{abc}	0.935^{a}	3.171^{ab}	8.725	7.354^{ab}
SEM	-	0.002	0.011	0.042	0.411	-	0.729
n=30							
Tf	-	0.032	0.171^{c}	1.011^{a}	2.817^{a}	-	6.614^{a}
SEM	-	0.001	0.010	0.047	0.281	-	0.281
n=30							
Tfi	-	0.024^{a}	0.190^{c}	1.392^{d}	4.606^{c}	-	7.549^{b}
SEM	-	0.002	0.011	0.048	0.287	-	0.251
n=30							
Tfiv	-	0.023^{a}	0.162^{bc}	1.239^{bcd}	4.224^{bc}	-	7.105^{b}
SEM	-	0.002	0.0.0	0.077	0.492	-	0.350
n=30							
Bs	-	0.023^{a}	0.126^{a}	1.305^{cd}	4.890^{c}	-	7.216^{ab}
SEM	-	0.002	0.010	0.121	0.310	-	0.191
n=30							
Bst	-	0.022^{a}	0.168^{c}	1.138^{abc}	4.368^{c}	-	7.116^{ab}
SEM	-	0.002	0.007	0.064	0.254	-	0.212
n=30							

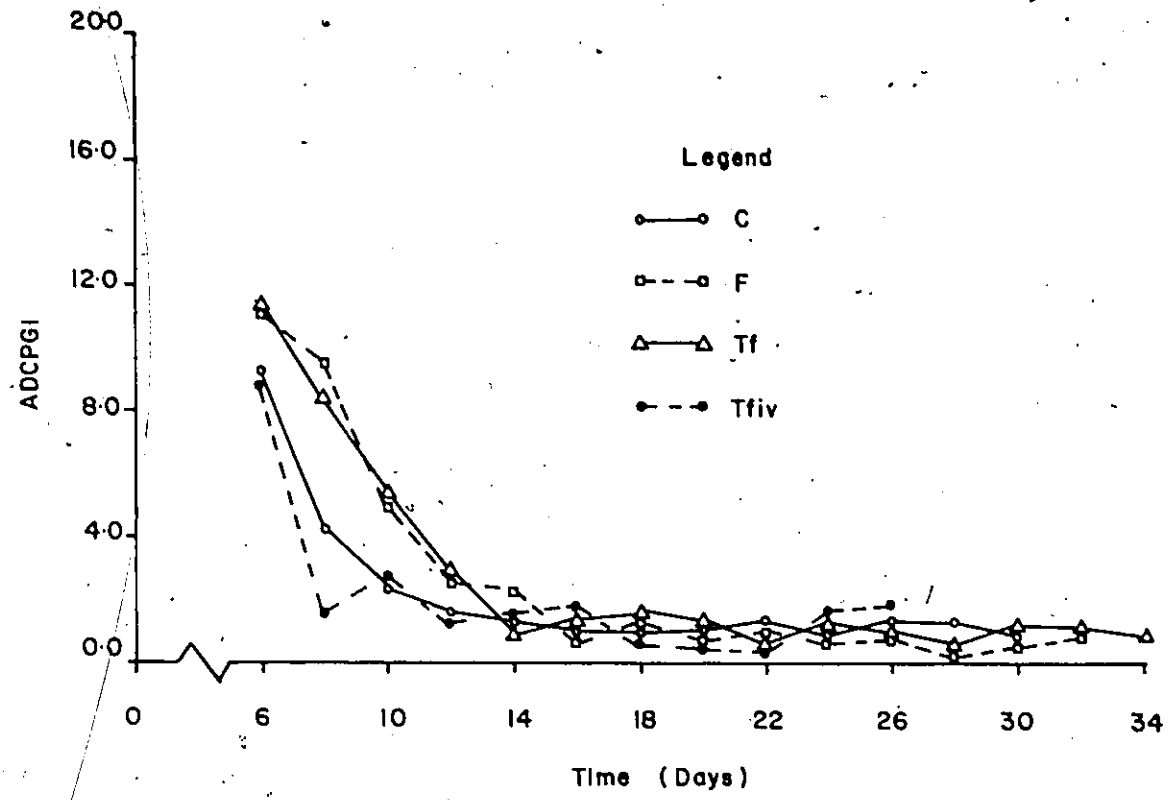
the insects' body weight during the first ten days of larval development (Fig. 3). This amount rapidly decreased in an exponential-like fashion as the population grew older. Diets Tfi and Bst showed the highest initial ADCPGI values of about 16.0 g of diet per gram insect. The ADCPGI at the beginning of each instar (Table 6) shows similar characteristics as Fig. 3. Second instar larvae of all diet groups have large ADCPGI values, particularly groups Tfi and Bst (13.25 g diet/g insect and 14.85 g diet/g insect respectively). There is no statistical difference in the third and fourth instar ADCPGI values for any of the diet groups.

1.2 Pupal Development:

Samples Tf, Bs, and Bst had the greatest number of insects pupating (80.0%, 66.7%, and 90.0% respectively) (Table 7). Fifty percent of the larvae in group Tfi pupated. Approximately 35% of the larvae in groups C, F, and Tfiv reached the prepupal stage.

The number of days required for a last instar larva to moult and for the pupa to fully tan varied from 6.0 (C) to 7.56 (Tfiv). There was no significant difference among groups (1) C, F, Tfi, and Bst and (2) F, Tf, Tfi, Tfiv, Bs, and Bst. The range of days till fully tanned was determined by taking the difference from the dates the first and last pupa became

Fig. 3. Amount of diet consumed per gram insect during development of tobacco hornworm larvae reared on different meridic diets under non-diapause conditions.


20·0
16·0
12·0
8·0
4·0
0·0
ADCPGI
Legend
C
F
Tf
Tfiv
0
6
10
14
18
22
26
30
34
Time (Days)

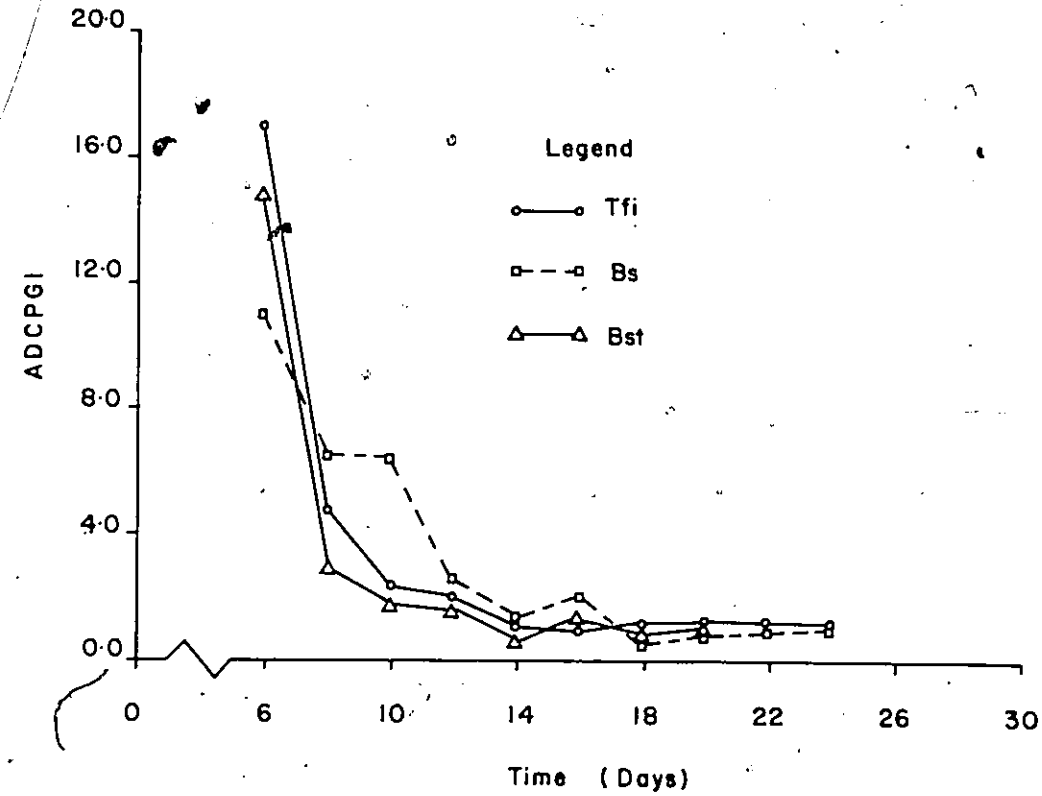

20·0
16·0
12·0
8·0
4·0
0·0
ADCPGI
Legend
Tfi
Bs
Bst
0
6
10
14
18
22
26
30
Time (Days)

Table 6. Amount of diet consumed per gram insect at the beginning of each larval stadium. Mean values are presented with the standard error of the mean (SEM). Numbers in a column followed by a similar letter are not statistically different from each other using a Duncan's Multiple Range Test.

Diet	ADCPGI at the Beginning of Instar				
	II	III	IV	V	VI
C	9.35^{ab}	2.82^{ab}	1.35^{a}	0.87^{a}	-
SEM	2.01	0.14	0.09	0.10	-
n=25					
F	6.78^{a}	4.25^{b}	1.44^{a}	1.10^{a}	0.50
SEM	1.22	0.60	0.13	0.08	-
n=30					
Tf	5.06^{a}	3.04^{ab}	1.14^{a}	0.81^{a}	-
SEM	0.42	0.18	0.06	0.10	-
n=30					
Tfi	13.25^{bc}	2.06^{a}	1.19^{a}	1.19^{a}	-
SEM	1.52	0.14	0.06	0.06	-
n=30					
Tfiv	9.03^{ab}	1.62^{a}	1.48^{a}	1.24^{a}	-
SEM	1.29	0.16	0.25	0.12	-
n=30					
Bs	11.33^{bc}	3.97^{b}	1.29^{a}	0.95^{a}	-
SEM	1.58	1.00	0.09	0.06	-
n=30					
Bst	14.85^{c}	3.97^{b}	1.46^{a}	1.04^{a}	-
SEM	1.30	0.27	0.07	0.08	-
n=30					

fully tanned and sclerotized. The ranges were similar for all diets tested (about seven days).

Group F had the shortest pupal development time of 28.0 days whereas group C required 36.0 days. There was no significant difference amongst samples (1) Tfi, Tfiv, and Bs and (2) C, Tf, and Tfiv.

The weight at the beginning of pupation ranged from 6.614 g (Tf) to 7.814 g (C). There was no statistical difference between groups (1) F, Tf, Tfiv, Bs, and Bst and (2) C, F, Tf, Tfiv, Bs, and Bst. There was no instance of larvae pupating before reaching a body weight of at least 5.0 g except for groups Tf and Bst (4.2% and 3.7% respectively). The body weights of pupae prior to eclosion ranged from 2.451 g (Bst) to 3.089 g (C). Statistically samples in groups (1) F, Tf, Tfi, Tfiv, Bs, and Bst and (2) C, F, Tf, Tfi, Tfiv, and Bs were not different from each other.

The pupal sex ratio (females (F)/males (M)) was least for groups C and F (0.80) and greatest for group Tfi (3.33).

It was convenient to monitor pupal mortality in two stages; (1) prepupal mortality and (2) mortality of tanned pupae. Prepupal mortality included insects which had died after the initiation of the wandering stage and before they were fully

tanned and sclerotized pupae. Tanned pupae which had died prior to eclosion were scored as tanned pupal mortalities. Diet group C exhibited equivalent prepupal and tanned pupal mortalities. Groups F, Tf, Tfiv, Bs, and Bst suffered the majority of their mortalities as fully formed pupae. Group Tfi encountered more prepupal than tanned pupal mortalities. Sample Tfi tended to have the lowest incidence of pupal mortalities.

Pupal deformities were minor or major. Minor deformities did not seem to affect the insect's ability to emerge from the pupal case, unfold its wings, or coil the proboscis. Minor deformities included a flattened, crooked, split, or shortened tongue case, the inability to shed the larval skin from the abdomen of the pupal case, or incomplete suture lines (Plate 3). Major deformities included incomplete formation of the pupal case, that is large spaces between the wing pads or between the thorax and abdomen which would not tan properly. Other major pupal deformities included outgrowths or depression along the body surface, the old larval cuticle still attached to the head, thorax, and abdominal regions of the pupa, and a greatly distorted or reduced tongue case. Insects with major deformities usually could not completely shed the pupal case or failed to emerge as an adult. As a result of a major deformity, adults could not expand their wings or had portions of the old pupal case still attached to certain regions of

their body. Insects with major deformities commonly could not retrieve their proboscis from the pupal case or had pieces of the old pupal cuticle remaining around the head region. The proportion of pupae with either minor or major pupal deformities ranged from 35% (Bs) to 6.7% (Tfi). There was no significant difference between samples in groups (1) F and Tfi, (2) C, F, Tf, and Tfiv, (3) C, Tf, and Tfiv, and (4) C, Tf, Bs, and Bst.

1.3 Adult Development:

All diet groups had more than 50% of their pupae moulting to the adult stage (Table 8). Samples within groups (1) F, Tfiv, and Bs, (2) F, Tf, Bs, and Bst, and (3) C, Tf, Tfi, Bs, and Bst were not significantly different from each other.

The mean number of days spent as an adult varied from 4.4 (F) to 7.05 (Bst). Samples within groups (1) C, F, Tf, Tfi, Tfiv, and Bs and (2) C, Tf, Tfi, Tfiv, Bs, and Bst were statistically similar. The range of days as an adult was calculated by subtracting the day the first adult emerged from the pupa from the day the last adult died. The range of days varied from 2.0 (F) to 8.0 (Tfiv and Bs).

Adult deformities included individuals with crumpled wings or those with parts of their pupal case still attached to the

Plate 111. Pupal deformities. a: normal pupa; b,c,d, pupae with minor tongue case deformities; e,f: major pupal deformities.

a

b

c

d

e

f

Table 7. Pupal characteristics of tobacco hornworms reared on seven different diets under non-diapause conditions. Pup. (%): percentage of insects pupating. Max. Pup. Wt. (g): mean body weight at the beginning of pupation. Min. Pup. Wt. (g): mean body weight at the end of pupation. DTT (days): mean number of days till the pupa had completely tanned. PM (%): total pupal mortality (%). PPM (%): pre-pupal mortality (%). TPM (%): tanned pupal mortality (%). Def. Pup. (%): the percentage of deformed pupae. DAP (days): the mean number of days spent in the pupal stage. PSR (F/M): pupal sex ratio (females to males). The numbers in brackets are the minimum and maximum time needed to complete pupal development (range). Numbers in a column followed by a similar number are statistically similar (difference of percentage). Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test).

Diet	Pup. (%)	Max. Pup. Wt.(g).	Min. Pup. Wt.(g).	DTT (days)	PM (%)	PPM (%)	TPM (%)	Def Pup. (%)	DAP (days)	FSR (F/M)
C SEM n=10	$40.0^{1,2}$	7.814^{b} 0.035	3.089^{b} 0.145	6.00^{a} 0.33	20.0^{1}	$10.0^{1,2}$	$10.0^{1,2}$	$20.0^{2,3,4}$	36.00^{b} 0.66 (28-40)	0.80
F SEM n=9	30.0^{1}	7.354^{ab} 0.729	2.724^{ab} 0.168	7.00^{ab} 0.38	44.4^{2}	$11.1^{1,2}$	33.3^{4}	$12.5^{1,2}$	28.00 1.55 (22-30)	0.80
Tf SEM n=24	80.0^{4}	6.614^{a} 0.281	2.644^{ab} 0.154	7.30^{6} 0.03	25.8^{1}	$8.7^{1,2}$	$17.1^{2,3}$	$21.2^{2,3,4}$	35.39^{b} 0.24 (30-40)	0.91
Tfi SEM n=15	$50.^{2,3}$	7.549^{b} 0.251	2.564^{ab} 0.118	6.62^{ab} 0.27	20.0^{1}	$13.3^{1,2}$	6.7^{1}	6.7^{1}	32.33^{a} 0.54 (30.36)	3.33
Tfiv SEM n=12	$6.7^{1,2}$	7.105^{b} 0.350	2.825^{ab} 0.196	7.56^{b} 0.56	43.3^{2}	18.3^{2}	$25.0^{3,4}$	$16.7^{2,3}$	34.33^{ab} 1.96 (32-44)	2.00
Bs SEM n=20	$66.7^{3,4}$	7.216^{ab} 0.191	2.805^{ab} 0.087	7.20^{b} 0.23	25.6^{1}	0.0	$25.6^{3,4}$	35.0^{4}	32.14^{a} 0.49 (28-36)	1.50
Bst SEM n=27	90.0^{4}	7.116^{ab} 0.212	2.451^{a} 0.229	6.88^{ab} 0.23	$29.6^{1,2}$	7.4^{1}	$22.2^{3,4}$	$25.9^{3,4}$	32.00 0.57 (28-36)	1.00

dorsal surface of the head and/or thorax. Quite often adults with deformities emerged from pupae which had been previously scored as having major deformities. Instances of adult deformities were observed in groups Tfiv, Bs, and Bst (16.2%, 7.1%, and 5.6% respectively).

The adult sex ratio (F/M) ranged from 0.25 (F) to 5.0 (Tfiv).

Groups C, F, Tf, Tfiv, and Bs laid less than one egg per female per two days. Females of groups Tfi and Bst oviposited about 30 eggs per female per two days. The fecundity rate (mean number of eggs produced per female per two days) ranged from approximately 20 (C, F, Tf, Tfiv, Bs, and Bst) to about 60 (Tfi).

Since females of groups C, F, and Tfiv had an ovipositioning rate of zero, the fertility (percent egg hatch) of these groups was also zero. Groups Tf, Tfi, Bs, and Bst displayed a fertility of about 80%.

1.4 Total Development Time:

The total time required for M. sexta to successively complete development was calculated by adding the mean number of days as a larva, pupa, and adult (Table 9a). A minimum

Table 8. Development of tobacco hornworm adults reared on different diets under non-diapause conditions. Eggs laid /F/ 2 days: mean number of eggs oviposited per female per two days (oviposition rate). Fec. /F/ 2 days: mean number of days spent in the adult stage. The numbers in brackets are the minimum and maximum time (days) spent in the adult stage. ASR (F/M): ratio of female adults to male adults. Numbers in a column followed by a similar number are statistically similar (differences of percentages). Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test).

Diet	Adults (%)	Eggs Laid /F/ 2 days	Fec/F /2 days	Fertility (% Hatch)	DAA (days)	ASR (F/M)
C SEM n=8	80.0[3]	0.0[a] 0.0	24.2[a] 15.8	0.0[1]	6.00[ab] 0.76 (4-8)	1.00
F SEM n=5	55.6[1,2]	0.0[a] 0.0	32.0[a] 32.0	0.0[1]	4.40[a] 0.40 (4-6)	0.25
Tf SEM n=19	77.3[2,3]	1.3[a] 1.3	17.5[a] 7.2	74.0[2]	5.29[ab] 0.42 (4-8)	1.12
Tfi SEM n=12	80.0[3]	34.5[a] 20.5	58.6[a] 27.6	80.0[2]	5.83[ab] 0.58 (4-10)	3.00
Tfiv SEM n=7	54.6[1]	0.0[a] 0.0	12.8[a] 8.5	0.0[1]	5.00[ab] 1.13 (2-10)	5.00
Bs SEM n=14	70.0[1,2,3]	1.4[a] 0.8	21.3[a] 12.1	84.0[2]	5.71[ab] 0.78 (2-10)	1.80
Bst SEM n=19	70.4[2,3]	28.1[a] 16.6	33.8[a] 18.9	82.0[2]	7.05[b] 0.39 (4-10)	0.73

average of 54.0 days was observed for individuals of group Bs. A maximum average of 57.4 days was recorded by insects of group F. There was no statistical difference between individuals of groups (1) C, F, Tfi, Tfiv, Bs, and Bst and (2) F, Tf, and Tfiv. The difference between the number of days needed by insects to develop from a first instar larva to the end of the adult stage (range) varied from 10 (C, Tfi, and Tfiv) to 14 (Bs).

2. Reproduction Experiment:

Five, six adult ratios of newly eclosed females (F) and males (M) were placed in mating cages and allowed to reproduce. The 3F/3M and 1F/5M groups reached their oviposition peaks four days after the experiment had begun (343 eggs per female and 33 eggs per female respectively) (Fig. 4). The 2F/4M and 4F/2M ratios recorded maximums of 230 eggs per female and 204 eggs per female on day 6. The 5F/1M group had a small peak of 43 eggs per female on day 4 and a second peak of 98 eggs per female on day 12. Eggs laid after day 8 by females of the 5F/1M group were infertile.

The 3F/3M and 4F/2M displayed the greatest rates of egg production (140 and 132 eggs/F/2 days respectively) and oviposition (124 and 100 eggs/F/2 days respectively) (Table 9b). The 2F/4M sample had a medium fecundity and oviposition rate

Fig. 4. The oviposition rates of females of different sex ratios.

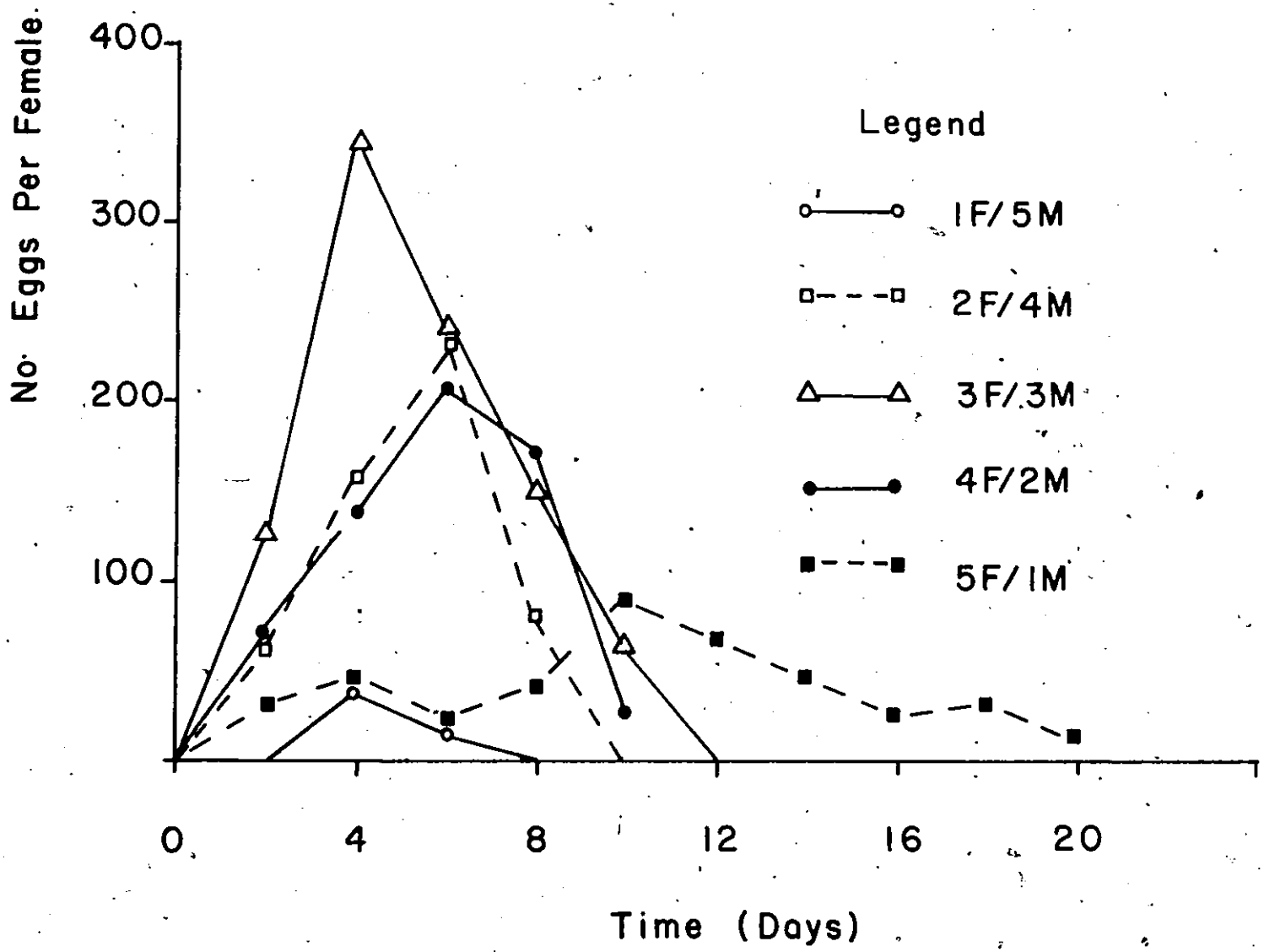

No. Eggs Per Female
400
300
200
100
0
4
8
12
16
20
Time (Days)
Legend
1F/5M
2F/4M
3F/3M
4F/2M
5F/1M

Table 9. Total developmental time of tobacco hornworms reared on one of the seven meridic diets. DAL: mean number of days as a larva. DAP: mean number of days as a pupa. DAA: mean number of days as an adult. Total: total development time (mean number of days). Numbers under the means are the standard error of the means. The numbers in brackets are the range of days needed for insects to develop in each stage. Numbers in a column followed by a similar letter are not statistically different from each other (Duncan's multirange analysis of variance).

Diet	DAL	DAP	DAA	TOTAL
C	18.50^{abc}	35.00^{c}	6.00^{ab}	55.75^{a}
SEM	0.63	0.65	0.76	1.64
n=25	(16-22)	(33-37)	(4-8)	(49-59)
F	19.20^{bc}	33.80^{bc}	4.40^{b}	57.40^{ab}
SEM	1.20	1.36	0.40	2.04
n=30	(16-22)	(29-37)	(4-6)	(51-63)
Tf	19.88^{c}	34.41^{c}	5.53^{ab}	59.71^{b}
SEM	0.61	0.61	0.40	0.84
n=30	(18-26)	(30-40)	(4-8)	(55-67)
Tfi	18.00^{ab}	31.33^{ab}	5.83^{ab}	55.17^{a}
SEM	0.00	0.54	0.58	0.97
n=30	(18)	(29-35)	(4-10)	(51-61)
Tfiv	17.67^{ab}	33.33^{abc}	5.00^{ab}	56.00^{ab}
SEM	0.33	1.96	1.13	1.91
n=30	(16-18)	(31-43)	(2-10)	(53-63)
Bs	17.14^{a}	31.14^{ab}	5.71^{ab}	54.00^{a}
SEM	0.35	0.49	0.78	1.27
n=30	(16-20)	(27-35)	(2-10)	(47-61)
Bst	18.11^{ab}	30.79	7.05^{a}	55.42^{a}
SEM	0.32	0.53	0.39	0.83
n=30	(16-22)	(27-35)	(4-10)	(49-61)

(103 and 88 eggs/F/2 days). The two extreme sex ratios of 1F/5M and 5F/1M recorded low rates of egg production (47 and 68 eggs/F/2 days respectively) and egg deposition (9 and 40 eggs/F/2 days).

It is interesting to note that as the ratio of females to males deviates from 3F/3M, the proportion of the total egg production retained within the females' abdomens increases.

The fertility of the eggs deposited decreased as the relative number of males decreased.

A polynomial regression for the fecundity, oviposition, and fertility data was performed. The 4F/2M ratio displayed the greatest fecundity in this model (Fig. 5). The 3F/3M sample laid the greatest number of eggs per female. Fertility is directly proportional to the number of males present (Fig. 6). The experimental values and predicted values differ by a large margin in some cases. It should be emphasized that the regression analysis was performed on a small sample size and, as a result, may not be an accurate representation of the experimental data.

3.0 Sublethal Toxicological Studies:

The population of each dosed group consisted of a mixture

Table 9b. Reproductive performance of five different adult sex ratios. Eggs Laid/F/2days: oviposition rate. Fec/F/2 days: fecundity rate. % Egg Prod. Retain: the proportion of the total egg production not oviposited per female. Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test).

Ratio (F/M)	Eggs Laid /F/ 2 days	Fec/F /2 days	% Egg. Prod. Retained	Fertility (% Hatch)
1F/ M	9.4	46.6	79.8	93.8
SEM	6.5	35.4		6.3
2F/4M	87.5[a]	I02.7[a]	14.8	64.0
SEM	36.8	39.7		7.5
3F/3M	124.3[b]	139.8[b]	11.1	69.0
SEM	56.0	51.3		13.9
4F/2M	99.5[ab]	131.5[ab]	24.4	48.4
SEM	33.0	38.1		7.5
5F/1M	39.0	67.9	41.2	23.6
SEM	9.2	14.5		12.4

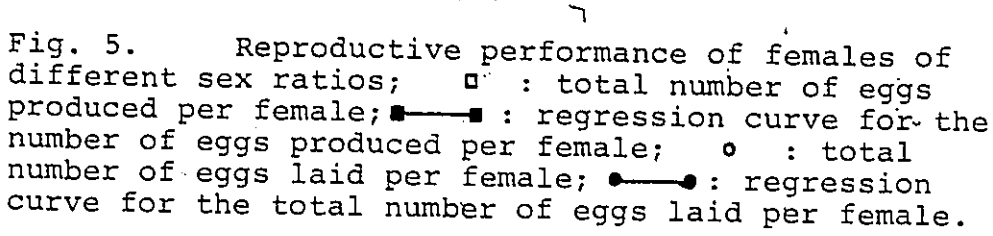


Fig. 5. Reproductive performance of females of different sex ratios; □ : total number of eggs produced per female; ■——■ : regression curve for the number of eggs produced per female; ○ : total number of eggs laid per female; ●——● : regression curve for the total number of eggs laid per female.

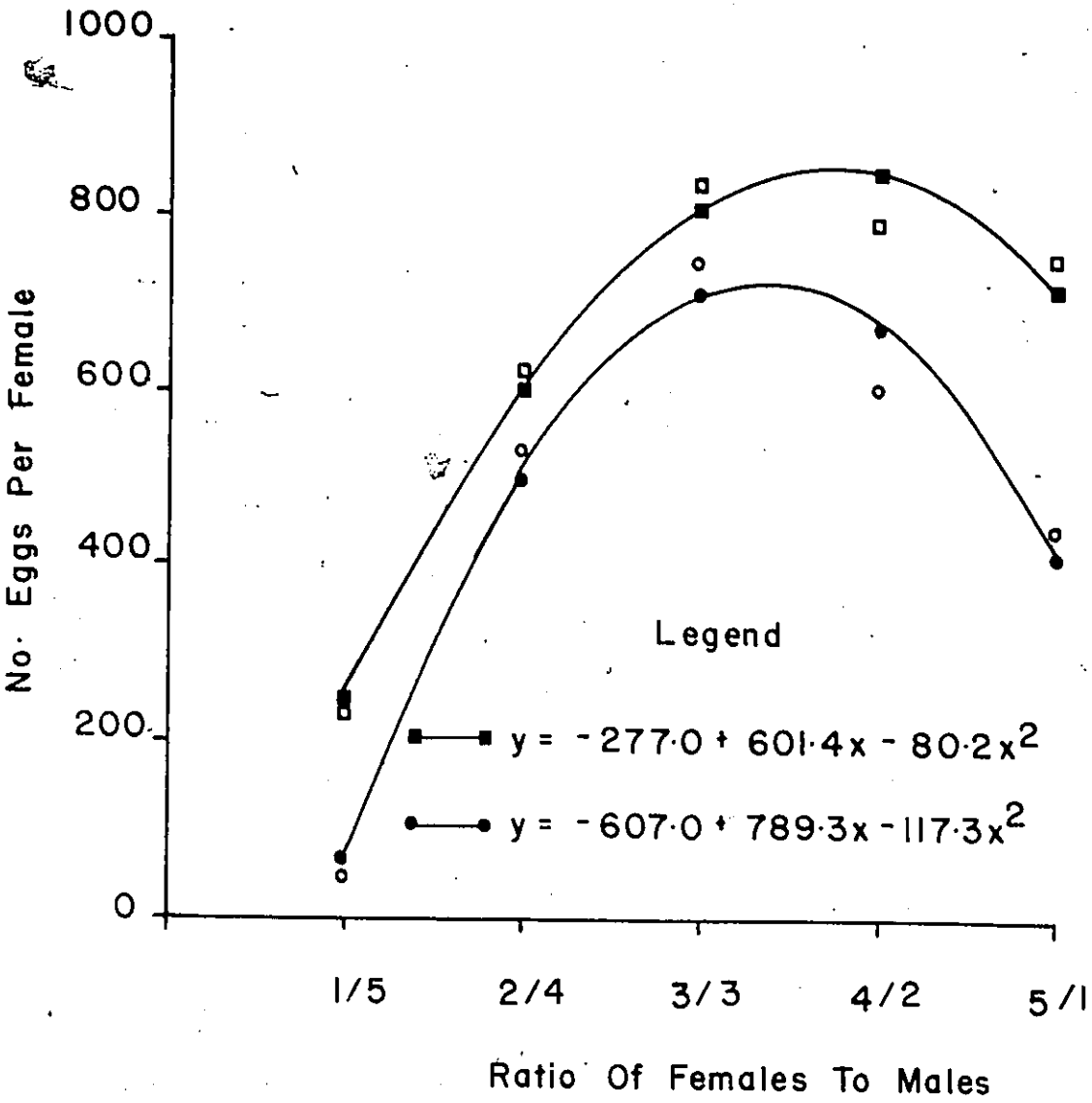
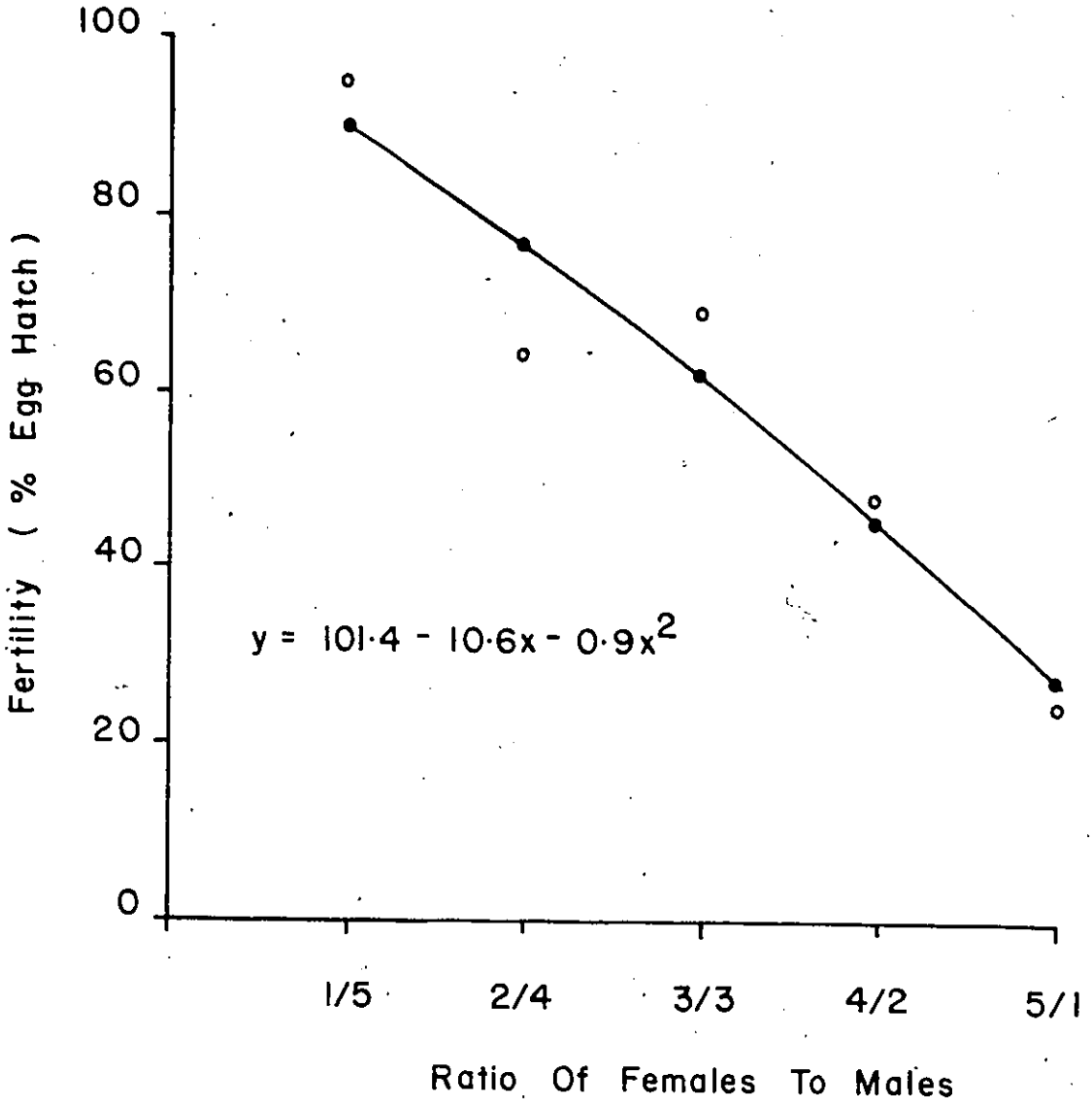

1000
800
600
400
200
0
No. Eggs Per Female
Legend
y = -277·0 + 601·4x - 80·2x²
y = -607·0 + 789·3x - 117·3x²
1/5
2/4
3/3
4/2
5/1
Ratio Of Females To Males

Fig. 6. The fertility of eggs laid by females at different sex ratios. o experimental data; ●——● regression curve.


100
80
60
40
20
0
Fertility (% Egg Hatch)
y = 101·4 - 10·6x - 0·9x²
1/5
2/4
3/3
4/2
5/1
Ratio Of Females To Males

of second and third, or second, third, and fourth instar larvae (Table 10). Each group had more than 50% of its population in the third stadium. The results of this set of experiments will be presented as larval, pupal, and adult development of the whole population, and larval and pupal development of insects treated during the second, third, and fourth stadia.

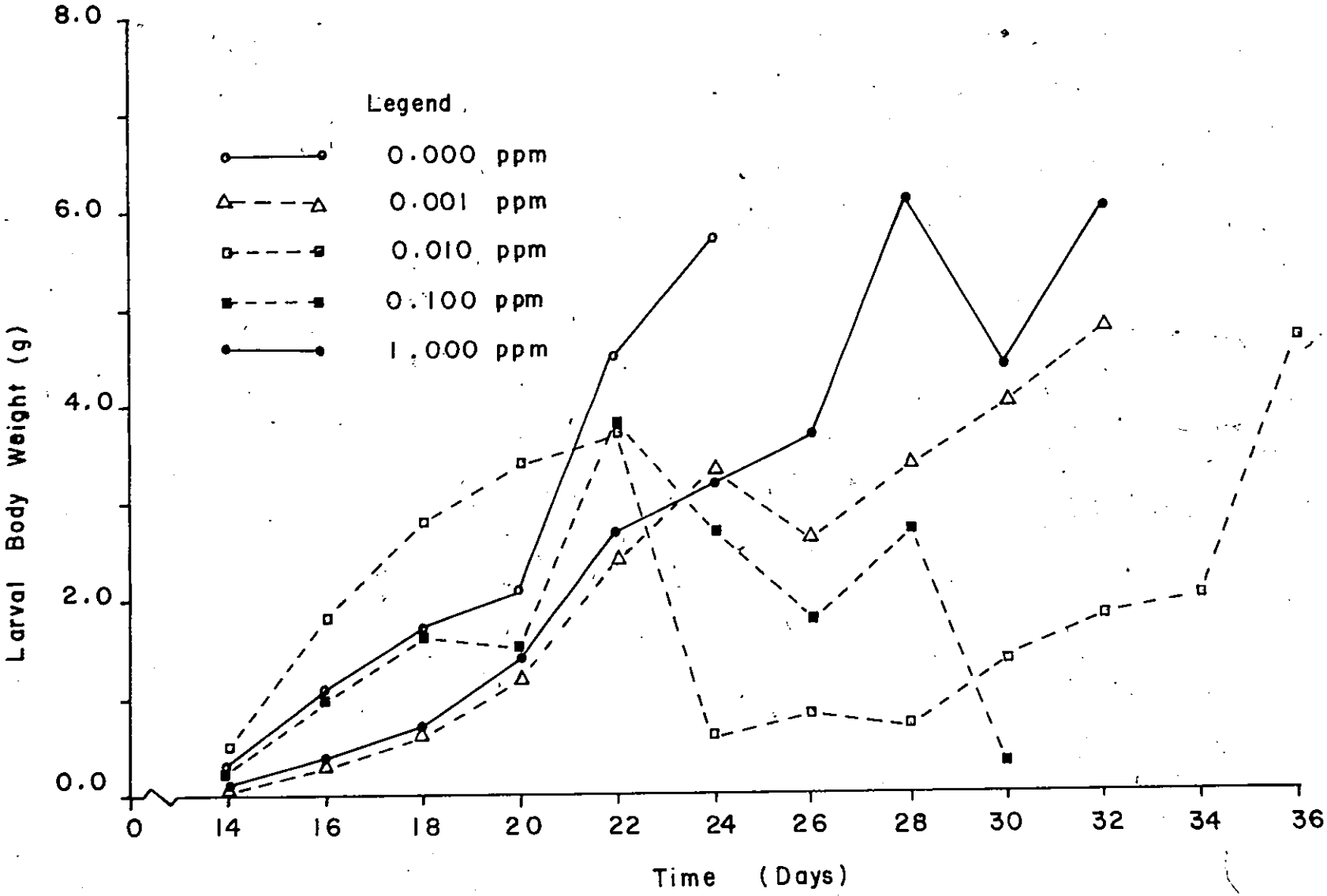
3.1 Larval Development of the Population:

Oral, sublethal doses of fenitrothion disrupted larval development (Fig. 7). The control population grew synchronously to the pupal stage and had a single body weight peak of 5.718 g. Samples treated with 0.001 ppm, 0.01 ppm, and 1.0 ppm concentrations displayed biphasic growth curves with body weight peaks of 3.272 g and 4.847 g, 3.657 g and 4.696 g, and 6.064 g and 5.978 g respectively. A triphasic growth curve was observed for the 0.1 ppm group with body weight peaks of 1.743 g, 3.764 g, and 2.708 g. the results of a one way analysis of variance (Duncan's Multiple Range Test) for the body weight data for each day is presented in Table 2, Appendix A. The body weights of the control group, 0.001 ppm, 0.1 ppm, and 1.0 ppm were statistically similar on the day of treatment. The 0.100 ppm sample's body weight was slightly greater than that of the control. This trend continued until day 22. From day 22 to day 24 the mean body

Table 10. The instar composition of each group on the day of treatment.

Treatment (ppm)	Percent of Population in Each Instar When Dosed		
	Instar		
	II	III	IV
Control n=30	0.0	80.0	20.0
0.001 n=30	14.3	81.0	4.8
0.010 n=30	0.0	56.0	44.0
0.100 n=30	0.0	84.0	16.0
1.000 n=30	22.7	72.7	4.5

Fig. 7. Development of a population of tobacco hornworm larvae exposed to sublethal doses of fenitrothion. The larvae were reared under non-diapause conditions.


Legend
0.000 ppm
0.001 ppm
0.010 ppm
0.100 ppm
1.000 ppm
Larval Body Weight (g)
8.0
6.0
4.0
2.0
0.0
0
14
16
18
20
22
24
26
28
30
32
34
36
Time (Days)

weight of the control group was significantly greater than those of the treated groups. There was no significant difference between any of the dosed groups after day 26.

Initial knockdown (% mortality) was not significantly different from the controls (Table 11).

There seems to be a direct relationship between the lower doses of fenitrothion and larval mortality (Table 12). The control group experienced a mortality of 5.0% following starvation and treatment with 10 l of 99% ethanol. Mortalities recorded for the 0.001 ppm, 0.01 ppm, and 0.1 ppm groups were statistically similar (14.3%, 16.0%, and 24.0% respectively). The 1.0 ppm sample's mortality was the greatest (45.5%). In all cases, the majority of the mortalities occurred in the third stadium. The 1.0 ppm population was the only group to exhibit second instar mortalities. All other treated groups experienced fourth and in one instance (0.01 ppm) fifth instar mortalities. Mortality during the course of larval development following treatment with fenitrothion is presented in Fig. 8.

With the exception of groups 0.001 ppm and 1.0 ppm, the time spent in each stadium did not vary greatly (Table 13). Larvae of the 0.001 ppm and 1.0 ppm concentrations spent more time in the second instar compared to the control. It should

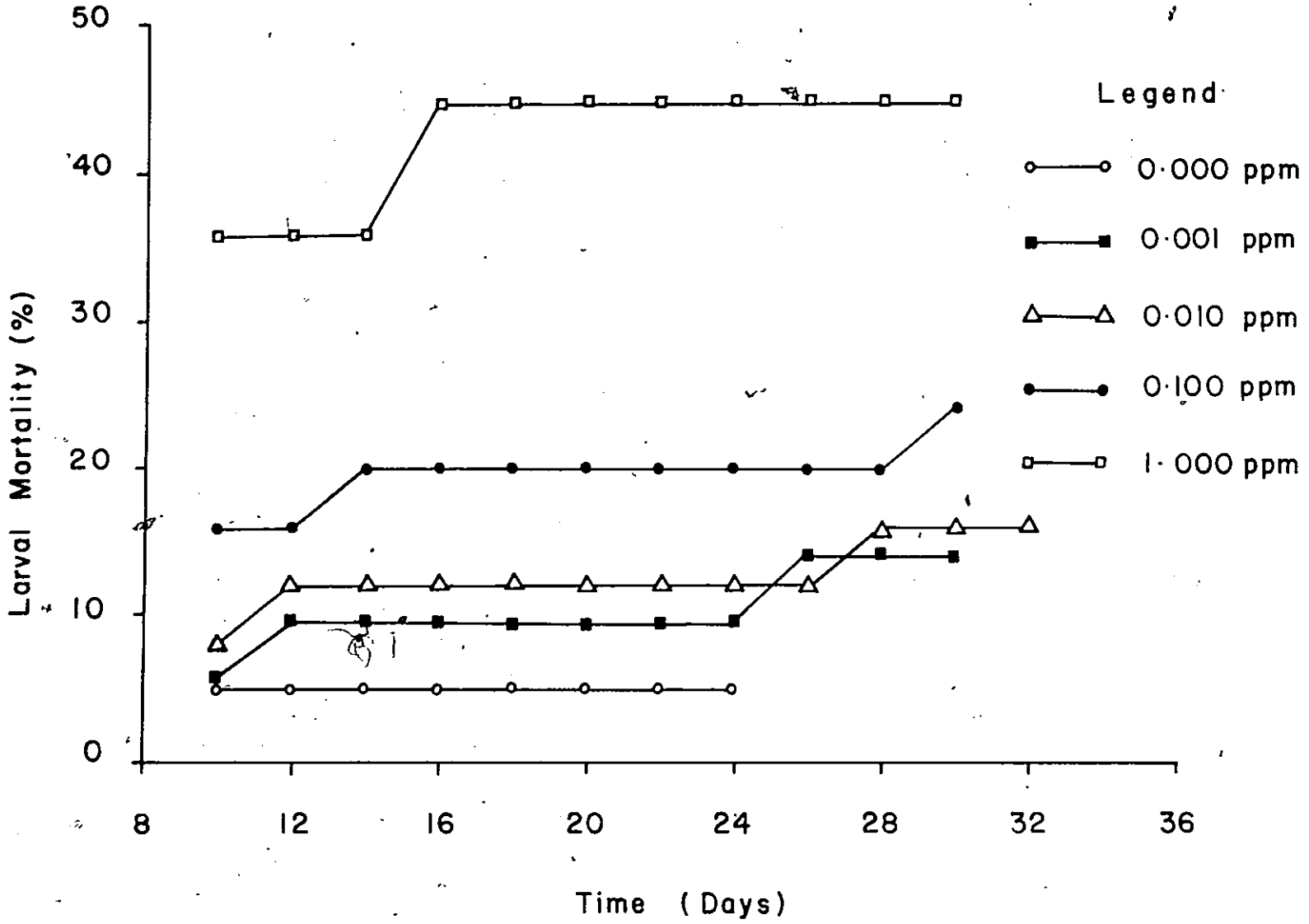
Table 11. The knockdown (KD) effect of oral sublethal doses of fenitrothion on tobacco hornworm larvae 48 hours after treatment. Numbers in a column followed by a similar letter are not statistically different from each other (differences of percentages).

Treatment (ppm)	KD (%) After 48 Hours
Control n=30	5.0[a]
0.001 n=30	4.8[a]
0.010 n=30	4.0[a]
0.100 n=30	16.0[ab]
1.000 n=30	36.4[b]

Table 12. Cummulative instar mortality for tobacco hornworm larvae treated with sublethal doses of fenitrothion. The numbers in brackets represent the proportion each instar mortality contributes to the total larval mortality. Numbers in a column followed by the same letter are not significantly different from each other (differences of percentages).

Treatment (ppm)	Cummulative Instar Mortality (%)					
	I	II	III	IV	V	Total
Control n=30	0.0[a] (0.0)	0.0[a] (0.0)	5.0[a] (100.0)	0.0[a] (0.0)	0.0[a] (0.0)	5.0[a]
0.001 n=30	0.0[a] (0.0)	0.0[a] (0.0)	9.5[ab] (66.6)	4.8 (33.4)	0.0[a] (0.0)	14.3[a]
0.010 n=30	0.0[a] (0.0)	0.0[a] (0.0)	8.0[a] (50.0)	4.0[a] (25.0)	4.0[a] (25.0)	16.0[a]
0.100 n=30	0.0[a] (0.0)	0.0[a] (0.0)	20.0[ab] (83.3)	4.0[a] (16.7)	0.0[a] (0.0)	24.0[ab]
1.000 n=30	0.0[a] (0.0)	13.6[a] (30.0)	31.8[b] (70.0)	0.0[a] (0.0)	0.0[a] (0.0)	45.5[b]

Fig. 8. Mortality of tobacco hornworm larvae exposed to different concentrations of fenitrothion.


50
40
30
20
10
0
Larval Mortality (%)
8
12
16
20
24
28
32
36
Time (Days)
Legend
0·000 ppm
0·001 ppm
0·010 ppm
0·100 ppm
1·000 ppm

be noted that both of these groups had larvae in the second stadium when dosed. The 1.0 ppm sample also needed significantly more time to reach the prepupal stage whereas the 0.01 ppm and 0.1 ppm samples took the same time as the control group. The control group required a range of six days to complete larval development (Table 13). All treated samples had a considerably larger range 0.001 ppm: 12 days, 0.01 ppm: 18 days, 0.1 ppm: 10 days, and 1.0 ppm: 12 days).

Regressive moults were recorded for insects of the 0.001 ppm, 0.1 ppm, and 1.0 ppm samples (10%, 8%, and 5% respectively).

The weight at the end of each instar (Table 14) gives an indication of the relative weights of each group prior to moulting. Statistically most treated groups were not different from the control sample. However, treated insects in the third and fourth stadia and the prepupal stage tended to have slightly larger body weights than did the control group. This trend was reversed for the fifth stadium.

The amount of diet consumed per gram insect (ADCPGI) decreases as the insect grows (Table 15). The correction factor used in the diet experiments was not applied for the toxicology experiments. The amount of diet given to each insect was too small, consequently the correction factor

Table 13. Mean number of days spent in each instar and total development time of tobacco hornworm larvae treated with different concentrations of fenitrothion. Numbers in brackets represent the difference of age (days) between the first and last larva in each group to reach the prepupal stage. Numbers in a column followed by a similar number are not significantly different from each other. (Duncan's Multiple Range Analysis of Variance).

Treatment (ppm)	Days in Instar					Days As Larva
	I	II	III	IV	V	
Control	4.00^{1}	$2.75^{1.2}$	6.21^{1}	4.84^{1}	6.11^{1}	23.26^{1}
SEM	0.000	0.37	0.57	0.35	0.24	0.51
n=30						(20-26)
0.001	4.20^{1}	6.40^{2}	$7.05^{1.2}$	4.67^{1}	6.22^{1}	26.22^{3}
SEM	0.20	1.39	0.60	0.40	0.28	0.68
n=30						(22-34)
0.010	4.00^{1}	2.18^{1}	5.39^{1}	4.18^{1}	7.24^{1}	22.19^{1}
SEM	0.00	0.18	0.54	0.47	0.43	0.85
n=30						(20-38)
0.100	4.00^{1}	$3.17^{1.2}$	6.40^{1}	5.37^{1}	6.00^{1}	$23.90^{1.2}$
SEM	0.000	0.58	0.72	0.53	0.10	0.58
n=30						(20-30)
1.000	4.00^{1}	7.00^{2}	9.00^{2}	5.00^{1}	6.83^{1}	$26.00^{2.3}$
SEM	0.00	1.00	0.80	0.91	1.03	1.04
n=30						(22-34)

Table 14. Weight (g) at the end of each instar of insects treated with different concentrations of fenitrothion. Maximum larval weight (g) is the weight of the larva prior to the non-feeding, wandering stage. Numbers in a column followed by a similar letter are statistically similar using Duncan's Multiple Range Test.

Treatment (ppm)	Weight (g) at the End of Instar					Maximum Larval Weight (g)
	I	II	III	IV	V	
Control	0.006^{1}	0.020^{1}	$0.135^{1.2}$	$1.075^{1.2}$	4.776^{1}	$7.098^{1.2}$
SEM	0.001	0.001	0.026	0.119	0.306	0.290
n=30						
0.001	0.006^{1}	0.032^{3}	$0.213^{2.3}$	1.247^{2}	4.603^{1}	6.674^{1}
SEM	0.001	0.003	0.030	0.108	0.322	0.277
n=30						
0.010	0.008^{1}	$0.026^{1.2.3}$	$0.167^{1.2.3}$	0.779^{1}	4.661^{1}	$7.272^{1.2}$
SEM	0.002	0.002	0.019	0.075	0.206	0.240
n=30						
0.100	0.006^{1}	$0.029^{2.3}$	0.109^{1}	1.308^{2}	4.707^{1}	$7.268^{1.2}$
SEM	0.003	0.003	0.010	0.098	0.288	0.237
n=30						
1.000	0.008^{1}	$0.024^{1.2}$	0.249^{3}	$1.075^{1.2}$	4.923^{1}	7.785^{2}
SEM	0.001	0.002	0.070	0.158	0.437	0.362
n=30						

produced too large an error. There was little statistical difference between the ADCPGI of the dosed groups and that of the control. An increase in the percent change in ADCPGI implies a decrease in the amount of diet eaten. All treated larvae in the fifth stadium consumed less diet with respect to the fourth stadium than did the control group. Also, 48-hours following treatment all treated groups except the 0.010 ppm sample showed an increase in the ADCPGI compared to the control sample.

3.2 Pupal Development of the Population:

The percentage of larvae pupating ranged from 95% (control) to 54.5% (1.000 ppm) (Table 16). The incidence of pupation appeared to be dose-dependent. As the concentration of fenitrothion increased, the number of insects pupating decreased.

There was little difference between the maximum pupal body weights of the control and the treated samples. All groups, except 0.001 ppm, had larger mean body weights relative to the control. Body weights prior to eclosion were similar for all samples. However, all treated groups displayed larger mean values compared to the control.

The mean number of days spent in the pupal stage (DAP)

Table 15. The amount of diet consumed per gram insect (ADCPGI) at the beginning of each instar for M. sexta larvae treated with different concentrations of fenitrothion. The numbers in brackets represent the percent change in ADCPGI for successive instars. It was calculated by dividing the difference of the older stadia ADCPGI value and that of the previous stadia and multiplying by 100. The percent change in the ADCPGI when dosed was calculated by taking the ADCPGI value 48 hours after treatment and dividing it by the ADCPGI prior to treatment and multiplying by 100. Numbers in a column followed by a similar number are not statistically different from each other (Duncan's Multiple Range Analysis of Variance).

Treatment (ppm)	ADCPGI at the Beginning of Instar				% Change in ADCPGI when Dosed
	II	III	IV	V	
Control	59.78[1]	14.61[1,2]	4.59[1]	1.12[1,2]	44.0
SEM	17.19	1.26	0.77	0.12	
n=30		(75.6)	(68.6)	(75.6)	
0.001	94.28[2]	13.25[1,2,3]	3.49[1]	1.03[1,2]	65.0
SEM	13.70	1.60	1.15	0.08	
n=30		(85.9)	(73.6)	(70.5)	
0.010	29.55[1]	12.00[2,3]	3.14[1]	1.48[3]	43.1
SEM	3.78	1.31	0.63	0.34	
n=30		(59.4)	(73.8)	(52.9)	
0.100	52.62[1]	9.39[3]	3.46[1]	0.91[2]	57.7
SEM	8.06	1.05	0.61	0.09	
n=30		(82.2)	(63.2)	(73.7)	
1.000	63.78[1,2]	16.42[1]	4.26[1]	1.25[1,2]	53.2
SEM	17.55	1.70	1.01	0.12	
n=30		(74.3)	(74.1)	(70.7)	

ranged from 28.59 (0.01 ppm) to 32.15 (control). All dosed groups spent less time in the pupal stage than the control group.

Pupal mortality was statistically similar for all groups tested. It ranged from 10.5% (0.01 ppm and 0.1 ppm) to 16.7% (0.001 ppm and 1.0 ppm).

The incidence of pupal deformities was as low as 5.6% (0.010 ppm) and as high as 25.0% (1.0 ppm). There was no significant difference among any of the groups tested.

The ratio of female pupae to male pupae (PSR) was least for the 0.1 ppm sample (0.46) and the greatest for the 0.001 ppm, 0.01 ppm and 1.0 ppm groups (1.00).

3.3 Adult Development of the Population:

All samples had more than 80% of their pupae surviving to the adult stage (Table 17). There was no statistical difference amongst the five groups.

The mean number of eggs laid per female per two days varied from 22.74 (0.001 ppm) to 47.32 (0.1 ppm). The mean number of eggs produced per female per two days ranged from 42.72 (0.001 ppm) to 61.49 (0.1 ppm). There was a trend for all the treated samples (except 0.001 ppm) to exhibit greater

Table 16. Pupal characteristics of M. sexta whose larvae were exposed to different concentrations of fenitrothion. DAP: mean number of days spent as a pupa. PSR: ratio of female pupae to male pupae. The numbers in brackets represent the difference (days) between the first and last pupa to reach the adult stage. Numbers in a column followed by a similar letter are not significantly different from each other (Duncan's Multiple Range Test). Numbers in a column followed by a similar number are statistically similar (difference of percentages).

Treatment (ppm)	Pupation (%)	Max. pupal wt. (g)	Min. pupal wt. (g)	DAP (days)	Pupal Mortality	Pupal Deformities	PSR (F/M)
Control SEM n=29	95.0^{2}	7.098^{ab} 0.290	2.828^{a} 0.103	32.15^{a} 1.37 (26-44)	13.3^{1}	10.5^{1}	0.89
0.001 SEM n=26	85.7^{2}	6.674^{a} 0.277	2.883^{a} 0.134	31.20^{ab} 0.70 (28-38)	16.7^{1}	16.7^{1}	1.00
0.010 SEM n=25	84.0^{2}	7.272^{ab} 0.240	3.046^{a} 0.114	28.59^{c} 0.51 (26-34)	10.5^{1}	5.6^{1}	1.00
0.100 SEM n=23	$76.0^{1,2}$	7.268^{ab} 0.237	2.865^{a} 0.106	29.41^{bc} 0.41 (28-32)	10.5^{1}	10.5^{1}	0.46
1.000 SEM n=16	54.5^{1}	7.785^{b} 0.362	2.898^{a} 0.238	30.60^{abc} 0.79 (28-36)	16.7^{1}	25.0^{1}	1.00

ovipositioning and fecundity rates than the control.

The proportion of the total egg production retained by females was as low as 23% (0.1 ppm) and as high as 46.7% (control, 0.001 ppm). Females of the 0.01 ppm, 0.1 ppm, and 1.0 ppm samples tended to retain a smaller proportion of their egg complement relative to the control group.

The fertility of the eggs laid was greater than 80% for all samples.

The mean number of days spent in the adult stage ranged from 5.80 (1.0 ppm) to 7.07 (control). Although not significantly different, all treated groups spent less time as adults compared to the control.

The adult sex ratio was least for the 0.1 ppm group (0.55) and greatest for the control group (1.0).

3.4 Total Development Time of the Population:

Insects in the 0.01 ppm, 0.1 ppm and 1.0 ppm groups required less time to complete development compared to the control (Table 18). Generally, these three groups needed more time to complete larval development but completed pupal and adult development faster than the control group.

Table 17. Reproductive biology of M. sexta adults whose larvae were exposed to sublethal doses of fenitrothion. Eggs laid/F/2 days: mean number of eggs oviposited per female per two days. Fec/F/2 days: mean number of eggs produced per female per two days. % eggs retained /F: the percentage of the egg production not oviposited by the female. Fert. (% Hatch): fertility of the eggs laid. DAA: mean number of days spent in the adult stage. ASR: ratio of female adults to male adults. SEM: standard error of the mean. Numbers in a column followed by a similar number are statistically similar (Duncan's Multiple Range Test). Numbers in a column followed by a similar number are statistically similar (difference of percentage).

Treatment (ppm)	Adults (%)	Eggs laid /F/2 days	Fec/F /2 days	% Eggs Retained/F	Fert. (% Hatch)	DAA (days)	ASR (F/M)
Control	86.7[1]	22.91[a]	42.96[a]	46.7	81.3[a]	7.07[a]	1.00
SEM		7.23	12.15		0.2	2.25	
n=25							
0.001	83.3[1]	22.74[a]	42.72[a]	46.7	83.1[a]	6.93[a]	0.75
SEM		6.63	12.20		12.1	2.37	
n=22							
0.010	89.5[1]	39.09[a]	56.59[a]	30.9	82.2[a]	6.12[a]	0.78
SEM		8.28	10.20		19.4	2.40	
n=22							
0.100	89.5[1]	47.32[a]	61.49[a]	23.0	88.4[a]	6.24[a]	0.55
SEM		9.70	11.10		3.1	2.82	
n=21							
1.000	83.3[1]	40.53[a]	60.53[a]	33.0	84.0[a]	5.80[a]	0.60
SEM		10.41	13.74		11.4	2.82	
n=13							

Table 18. Total development of tobacco hornworms who were dosed with different concentrations of fenitrothion during the larval stage. DAL: mean number of days spent in the larval stage. DAP: mean number of days spent in the pupal stage. DAA: mean number of days spent in the adult stage. Tot. Dev. Time: mean number of days from egg-hatch till adult mortality. Numbers in a column followed by a similar number are not statistically different from each other (Duncan's Multiple Range Test).

Treatment (ppm)	DAL (days)	DAP (days)	DAA (days)	Tot. Devel. Time (days)
Control	23.26^{a}	32.15^{a}	7.07^{a}	63.23^{a}
SEM	0.51	1.37	2.25	4.87
n=30				
0.001	26.22^{c}	31.20^{ab}	6.93^{a}	64.00^{a}
SEM	0.68	0.70	2.37	5.36
n=30				
0.010	22.19^{a}	28.59^{c}	6.12^{a}	55.12
SEM	0.85	0.51	2.40	4.44
n=30				
0.100	23.90^{ab}	29.41^{bc}	6.24^{a}	59.53^{b}
SEM	0.58	0.41	2.82	4.09
n=30				
1.000	26.00^{bc}	30.60^{abc}	5.80^{a}	61.80^{ab}
SEM	1.04	0.79	0.63	1.47
n=30				

3.5 Development of Larvae Dosed During the Second Stadium:

The 0.001 ppm and 1.0 ppm concentrations had larvae in the second stadium when dosed (Table 19). Third instar control larvae were included for the purpose of comparison. The control group developed synchronously and had a single body weight maximum of 5.718 g (Fig. 9). The 0.001 ppm sample developed asynchronously and displayed body weight peaks of 3.535 g and 6.206 g. No larvae in the 1.0 ppm group survived to the pupal stage. The initial knockdown of fenitrothion 48-hours after treatment was less than 7.0% for the control and 0.001 ppm groups and 60.0% for the 1.0 ppm sample (Table 19).

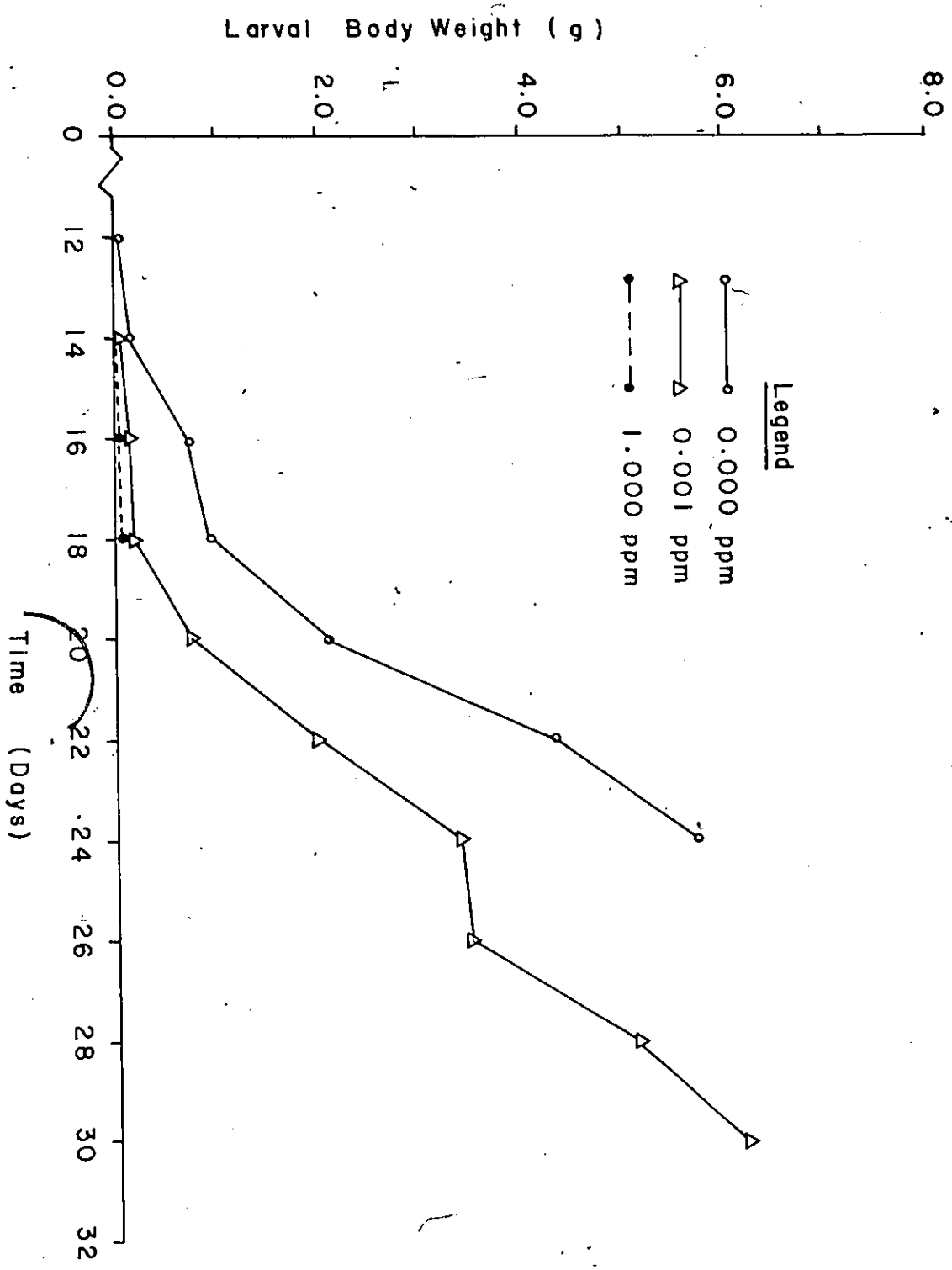
All of the control group's mortalities occurred during the third stadium (6.3%). All larvae in the 0.001 ppm group reached the prepupal stage. Sixty percent of the larvae in the 1.0 ppm sample died in the second stadium and the remaining 40.0% in the third.

Both the 0.001 ppm and 1.0 ppm groups required significantly more time to complete second instar development compared to the control (Table 20). The time needed to complete first, third, fourth, and fifth instar development was similar for all groups. Larvae of the 0.001 ppm sample took about five days longer to reach the pupal stage compared to the control.

Table 19. The knockdown (KD) effect of oral sublethal doses of fenitrothion on second instar tobacco hornworm larvae 48 hours after treatment. Numbers in a column followed by a similar letter are not significantly different from each other (difference of percentages).

Treatment (ppm)	KD (%) After 48 Hours
Control n=24	6.3[a]
0.001 n=4	0.0[a]
1.000 n=6	60.0

Fig. 9. Development of larvae treated during the second stadium with sublethal concentrations of fenitrothion.


Larval Body Weight (g)
0.0
2.0
4.0
6.0
8.0
0
12
14
16
18
20
22
24
26
28
30
32
Time (Days)
Legend
0.000 ppm
0.001 ppm
1.000 ppm

The difference between the time needed for the first and last larva in each group to reach the wandering stage was similar for the control and 0.001 ppm groups (4 and 6 days respectively).

Larvae of the 0.001 ppm sample tended to have larger mean body weights at the end of the third, fourth, and fifth stadia, and maximum larval body weight (Table 21).

As seen previously, the ADCPGI values decrease as the insect develops. With the exception of the third stadium, the mean ADCPGI values were similar for all groups (Table 22). Compared to the control both the 0.001 ppm and 1.000 ppm groups showed a decrease in the ADCPGI following treatment.

3.6 Pupal Development of Larvae Dosed During the Second Stadium:

As mentioned previously, only the 0.001 ppm and 1.000 ppm groups were treated during the second stadium. Third instar control larvae were used for comparison. No larvae in the 1.000 ppm group survived to the pupal stage.

The number of insects pupating was similar for the control group (93.8%) and the 0.001 ppm group (100.0%).

The mean body weights at the beginning and at the end of

Table 20. Mean number of days spent in each instar and total development time of tobacco hornworm larvae treated in the second stadium with different concentrations of fenitrothion. Numbers in brackets represents the difference in age of the first and last larva in each group to reach the prepupal stage. Numbers in a column followed by a similar letter are not statistically different from each other (Student's T-test).

Treatment (ppm)	Time (Days) Spent in Instar					Days as Larva
	I	II	III	IV	V	
Control	4.00[a]	2.75	7.07[a]	4.80[a]	5.87[a]	23.73
SEM	0.00	0.37	0.51	0.43	0.24	0.47
n=24						(22-26)
0.001	4.67[a]	8.00[a]	6.00[a]	3.33[a]	6.67[a]	28.67
SEM	0.67	3.06	2.31	0.68	0.67	1.76
n=4						(26-32)
1.000	4.00[a]	7.00[a]	-	-	-	-
SEM	0.00	1.00	-	-	-	-
n=6						-

Table 21. Mean body weight (g) at the end of each instar for second instar insects treated with different concentrations of fenitrothion. Maximum larval body weight is the weight of the larva prior to the non-feeding, wandering stage.

Treatment* (ppm)	Body Weight (g) at End of Instar					Maximum larval wt. (g)
	I	II	III	IV	V	
Control	0.006	0.020	0.132	1.250	4.888	7.220
SEM	0.001	0.001	0.033	0.108	0.365	0.334
n=24						
0.001	0.007	0.032	0.234	1.325	7.956	7.277
SEM	0.000	0.018	0.041	0.307	1.096	0.875
n=4						
1.000	0.008	0.014	-	-	-	-
SEM	0.001	0.033	-	-	-	-
n=6						

Table 22. The amount of diet consumed per gram insect (ADCPGI) at the beginning of each instar for M. sexta larvae treated with different concentrations of fenitrothion during the second stadium. Numbers in brackets are the percent change in ADCPGI for successive instar. It was calculated by dividing the difference of the older stadia ADCPGI and that of the previous stadia by the previous stadia and multiplying by 100. The percent change in ADCPGI when dosed was calculated by dividing the ADCPGI value 48 hours after treatment by the ADCPGI value prior to treatment and multiplying by 100. Numbers in a column followed by a similar letter are statistically different from each other (Student's t-test).

Treatment (ppm)	ADCPGI at Beginning of Instar				% Change in ADCPGI When Dosed
	II	III	IV	V	
Control	59.78	14.58[a]	4.59	0.96	54.7
SEM	17.19	1.14	0.77	0.12	
n=24	(-)	(75.6)	(68.6)	(79.1)	
0.001	86.42	7.15[a]	2.06	1.27	12.8
SEM	14.89	2.07	0.32	0.02	
n=4	(-)	(91.7)	(71.2)	(38.3)	
1.000	63.78	-	-	-	30.6
SEM	17.56	-	-	-	
n=6					

the pupal stage were larger in the 0.001 ppm group (7.277 g and 3.273 g) than in the control group (7.196 g and 2.903 g).

The 0.001 ppm sample required 34 days to complete pupal development whereas the control group required 31.64 days.

There was no mortality in the control sample but it reached 33% in the 0.001 ppm sample. A total of 6.7% of the control group had pupal deformities whereas the 0.001 ppm group had 0%.

The ratio of female pupae to male pupae was 0.75 for the control group and 0.5 for the 0.001 ppm sample.

3.7 Development of Larvae Dosed During the Third Stadium:

Insects of all dosed populations developed asynchronously (Fig. 10). The control group had a body weight maximum of 5.718 g. All treated samples displayed biphasic growth curves with body weight peaks of 3.092 g and 4.847 g (0.001 ppm), 3.675 g and 4.696 g (0.01 ppm), 3.730 g and 2.708 g (0.1 ppm), and 6.064 g and 5.978 g (1.0 ppm). There was no significant difference in the mean body weights among any of the samples from day 14 to day 18 (Table 3, Appendix A). The control group's mean body weights were greater than those of the treated groups from day 20 to day 22. There was no statistical difference in mean body weights among the four dosed groups after

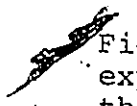
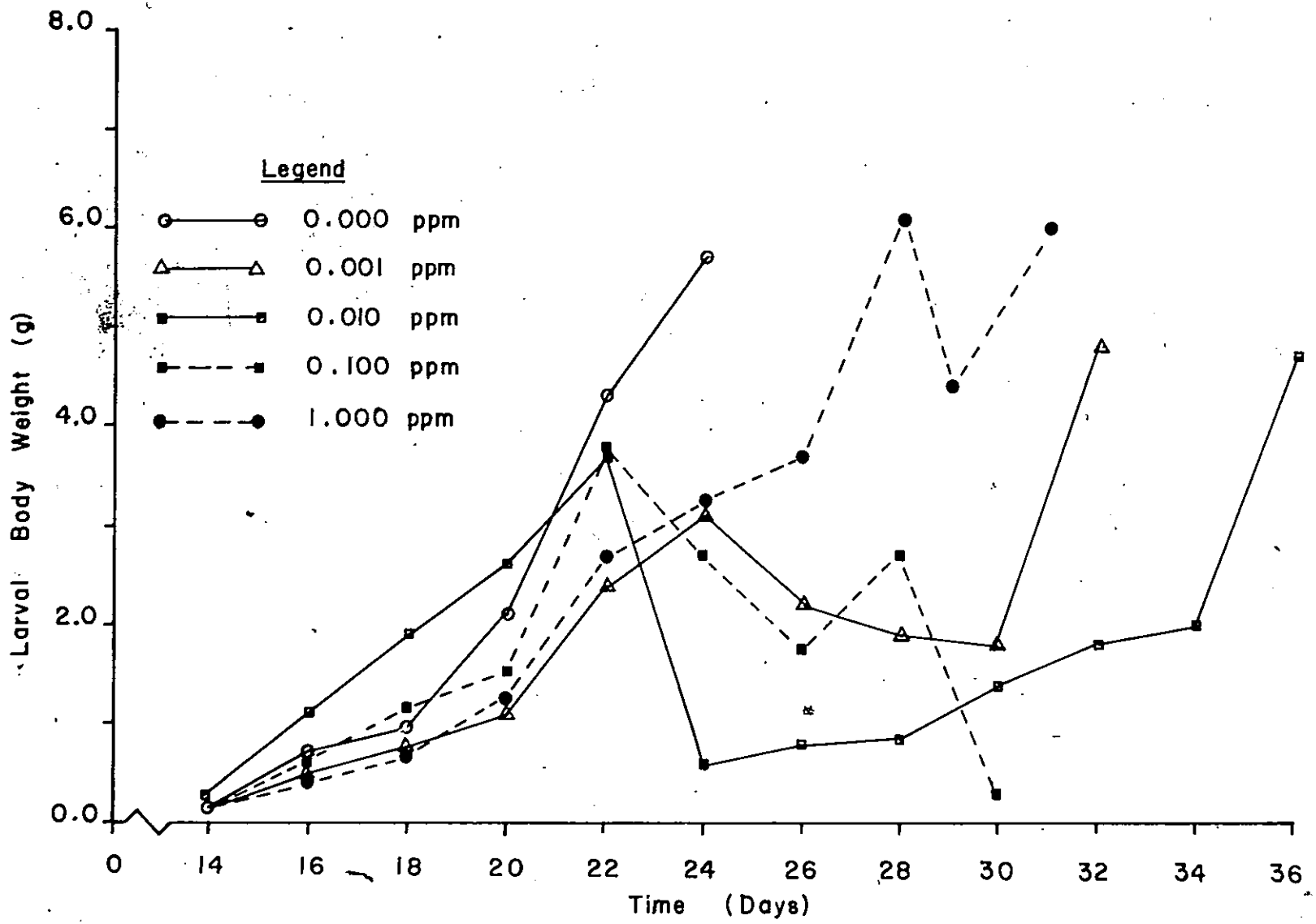


Fig. 10. Development of tobacco hornworm larvae exposed to sublethal doses of fenitrothion during the third stadium.


Legend
0.000 ppm
0.001 ppm
0.010 ppm
0.100 ppm
1.000 ppm
Larval Body Weight (g)
8.0
6.0
4.0
2.0
0.0
0
14
16
18
20
22
24
26
28
30
32
34
36
Time (Days)

day 22.

The initial knockdown (% mortality) 48-hours after treatment was statistically similar for all groups (Table 23). However, as the concentration of fenitrothion was increased the incidence of initial larval mortality also increased.

As with the KD data, total larval mortality also seemed to follow a dose-response relationship (Table 24). The control group displayed the lowest mortality (6.3%) whereas the 1.0 ppm sample had the highest mortality (31.3%). All groups encountered at least 66.7% of their total mortality during the third stadium. The 0.01 ppm sample lost 7.1% of its larvae during the fifth stadium and the 0.1 ppm group incurred mortalities during the fourth stadium (4.8%).

With the exception of fourth instar larvae of the 0.01 ppm group, time spent in each instar did not differ greatly (Table 25). Treated groups required more time to complete the third and fifth stadia. The mean number of days spent as a larva was greater for all treated groups except for the 0.010 ppm sample (Table 25). The range of days needed to complete larval development was narrow for the control group (4 days) and considerably larger for the dosed groups (0.001 ppm and 0.100 ppm (10 days), 0.010 ppm (18 days), and 1.000 ppm (12 days)).

Table 23. The knockdown (KD) effect of oral sublethal doses of fenitrothion on third instar tobacco hornworm larvae 48 hours after treatment.

Treatment (ppm)	KD (%) After 48 Hours
Control n=24	6.3
0.001 n=25	5.9
0.010 n=17	14.3
0.100 n=25	19.1
1.000 n=23	31.3

Table 24. Instar mortality of larvae treated with different concentrations of fenitrothion during the third stadium. The numbers in brackets represent the proportion each instar mortality contributes to the total larval mortality.

Treatment (ppm)	Instar Mortality (%)					Total larval mortality (%)
	I	II	III	IV	V	
Control SEM n=24	0.0 (0.0)	0.0 (0.0)	6.3 (100)	0.0 (0.0)	0.0 (0.0)	6.3
0.001 SEM n=25	0.0 (0.0)	0.0 (0.0)	11.8 (66.7)	5.9 (33.3)	0.0 (0.0)	17.7
0.010 SEM n=17	0.0 (0.0)	0.0 (0.0)	14.3 (66.8)	0.0 (0.0)	7.1 (33.2)	21.4
0.100 SEM n=25	0.0 (0.0)	0.0 (0.0)	23.8 (83.2)	4.8 (16.8)	0.0 (0.0)	28.6
1.000 SEM n=23	0.0 (0.0)	0.0 (0.0)	31.3 (100)	0.0 (0.0)	0.0 (0.0)	31.3

Table 25. Mean number of days spent in each instar and total development time of tobacco hornworm larvae treated with different concentrations of fenitrothion during the third stadium are presented with the standard error of the mean (SEM). Numbers in brackets represent the age (days) of the first and last larva to reach the prepupal stage. Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test).

Treatment (ppm)	Time (days) Spent in Instar					Days as larva
	I	II	III	IV	V	
Control	4.00^{a}	2.75^{ab}	7.07^{a}	4.80^{a}	5.87^{ab}	23.73^{a}
SEM	0.00	0.37	0.51	0.43	0.24	0.47
n=24						(22-26)
0.001	4.00^{a}	4.86^{b}	7.87^{ab}	5.00^{a}	6.00^{ab}	26.00^{a}
SEM	0.00	1.44	0.57	0.41	0.30	0.70
n=25						(24-34)
0.010	4.00^{a}	2.18^{a}	7.17^{a}	2.83	7.46^{b}	23.45^{a}
SEM	0.00	0.18	0.58	0.63	0.77	1.53
n=17						(20-38)
0.100	4.00^{a}	3.17^{ab}	7.50^{a}	4.80^{a}	5.73^{a}	24.67^{a}
SEM	0.00	0.58	0.65	0.47	0.38	0.54
n=25						(20-30)
1.000	-	-	9.46^{b}	4.55^{a}	6.36^{ab}	26.36^{a}
SEM	-	-	0.72	0.72	0.93	1.07
n=23						(22-34)

The mean body weights at the end of the first, second, and fifth stadia were statistically similar for all groups tested (Table 26). The 1.000 ppm sample had a larger mean body weight at the end of the third stadium compared to the other groups. The mean body weight of the 0.010 ppm group at the end of the fourth stadium was significantly less than those of the other samples. The 1.000 ppm was the only group to display a significantly larger maximum larval body weight compared to the control. It should be noted that the mean body weights at the end of the fifth stadium and the maximum larval body weight tended to increase with the concentration of fenitrothion.

The ADCPGI at the beginning of each stadium did not vary greatly (Table 27). All groups except the 0.01 ppm sample showed a slight decrease in the quantity of diet consumed 48-hours following treatment with fenitrothion compared to the control group.

3.8 Pupal Development of Larvae Dosed During the Third Stadium:

The number of insects surviving to the pupal stage ranged from 93.8% (control) to 68.8% (1.0 ppm) (Table 28). Although there was no significant difference among any of the samples, the number of insects pupating decreased as the concentration of fenitrothion increased.

Table 26. Mean weights (g) at the end of each instar for insects treated during the third stadium with different concentrations of fenitrothion. Maximum larval weight (g) is the mean weight of the larvae prior to the non-feeding, wandering stage. Numbers in a column followed by a similar letter are not statistically different from each other using a Duncan's Multiple Range Test.

Treatment (ppm)	Body Weight (g) at the End of Instar					Maximum larval wt. (g)
	I	II	III	IV	V	
Control	0.006[a]	0.020[a]	0.132[ab]	1.250[a]	4.880[a]	7.220[ab]
SEM	0.001	0.001	0.033	0.108	0.365	0.334
n=24						
0.001	0.006[a]	0.029[a]	0.210[ab]	1.258[a]	4.334[a]	6.410[a]
SEM	0.001	0.003	0.038	0.114	0.338	0.308
n=25						
0.010	0.008[a]	0.030[a]	0.170[ab]	0.242[b]	4.417[a]	6.870[ab]
SEM	0.002	0.003	0.037	0.098	0.362	0.299
n=17						
0.100	0.006[a]	0.028[a]	0.129[a]	1.343[a]	4.753[a]	7.322[ab]
SEM	0.000	0.003	0.025	0.115	0.360	0.288
n=25						
1.000	-	0.026[a]	0.262[b]	1.120[ab]	5.082[a]	7.759[b]
SEM	-	0.002	0.075	0.160	0.448	0.396
n=23						

Table 27. The ADCPGI at the beginning of each instar for third stadium larvae treated with different concentrations of fenitrothion. The numbers in brackets represent the percent change in the ADCPGI for successive instars. It was calculated by dividing the difference of the older ADCPGI value and that of the previous stadia by the previous stadia and multiplying by 100. The percent change in ADCPGI when dosed was calculated as above except the ADCPGI before treatment and 48 hours after treatment were used. Numbers in a column followed by a similar letter are statistically similar using a Duncan's Multiple Range Test.

Treatment (ppm)	ADCPGI at the Beginning of Instar				% Change in ADCPGI when dosed
	II	III	IV	V	
Control	59.78[a]	14.58[ab]	4.59[a]	0.96[a]	54.7
SEM	17.19	1.14	0.77	0.12	
n=24	(-)	(75.6)	(68.5)	(79.1)	
0.001	99.99	14.15[ab]	3.82[a]	0.93[a]	53.1
SEM	20.45	1.82	1.41	0.09	
n=25	(-)	(85.8)	(73.0)	(75.7)	
0.010	29.55[a]	10.62[a]	3.03[a]	1.47[b]	56.5
SEM	3.78	1.16	0.57	0.16	
n=17	(-)	(64.1)	(71.5)	(51.5)	
0.100	52.62[a]	10.54[a]	3.45[a]	0.89[a]	36.4
SEM	8.06	1.23	0.61	0.09	
n=25	(-)	(80.0)	(67.3)	(74.2)	
1.000	-	16.07[b]	4.57[a]	1.17[ab]	47.3
SEM	-	1.84	0.96	0.09	
n=22	(-)	(-)	(71.6)	(74.4)	

There was no statistical difference between the maximum pupal body weight of the control group and the four treated groups. The 0.001 ppm and 0.01 ppm samples had mean body weights smaller than the control whereas the maximum pupal body weights of the 0.1 ppm and 1.0 ppm groups were larger than the control. The maximum pupal body weights of the dosed groups increased as the level of fenitrothion was increased. The minimum pupal body weights were statistically similar for all samples. All treated groups, except the 0.1 ppm group, had lower mean body weights than the control prior to eclosion.

The mean number of days required to complete pupal development varied from 28.67 (0.01 ppm) to 31.64 (control). Compared to the control group, all treated groups spent less time in the pupal stage.

Pupal mortality ranged from 0% (control and 0.01 ppm) to 18.2% (1.0 ppm).

The 0.01 ppm had the least number of pupal deformities (0%) whereas the 1.0 ppm group incurred the greatest number (27.3%).

The ratio of female pupae to male pupae varied from 0.50 (0.1 ppm) to 1.20 (1.0 ppm).

Table 28. Pupal characteristics of M. sexta whose larvae were exposed to different concentrations of fenitrothion during the third instar. DAP: mean number of days as a pupa. PSR: ratio of female pupae to male pupae. The numbers in brackets represent the difference (days) between the first and last pupa to reach the adult stage. Numbers in a column followed by a similar letter are not statistically different from each other (Duncan's Multiple Range Test). Numbers in a column followed by the same number are statistically simmilar (differences of percentages).

Treatment (ppm)	Pupation (%)	Max. Pupal Wt. (g)	Min. Pupal Wt. (g)	DAP (days)	Pupal Mortality	Pupal Deformities	PSR (F/M)
Control SEM n=23	93.8[1]	7.196[ab] 0.322	2.903[a] 0.077	31.64[ab] 1.00 (26-38)	0.0[1]	6.7[1,2]	0.75
0.001 SEM n=20	82.4[1]	6.511 1.740	2.769[a] 0.140	30.83[ab] 0.63 (28-36)	14.3[1]	21.4[1,2]	1.00
0.010 SEM n=13	78.6[1]	6.873[ab] 0.298	2.728[a] 0.103	28.67[b] 0.75 (26-34)	0.0[1]	0.0[1]	1.50
0.100 SEM n=18	71.4[1]	7.322[ab] 0.288	2.912[a] 0.118	29.54[ab] 0.46 (28-32)	13.3[1]	13.3[1,2]	0.50
1.000 SEM n=16	68.8[1]	7.759[b] 0.396	2.870[a] 0.264	30.89[ab] 0.26 (28-36)	18.2[1]	27.3[2]	1.20

3.9 Development of Larvae Treated During the Fourth Stadium:

The control group developed synchronously and had a body weight maximum of 4.354 g 18-days after eclosion (Fig. 11). The 0.1 ppm group had two body weight peaks of 3.698 g and 4.275 g. Larvae of the 0.001 ppm, 0.01 ppm and 1.0 ppm samples reached their body weight peaks (4.587 g, 5.156 g, and 3.159g, respectively) four to six days after the control group.

The initial knockdown (% mortality) 48-hours after treatment was zero for all groups tested. The 0.010 ppm sample was the only group to record instar mortalities (9.9% during the fourth stadium).

All dosed groups except 1.0 ppm spent more time than the control in the fourth stadium (Table 29). The 0.100 ppm group spent less time completing the fifth stadium than the other samples. All dosed groups required more time to complete larval development compared to the control group. The range of days needed to reach the prepupal stage was narrow for all groups.

All treated samples displayed larger mean body weights at the end of the fifth stadium compared to the control whereas that of the 1.0 ppm sample was less than the control group (Table 30). Compared to the control group, all treated samples

Fig. 11. Development of tobacco hornworm larvae treated with sublethal doses of fenitrothion during the fourth stadium

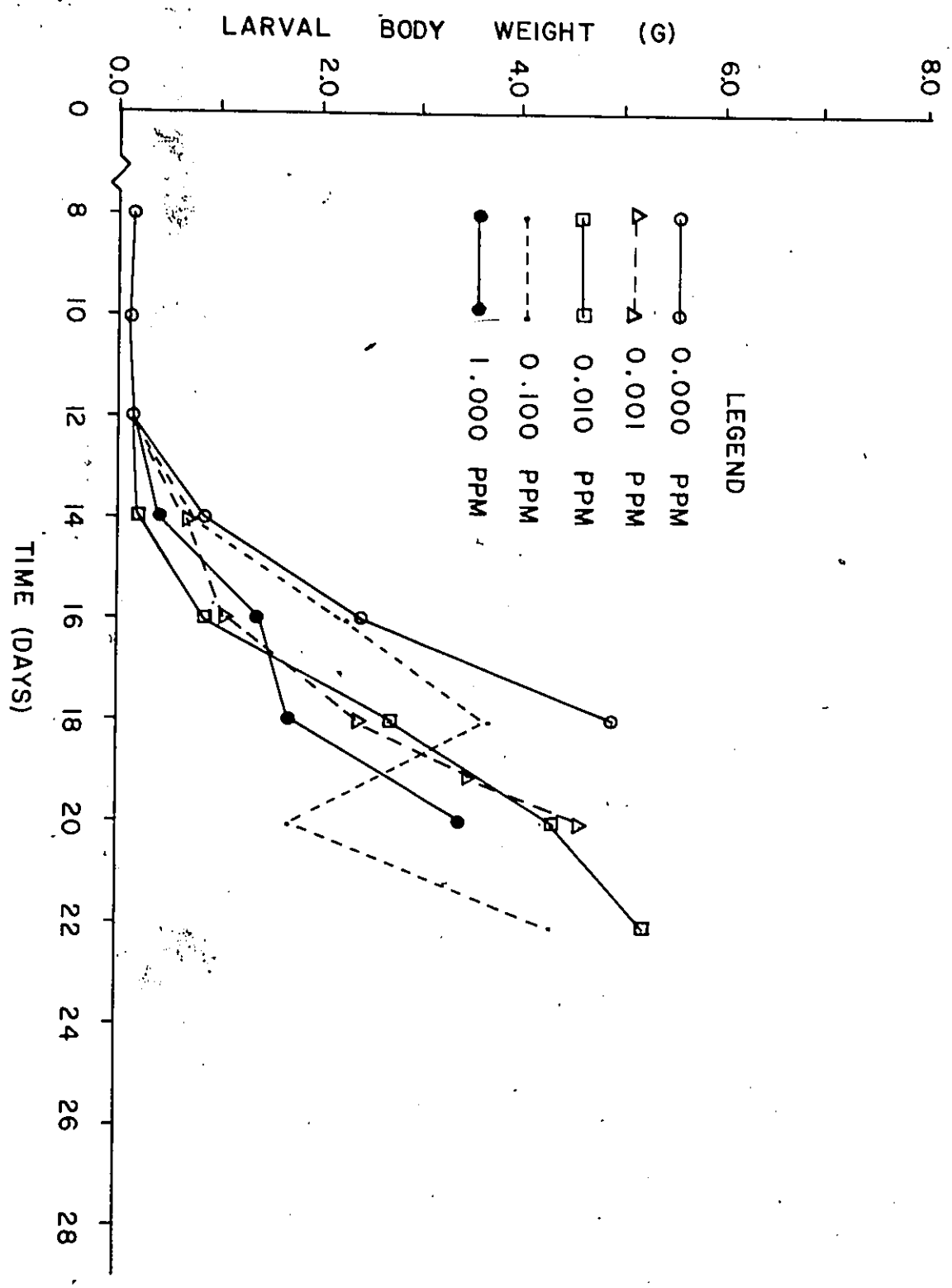

LARVAL BODY WEIGHT (G)
0.0
2.0
4.0
6.0
8.0
TIME (DAYS)
0
8
10
12
14
16
18
20
22
24
26
28
LEGEND
0.000 PPM
0.001 PPM
0.010 PPM
0.100 PPM
1.000 PPM

Table 29. Mean number of days spent in each instar and total development time of larvae treated with different concentrations of fenitrothion during the fourth stadium. Numbers in brackets represent the age (days) of the first and last larva to reach the prepual stage.

Treatment (ppm)	Time (days) Spent in Instar				Days as larva
	II	III	IV	V	
Control	-	3.00	5.00	7.00	20.00
SEM	-	0.58	0.58	0.58	0.00
n=6					(20)
0.001	-	4.00	6.00	8.00	22.00
SEM	-	-	-	-	-
n=1					(22)
0.010	-	3.27	5.80	7.00	20.80
SEM	-	0.30	0.20	0.33	0.33
n=13					(20-22)
0.100	-	2.00	7.50	5.50	21.00
SEM	-	0.00	1.50	0.50	1.00
n=5					(20-26)
1.000	-	4.00	4.00	10.00	22.00
SEM	-	-	-	-	-
n=1					(22)

had greater maximum larval body weights.

The ADCPGI at the beginning of the fourth stadium could not be calculated (Table 31). Fifth instar ADCPGI values were similar for all groups. All treated groups except the 0.100 ppm sample showed a slight depression in the ADCPGI 48-hours following treatment.

3.10 Pupal Development of Larvae Treated During the Fourth Stadium:

The number of pupae within each group ranged from 1 (0.001 ppm and 1.0 ppm) to 10 (0.01 ppm). Due to the small sample sizes, no statistical analyses were performed.

The percentage of fourth instar larvae pupating was greater than 90% for all groups (Table 32).

The maximum pupal body weights ranged from 6.643 g (control) to 8.072 g (1.0 ppm). The body weights increased as the concentration of fenitrothion increased. Body weights prior to eclosion was a low as 2.415 g (control) and as high as 3.547 g (0.01 ppm). All treated groups had larger mean body weights than the control.

The amount of time spent in the pupal stage varied from 28 days (1.0 ppm) to 35 days (control). All treated groups

Table 30. Mean body weights (g) at the end of each instar for insects treated during the fourth stadium with various concentrations of fenitrothion. Maximum larval weight (g) is the mean weight of the larvae prior to the wandering stage.

Treatment (ppm)	Body Weight (g) at the End of Instar					Maximum larval weight (g)
	I	II	III	IV	V	
Control	-	0.021	0.146	0.417	4.354	6.638
SEM	-	0.005	0.023	0.142	0.514	0.601
n=6						
0.001	-	0.023	0.191	0.751	4.857	7.153
SEM		-	-	-	-	-
n=1						
0.010	-	0.021	0.164	0.812	4.939	7.714
SEM	-	0.003	0.011	0.162	0.250	0.344
n=13						
0.100	-	0.036	0.128	1.178	4.532	7.062
SEM	-	0.002	0.013	0.197	0.268	0.365
n=5						
1.000	-	0.016	0.104	0.441	3.359	8.072
SEM	-	-	-	-	-	-
n=1						

Table 31. The ADCPGI at the beginning of each instar for fourth instar larvae treated with different concentrations of fenitrothion. The percent change in ADCPGI when dosed was calculated by dividing the difference of the ADCPGI value prior and after treatment by the ADCPGI value prior to treatment and multiplying by 100. Fourth instar values could not be obtained because all larvae were moulting to the fourth instar when they were starved prior to dosage.

Treatment (ppm)	ADCPGI at the Beginning of Instar				% Change in ADCPGI When Dosed
	II	III	IV	V	
Control	-	14.70	-	1.71	66.1
SEM	-	4.17	-	0.13	
n=6					
0.001	-	16.56	-	1.53	47.6
SEM	-	-	-	-	
n=1					
0.010	-	13.17	-	1.50	57.9
SEM	-	2.25	-	0.09	
n=13					
0.100	-	5.41	-	1.21	67.2
SEM	-	0.61	-	0.02	
n=5					
1.000	-	6.59	-	2.35	64.3
SEM					
n=1	-	-	-	-	

required less time to complete pupal development than the control group.

The only control group and the 0.01 ppm group incurred pupal mortalities (50% and 20%, respectively) and pupal deformities (25% and 10%, respectively).

The ratio of female pupae to male pupae varied from 0/1 to 1/0 (1.0 ppm and 0.001 ppm, respectively) to 2.00 (control).

4.0 Larval Development of the F_1 Generation:

Synchronous growth curves were recorded by groups 0.001 and 0.01 ppm (Fig. 12). Both had a single body weight maximum of 4.948 g and 4.066 g respectively. Larvae of the control and 0.1 ppm samples displayed biphasic growth curves with body weight peaks of 2.972 g and 6.036 g, and 3.415 g and 6.032 g respectively. The 1.0 ppm group had three body weight peaks (2.788 g, 3.097 g, and 1.749 g).

Larval mortality varied from 4% (0.01 ppm) to 52% (1.0 ppm) (Table 33). With the exception of the 0.01 ppm sample, mortality of all treated groups was higher than the control group.

Incomplete ecdysis was common in all treated groups.

Table 32. Pupal characteristics of M. sexta whose larvae were treated with sublethal dosages of fenitrothion during the fourth stadium. DAP: mean number of days as a pupa. PSR: the ratio of female pupae to male pupae. The numbers in brackets represent the difference (in days) between the first and last pupa to reach the adult stage.

Treatment (ppm)	Pupation (%)	Max. Pupal Wt. (g)	Min. Pupal Wt. (g)	DAP (days)	Pupal Mortality (%)	Pupal Deformities (%)	PSR (F/M)
Control	100.0	6.643	2.415	35.00	50.0	25.0	2.00
SEM		0.598	0.543	9.00			
n=6				(26-44)			
0.001	100.0	7.153	3.547	30.00	0.0	0.0	1/0
SEM		-	-	-			
n=1				(-)			
0.010	90.9	7.717	3.405	28.50	20.0	10.0	0.60
SEM		1.086	0.120	0.73			
n=12				(26-32)			
0.100	100.0	7.062	2.709	29.00	0.0	0.0	0.33
SEM		0.365	0.253	1.00			
n=5				(28-32)			
1.000	100.0	8.072	3.151	28.00	0.0	0.0	0/1
SEM		-	-	-			
n=1				(-)			

Fig. 12. Development of F_1 generation larvae whose P_1 generation was reared under non-diapause conditions and exposed to sublethal doses of fenitrothion.

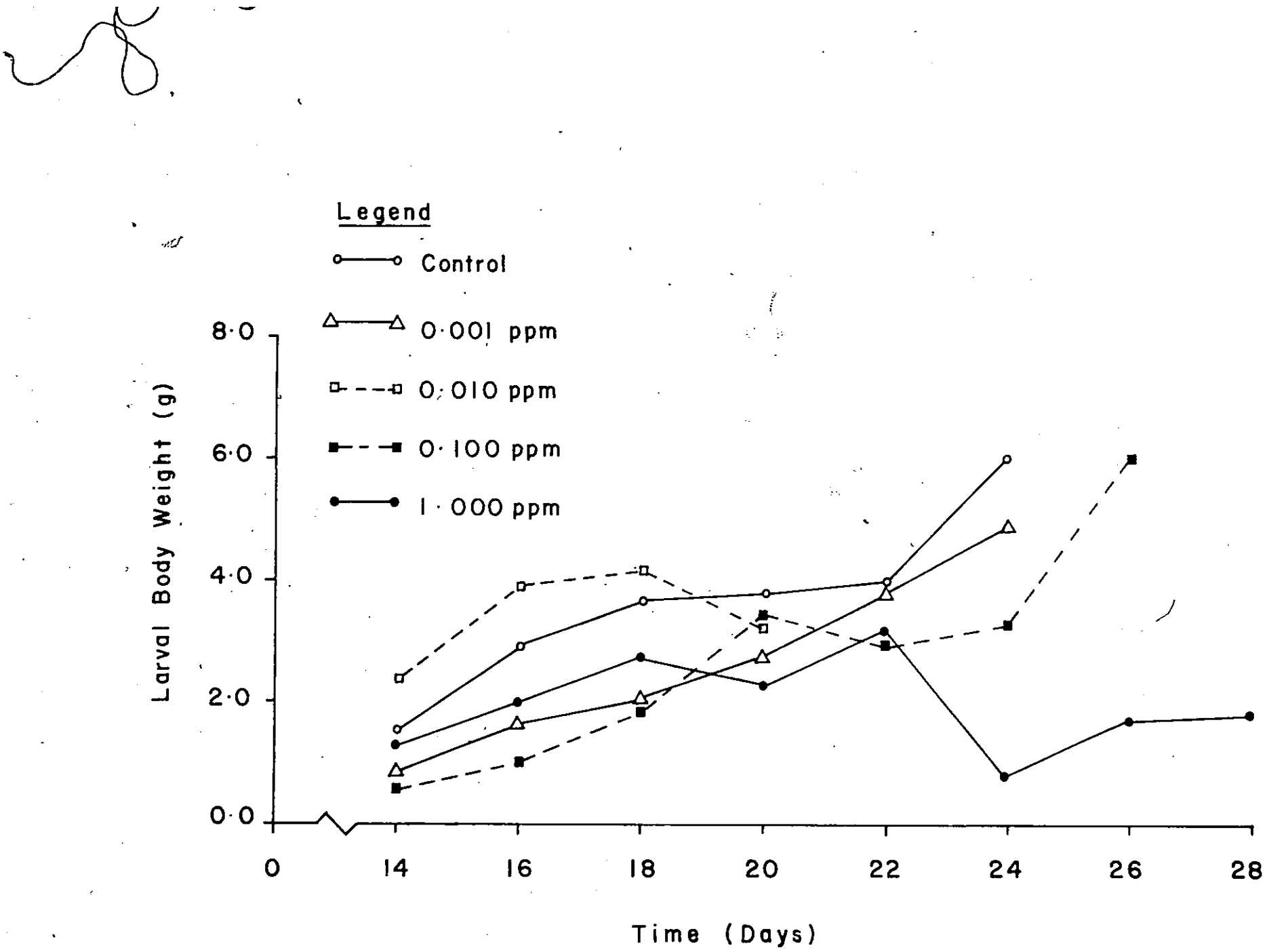

Legend
Control
0·001 ppm
0·010 ppm
0·100 ppm
1·000 ppm
Larval Body Weight (g)
8·0
6·0
4·0
2·0
0·0
0
14
16
18
20
22
24
26
28
Time (Days)

Table 33. The development of F1 generation M. sexta larvae whose parental generation was treated with sublethal doses of fenitrothion. Numbers in a column followed by a similar number are not statistically different from each other (difference of percentages). Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test). The sample size of all groups was n=25.

Parents Treatment (ppm)	Larval Mortality (%)	Maximum Larval Wt. (g)	Days As Larva	Larvae Pupating Before 5.0 (g) (%)
Control	15.5[1.2]	7.998	20.82[a]	0.0[1]
SEM		0.246	0.69	
0.001	36.0[2.3]	6.845[a]	21.50[a]	12.0[1]
SEM		0.521	0.70	
0.010	4.0[1]	6.660[a]	18.50	12.0[1]
SEM		0.296	0.37	
0.100	36.0[2.3]	6.656[a]	22.38[a]	12.0[1]
SEM		0.401	0.52	
1.000	52.0[3]	6.701[a]	20.67[a]	4.0[1]
SEM		0.375	0.71	

There was one instance where a larva of the 1.0 ppm group was unable to completely shed its cuticle. Haemolymph filled the cavity between the old and the new cuticle (see Plate 4). The haemolymph disappeared after a day. The larva died two days later.

The control group had the highest mean larval body weight (7.998 g) (Table 33). This was statistically larger than the four treated samples.

There was no significant difference in the mean number of days spent in the larval stage among any of the groups.

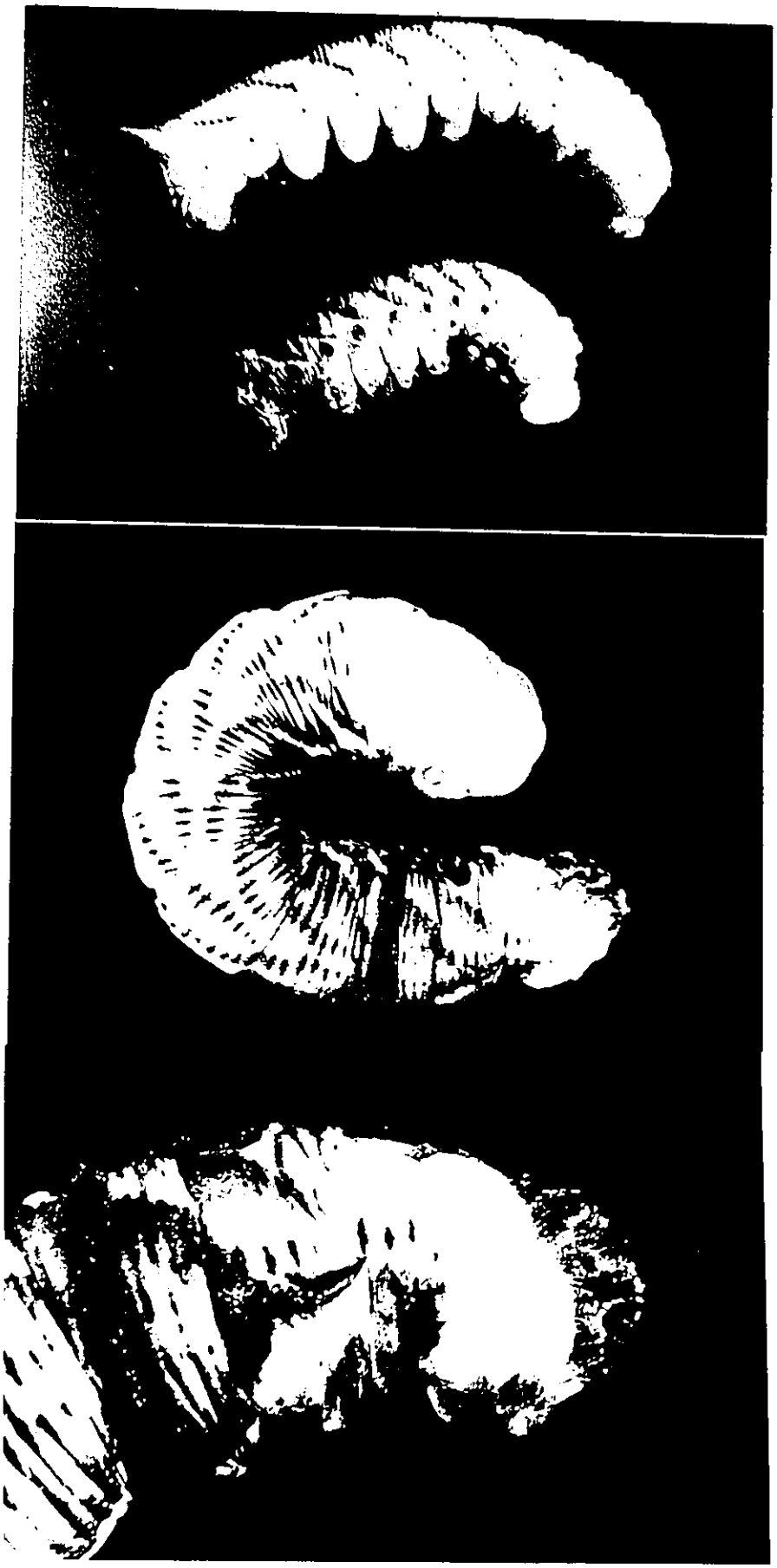
All treated samples had instances where larvae pupated prior to reaching a minimum larval weight of 5.0 g. The values varied from 4% (1.0 ppm) to 12% (0.001 ppm, 0.01 ppm, and 0.1 ppm).

4.1 Pupal Development of the F_1 Generation:

The proportion of larvae reaching the pupal stage varied from 48% (1.0 ppm) to 96% (0.01 ppm) (Table 34).

The mean maximum pupal body weights of all treated groups were statistically smaller than the control group. The mean minimum pupal body weights of the treated groups also tended

Plate lV. Larval deformities. a: normal larva (top) shedding smaller head capsule. A regressive moult (below). Note the larger head capsule being shed. b: incomplete larval ecdysis with haemolymph filling the space between old and new cuticle. c: a closeup of b.


a
b
c

to be smaller than the control.

The mean number of days spent in the pupal stage was as low as 35.5 days (0.1 ppm) and as high as 39.14 (control). All treated groups took less time to complete pupal development relative to the control sample.

Pupal mortality was 25% or less for the control, 0.001 ppm, 0.1 ppm, and 1.0 ppm groups and 63.3% for the 0.01 ppm sample.

Pupal deformities ranged from 0% (1.0 ppm) to 22.2% (control).

All groups had a greater number of female pupae than male pupae. The ratios varied from 1.33 (0.1 ppm) to 3.0 (1.0 ppm).

4.3 Adult Development of the F_1 Generation:

The 0.01 ppm sample had the least number of pupae surviving to the adult stage (36.7%) (Table 35). All other samples had 75% or more adult emergence.

The mean number of eggs laid per female per two days was 12.92 for the control. The 0.001 ppm and 0.01 ppm group values' were less than the control's (0.0 and 0.28 respectively) and the 0.1 ppm and 1.0 ppm groups values' were greater than the

Table 34. The development of F_1 generation M. sexta pupae whose parental generation was exposed to sublethal doses of fenitrothion. PSR: ratio of female pupae to male pupae. Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test). Numbers in a column followed by a similar number are statistically similar (differences of percentages).

Parents Treatment (ppm)	Pupation (%)	Maximum Pupal Wt. (g)	Minimum Pupal Wt. (g)	Days As Pupae	Pupal Mortality (%)	Pupal Deformities (%)	PSR (F/M)
Control	$84.6^{2,3}$	7.998	2.803^{a}	39.14^{a}	22.2^{1}	22.2^{1}	2.33
SEM		0.246	0.135	1.44			
n=21							
0.001	$64.0^{1,2}$	6.845^{a}	2.727^{a}	38.17^{a}	25.0^{1}	6.3^{1}	1.17
SEM		0.521	0.197	1.19			
n=16							
0.010	96.0^{3}	6.660^{a}	2.779^{a}	36.00^{a}	63.3	9.1^{1}	1.50
SEM		0.296	0.359	3.06			
n=24							
0.100	$64.0^{1,2}$	6.656^{a}	2.698^{a}	35.50^{a}	25.0^{1}	12.5^{1}	1.33
SEM		0.401	0.240	1.40			
n=16							
1.000	48.0^{1}	6.701^{a}	2.369^{a}	36.00^{a}	20.0^{1}	0.0^{1}	3.00
SEM		0.375	0.032	3.06			
n=12							

control's (66.79 and 47.2 respectively). The mean number of eggs produced per female per two days showed similar trends. The values of the 0.001 ppm and 0.01 ppm groups (8.42 and 22.19 respectively) were less than the control (32.59) whereas the 0.1 ppm and 1.0 ppm were greater than the control (87.32 and 59.63 respectively). The proportion of the egg production total retained by females varied from 100% (0.001 ppm) to 20.8% (1.0 ppm). The 0.001 ppm and 0.01 ppm samples were lower than the control group and the 0.1 ppm and 1.0 ppm groups had values greater than the controls.

Fertility of the eggs laid ranged from 0% (0.001 ppm and 0.01 ppm) to 84.0% (1.0 ppm).

Although not statistically different, all treated samples spent more time in the adult stage compared to the control group.

The proportion of female adults to male adults was as low as 3/0 (0.01 ppm) and as high as 3.0 (0.001 ppm).

4.4 Total Development Time of the F_1 Generation:

Insects of all treated groups tended to take slightly more time to complete development than did the control (Table 36). In general, treated groups required about the same time

Table 35. Development of F_1 generation *M. sexta* adults whose parental generation was exposed to sublethal concentrations of fenitrothion. Eggs Laid/F/2 days: mean number of eggs oviposited per female per two days. Fec/F/2 days: mean number of eggs produced per female per two days. ASR: ratio of female adults to male adults. Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test). Numbers in a column followed by a similar number are statistically similar (differences of percentages).

Parents Treatment (ppm)	Adults (%)	Eggs Laid/ F/2 days	Fec./F /2 days	% Egg Production Retained	Fertility (% Hatch)	Days As Adult	ASR (F/M)
Control	77.8[1]	12.92[a]	32.59[a]	60.4	66.0[2]	7.71[a]	2.50
SEM		5.73	13.28			1.87	
n=16							
0.001	75.0[1]	0.00[a]	8.42[b]	100.0[1]	0.0[1]	15.33[a]	3.00
SEM		0.00	4.51			2.17	
n=12							
0.010	36.7	0.28[a]	22.19[ab]	98.7[1]	0.0[1]	16.67[a]	3/0
SEM		0.22	11.54			0.67	
n=9							
0.100	75.0[1]	66.79	87.32	23.5[2]	50.0[2]	11.50[a]	1.00
SEM		15.23	20.64			1.64	
n=12							
1.000	80.0[1]	47.20	59.63	20.8[2]	84.0[2]	13.50[a]	1.40
SEM		11.42	14.13			2.17	
n=10							

to complete larval development, less time to complete pupal development, and more time to complete adult development in relation to the control sample.

Table 36. Development of F_1 generation M. sexta adults whose parental generation was exposed to sublethal concentrations of fenitrothion. Numbers in a column followed by a similar letter are statistically similar (Duncan's Multiple Range Test).

Parents Treatment (ppm)	Days As Larva	Days As Pupa	Days As Adults	Total Development Time (Days)
Control	20.82[a]	39.14[a]	7.71[a]	71.14[a]
SEM	0.69	1.44	1.87	
n=25				
0.001	21.50[a]	38.17[a]	15.33[a]	77.00[a]
SEM	0.70	1.19	2.17	2.49
n=25				
0.010	18.50	36.00[a]	16.67[a]	71.33[a]
SEM	0.37	3.06	0.67	2.40
n=25				
0.100	22.38[a]	35.50[a]	11.50[a]	71.17[a]
SEM	0.52	1.40	1.64	2.76
n=25				
1.000	20.67[a]	36.00[a]	13.50[a]	73.50[a]
SEM	0.71	3.06	2.17	5.12
n=25				

DISCUSSION

1. The Growth of the Tobacco Hornworm on Different Diets:

Tobacco hormworm larvae were reared most successfully on diets Tfi, Bs, and Bst. Of the seven diets tested, only larvae of group Tfi developed synchronously. With the exception of larval mortality and mean number of days spent in the larval stage, insects reared on Tfi fared better than those reared on Bs or Bst.

Larval mortality was high for most groups including Tfi. The small plastic rearing vials used in this experiment limited the larva's space and were poorly ventilated. Condensation would build up in the vial either drowning the larvae or promoting the growth of microbial contaminants. Mortality of control larvae reared in larger, better ventilated vials on diet Tfi in the toxicology experiments was low (see Table 12).

The addition of water to Tf resulted in an increased ADCPGI. Insects tend to consume more diet when the nutrients are diluted (Vanderzant, 1969). The addition of tobacco to the diets also increased the ADCPGI. Tobacco had a phagostimulatory effect on tobacco hornworm larvae. This insect has been successfully reared on tobacco-free diets for many years (Bell and Joachim, 1976). However, the larvae lose their specificity to tobacco and, in addition, can no longer survive on tobacco plants or

tobacco based diets (Dethier, personal communication; Jermy et al, 1968).

The greater incidence of pupal mortalities and deformities in groups Bs and Bst could have been the result of excessive or inadequate levels of essential nutrients (House, 1965).

Adults of Tfi had the greatest reproductive potential of all the diets tested. Reduced adult yield, ecdysis failure, mortalities, and deformities of insects reared on an inadequate diet is well documented (Chippendale and Beck, 1965 ; Dadd, 1960; Navon, 1978; Rock, 1967; Vanderzant et al, 1962). It is clearly evident from the data that ingredients of Tfi best satisfied the nutritional requirements of the tobacco hornworm and promoted the insects' growth and development better than the other diets tested.

2. Adult Sex Ratio and Reproductive Performance:

The optimum sex ratio for laboratory reared tobacco hornworm adults was 3F/3M. This ratio displayed the highest rates of oviposition and egg production of the five groups tested (Table 9) and is in agreement with the work of Otake and Sakuratami (1972). The percentage of the total egg production retained in the abdomens of females of the 3F/3M group was least. Females of the 2F/4M and 4F/2M samples also had good

oviposition and fecundity rates.

The fertility of the eggs laid was dependent on the number of males present in the group (Table 9). This agrees with the findings of Burton (1969), Guerra, et al (1972), Jones et al (1975, 1979), and Otake and Sakuratami (1972).

Females of the 1F/5M and 5F/1M ratios showed some interesting trends. Mated females of the 5F/1M group deposited fertile eggs within the first 8 days of emergence (Fig. 4). After this period infertile eggs were laid. The first peak (day 4) of the 5F/1M group corresponds with the fertile egg deposition peak of the 1F/5M sample. The second peak (day 10) is related to the activity of virgin females. Wigglesworth (1966) stated that virgin females produced the same number of ripe eggs as mated females and that copulation was the stimulus for oviposition. Females of the 5F/1M group may have delayed oviposition because of the absence of the copulation stimulus. Eight days after emergence, an overriding mechanism may have induced the females to begin laying infertile eggs.

Jones et al (1979) found that large numbers of males relative to the number of females decreased female longevity. This appears to be the case with the 1F/5M group. The longer life span of females of the 5F/1M group (about 14 days) could be attributed to the low number of males present. However, it is

apparent from Fig. 4 that virgin females reach their oviposition peak after mated females and live longer than mated females. Therefore, the longevity of females appears to be related to the mating status of the female rather than the number of males present.

The percentage of the total egg production retained by females was highest for the 1F/5M and 5F/1M samples. The higher percentage shown by the 5F/1M group could be attributed to the virgin females in the sample as Wigglesworth (1966) had observed. The female of the 1F/5M group laid fertile eggs. The large proportion of the total egg production retained by this female may have been the result of a stress related impairment of reproductive behavior that Guerra et al (1972) proposed.

3.0 The Sublethal Effects of Fenitrothion on the Development of the P_1 Generation:

3.1 Development of the Population:

Sublethal doses of fenitrothion had a definite effect on M. sexta development. All treated populations developed asynchronously to the pupal stage (Fig. 7). In general, the mean number of days needed to complete larval development was greater for most treated groups compared to the control (Table 13). Associated with the treated groups' longer development time was the larger mean body

weights at the end of the larval stage (Table 14). The ADCPGI was reduced for all treated groups 48 hours after treatment with fenitrothion (Table 15).

The reduced rate of development of the treated groups was probably the result of a decrease in food consumption (Turunen, 1972, 1975, 1977) and poor food utilization (Tanton and Khan, 1978c). Fenitrothion could have disrupted the midgut epitheluim so that the efficiency of food absorption and utilization was reduced. Many treated larvae were observed discharging partially digested semi-liquid wastes both orally and rectally. The damaged disgestive system could also have had an inhibitory influence on feeding.

The treated larvae required more time to surpass the minimum fifth instar body weight required for pupation to be initiated (Table 13) (Nijhout, 1975; Nijhout and Williams, 1975a,b). The dose-dependent increase of the groups' maximum larval body weight (Table 14) could be the result of an inhibition of JH metabolism (Pratt, 1975) or some other impairment of the insect's endocrine system.

As might be predicted, mortality was dose-dependent. The regressive moults recorded in groups 0.001, 0.1 and 1.0 ppm indicate that fenitrothion affected some larvae of each group more than others.

Pupae of all samples recorded similar body weights prior to adult eclosion (Table 16). The mean number of days spent in the pupal stage was less for the dosed groups with respect to the control. There also may be a critical minimum weight pupae must reach prior to emergence. The control group had similar mortalities and deformities as the treated groups. It is more likely that these effects are the result of the stress involved with starvation and general rearing rather than latent toxic effects of fenitrothion.

Adults of treated larvae recorded a greater reproductive potential compared to the control adults (Table 17). The increase in the rates of oviposition and fecundity of the treated groups tended to be dose-dependent. These results are consistent with the findings of Hodjat (1971) and Honek and Novak (1978). The reduction in the proportion of the total egg production retained by females of the treated groups could be attributed to the short life of these adults. As the concentration or the pesticide dose increased, the life-span of the adults decreased. The reduced longevity of the treated adults could have been the result of latent toxicity, an impairment of endocrine function, or an inadequate supply of protein, carbohydrate, or liquid reserves. This could follow from remarks made earlier on the absorption of food in treated larvae. As the fertility of all treated groups was high, mating did not appear to be affected.

The total development time of the treated impaired insects was generally shorter than the control. Treated larvae took longer than the control to develop, while treated pupae and adults required less time to complete development. Boles (1974), De Coursey and Webster (1952), Grosch (1975), Riviere (1974), and Zettler and Le Cato (1974) recorded similar results. It would seem that once larvae have overcome the detrimental effects of the insecticide, surviving individuals possess an adaptive mechanism that ensures the emergence of adults at roughly equal times. This is similar to the effect of naturally occurring alkaloids on the dark-sided cutworm (Devitt 1978).

3.2 Development of Insects Treated During the Second Stadium:

Only two groups contained second instar larvae at the time of treatment: 0.001 ppm and 1.0 ppm. The sample size of each group was small. Third stadium control insects were used as a comparison. It is recognized that using third instar control insects is not the most accurate approach for interpretation of the data but this still permits some comparison of treated and untreated insects.

The 1.0 ppm dose of fenitrothion was very toxic to second instar larvae whereas the 0.001 ppm dose was not. Compared to the third instar control data, second instar larvae of the 0.001 ppm sample required more time to complete larval development,

weighed more at the end of the pupal stage, and displayed a decrease in the ADCPGI 48 hours after treatment.

Pupae of the 0.001 ppm group weighed less than the control and took longer to complete pupal development.

3.3 Development of Insects Treated During the Third Stadium:

The population of all treated groups grew asynchronously (Fig. 10). Weight at the end of the larval stage was less than the control for the 0.001 and 0.01 ppm groups and greater than the control for the 0.1 and 1.0 ppm samples (Table 26). Maximum larval body weight increased with the concentration of fenitrothion. Presence of sublethal doses of fenitrothion in the developing larvae appears to offset the minimum larval body weight proposed by Nijhout (1975) and Nijhout and Williams (1975 a,b). Sublethal doses could also have affected the initiation of pupation by interfering with the insect's endocrine system. Low doses of fenitrothion had no apparent effect on the ADCPGI (Table 27). A greater degree of intestinal tract damage in the 0.1 and 1.0 ppm groups may have caused the depression of the ADCPGI valves. Mortality was dose-dependent (Table 24).

The mean number of days spent in the pupal stage of all treated groups was less than the control (Table 28). As suggested earlier, this could be a mechanism to compensate for the longer

larval development time of the treated samples and ensure synchronous eclosion. The higher incidence of mortalities and deformities of the 0.001, 0.1, and 1.0 ppm groups is consistent with the findings of Bond et al (1969), Bond and Upitis (1973), Gough (1939), and Loschiavo (1960).

3.4 Development of Larvae Treated During the Fourth Stadium:

The asynchronous development of the treated fourth instar larvae (Fig. 11) was less pronounced than for treated second and third instar larvae. However, treated fourth instar larvae still required more time to complete their development (Table 29) and weighed more at the end of the larval stage (Table 30) compared to the control sample. The low mortalities recorded by all treated groups indicates that fourth instar larvae are less sensitive to sublethal doses of fenitrothion ranging from 0.001 - 1.0 ppm or are better equipped to cope with the toxicant.

The pupal body weight prior to adult emergence of the dosed samples was greater than the control (Table 32). Consistent with previous results, the number of days required for treated pupae to complete development was less than the control. Fewer pupal deformities and mortalities were recorded in the fourth instar treated groups than in the third instar treated groups.

4.0 The Sublethal Effects of Fenitrothion on the Development of the F_1 Generation:

Sublethal doses of fenitrothion not only affected development of the P_1 generation but the F_1 generation as well. Larvae of all treated groups weighed less at the end of the larval stage compared to the control (Table 33). Most treated larval groups experienced a greater number of larval mortalities. The higher incidence of larvae initiating pupation at body weight lower than 5.0 g and the incidence of incomplete ecdysis of larvae of treated groups suggests an interference with the insect's endocrine system on a long-term basis.

As recorded with the P_1 generation, the weight of the treated pupae prior to adult emergence was less than the control group (Table 34). The development time of the treated pupae was consistently less than the control.

The oviposition and fecundity rates of the F_1 control group were less than the control values of the P_1 generation (Table 35). Adults of the 0.001 and 0.01 ppm groups recorded very low fecundity and egg laying rates whereas those of the 0.1 and 1.0 ppm groups was greater. The females of the 0.1 and 1.0 ppm groups managed to lay a larger proportion of their eggs compared to the other groups. All treated adults spent more time in the adult stage than did control adults.

Treated pupae tended to take less time to complete development whereas the treated adults displayed a greater longevity compared to the control. As a result, the life span of all treated groups was greater than the control group.

CONCLUSIONS

1. Diet experiments:

A wheat germ based diet with dried tobacco leaves added, and an increased water content (Tfi) best satisfied the nutritional requirements of the tobacco hornworm and was the most successful in promoting the growth and development of the tobacco hornworm.

2. Adult Sex Ratio and Reproductive Performance:

a) The optimum sex ratio for laboratory reared tobacco hornworm adults was 3F/3M.

b) The fertility of the eggs laid was dependent on the number of males present in the group.

c) Unmated females of the 5F/1M group began oviposition 8 days later than mated females and lived 14 days longer than mated females.

d) Females of the 1F/5M and 5F/1M samples retained a larger proportion of their egg complement compared to females of the other sex ratios.

3. Effects of Fenitrothion on the Development of the P_1 Generation:

a) All treated larvae displayed a lower growth rate than the control.

b) Larval mortality was dose-dependent.

c) Treated pupae required less time than the control, to complete development.

d) Treated adults exhibited a greater reproductive potential than the control. Therefore, fenitrothion, at low doses, appears to have a stimulatory effect on fecundity.

e) Total development time of all treated groups was less than the control.

4. Effects of Fenitrothion on the Development of the F_1 Generation:

a) Treated larvae weighed less at the end of the larval stage compared to the control.

b) Larval mortality of all treated groups was less than the control.

c) In treated larvae, the initiation of the pupal stage could occur at body weights of less than 5 g.

d) Pupae of the treated groups required less time than the control to complete development.

e) The reproductive potential of the 0.001 ppm and 0.01 ppm adults was less than the control whereas that of the 0.1 ppm and 1.0 ppm adults was greater than the control.

f) The time spent in the adult stage and total development time of the treated groups were greater than the control.

Further Studies

Most research projects generate more questions upon their completion than were initially asked before the work was begun. Some further studies should prove interesting and enlightening in the following areas: (1) repeat the diet experiments under better rearing conditions and for more generations in order to verify the results presented in this thesis, (2) it would be interesting to conduct a histological study of the digestive tract of the treated tobacco hornworm larvae so that the presence or absence of lesions can be determined. These studies might also prove useful in elucidating the amount of time required for the digestive tract to recover from sublethal doses of fenitrothion; (3) Investigate the sublethal effects of fenitrothion on the insect's endocrine system. One should attempt to correlate acetylcholinesterase inhibition with JH, ecdysone, and PTTH titers and how this inhibition would affect growth, ecdysis, initiation of the pupal and adult stage, and on the reproductive potential of the tobacco hornworm; (4) Study the F_1 generation of treated adults to determine whether any impairment of the endocrine system resulted from the parental generation being exposed to low doses of fenitrothion; (5) It is important that a protocol be established to separate the effects of stress of the perturbant and the effects of stress from the environment.

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APPENDIX

Fig. 13 The weight loss of Bst diet cubes after two days. The regression curves were used to correct errors in the ADCPGI calculations for the diet experiments. The regression equations of the other diets were:

C: $y = 45.82x^{-0.76}$

F: $y = 49.87x^{-0.79}$

Tf: $y = 50.33x^{-0.80}$

Tfi: $y = 63.55x^{-0.95}$

Tfiv: $y = 57.01x^{-0.80}$

Bs: $y = 53.42x^{-0.78}$

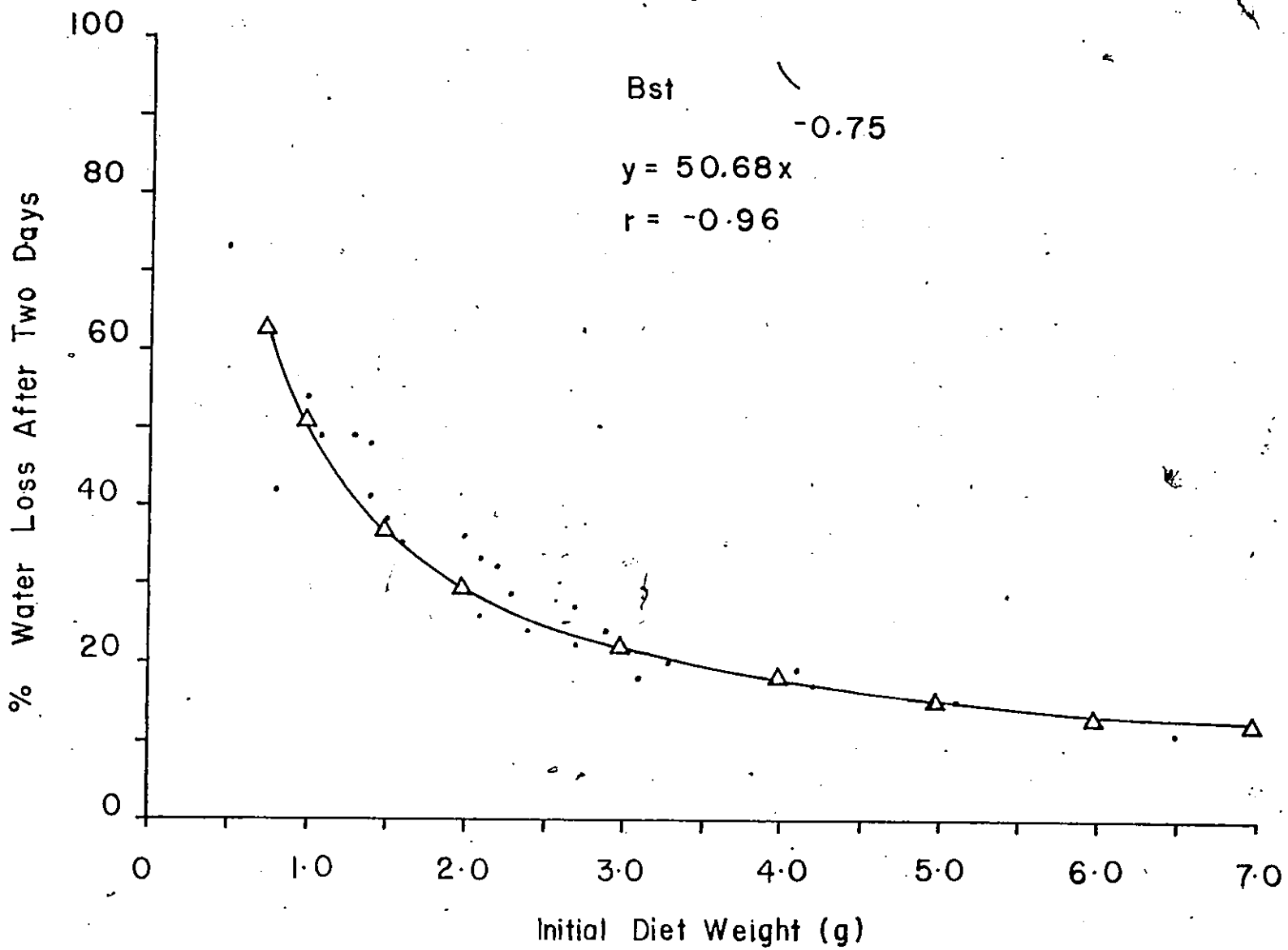
Bst
-0.75
y = 50.68x
r = -0.96
% Water Loss After Two Days
100
80
60
40
20
0
0
1·0
2·0
3·0
4·0
5·0
6·0
7·0
Initial Diet Weight (g)

Table 37 Body weights of tobacco hornworm larvae reared on seven meridic diets. Mean weights (g) are presented with the standard error of the mean (SEM). Numbers in a column followed by a similar letter are not statistically different from each other (Duncan's multiple range test).

	Time (days)														
Diet	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
C	0.02^{ab}	0.03^{ab}	0.11^{b}	0.21^{ab}	0.56^{ab}	1.04^{ab}	2.45^{ab}	1.37^{a}	1.02^{a}	0.92^{a}	1.10^{a}	0.85	2.70	1.62	
SEM	0.00	0.00	0.01	0.04	0.09	0.20	0.55	0.42	0.43	0.37	0.35	0.36	1.79	-	
F	0.01^{a}	0.02^{a}	0.07^{a}	0.13^{a}	0.37^{a}	0.62^{a}	1.26^{a}	1.83^{a}	2.37^{a}	2.07^{a}	1.09	1.6	3.12	5.10	3.73
SEM	0.00	0.00	0.01	0.02	0.08	0.12	0.43	0.54	0.45	1.35	-	-	-	-	-
Tf	0.01	0.03^{ab}	0.06^{a}	0.14^{a}	0.34^{a}	0.81^{a}	1.24^{a}	1.55^{a}	2.44^{a}	2.10^{a}	2.66^{a}	3.40	4.26	3.12	
SEM	0.00	0.00	0.00	0.01	0.04	0.07	0.15	0.26	0.33	0.74	1.06	0.14	1.30	-	
Tfi	0.02^{bc}	0.03^{bc}	0.12^{bc}	0.31^{ab}	0.93^{c}	1.77^{b}	4.11^{c}	0.80^{a}	0.23	0.19					
SEM	0.00	0.00	0.02	0.05	0.12	0.30	0.43	0.61	-	-					
Tfiv	0.02^{a}	0.04^{bc}	0.13^{bc}	0.28^{ab}	0.83^{bc}	1.57^{b}	3.12^{bc}	1.29^{a}	1.62^{a}	1.09^{a}	1.05^{a}	0.19			
SEM	0.00	0.00	0.01	0.04	0.10	0.24	0.51	0.29	0.67	0.81	0.81	-			
Bs	0.02^{bc}	0.06	0.13^{bc}	0.59	1.11^{c}	2.84	4.51^{c}	3.70^{a}	1.17^{a}	1.53^{a}	2.28^{a}	5.56			
SEM	0.00	0.01	0.01	0.11	0.16	0.35	0.68	1.12	0.66	0.57	1.02	0.05			
Bst	0.01^{a}	0.04^{c}	0.15^{c}	0.32^{b}	0.91^{c}	1.73^{b}	3.59^{bc}	3.61^{a}	3.30^{a}	3.48^{a}	4.88				
SEM	0.00	0.00	0.01	0.03	0.09	0.21	0.40	0.91	0.87	0.25	-				

Table 38 Mean body weight (g) during the course of larval development of tobacco hornworms exposed to different concentrations of fenitrothion. SEM: Standard error of the mean. Numbers in a column followed by a similar letter are not significantly different from each other using a Duncan's multiple range test. Day 4 represents the fourth day after emergence from the egg.

	Time (days)																
	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
0.000	0.01[a]	0.02[a]	0.06[a]	0.05[a]	0.07[a]	0.29[a]	1.02[a]	1.67[ab]	2.08[a]	4.53[a]	5.72						
SEM	0.00	0.00	0.01	0.01	0.01	0.07	0.18	0.35	0.23	0.45	0.42						
0.001	0.001[c]	0.02[a]	0.04[a]	0.04[a]	0.04[a]	0.13[a]	0.42[bc]	0.73[ac]	1.42[a]	2.70[ab]	3.24[a]	3.73[a]	6.06[a]	4.37	5.98		
SEM	0.00	0.00	0.01	0.01	0.00	0.01	0.03	0.11	0.15	0.32	0.51	0.69	0.99	1.44	-		
0.010	0.01[bc]	0.03	0.11	0.07	0.13	0.54	1.79	2.78	3.38	3.68[ab]	0.63[a]	0.79[a]	0.69[a]	1.38	1.85	2.01	4.70
SEM	0.00	0.00	0.01	0.01	0.02	0.09	0.22	0.34	0.51	1.37	0.28	0.06	0.05	-	-	-	-
0.100	0.01[ab]	0.02[a]	0.07[a]	0.05[a]	0.07[a]	0.28[a]	0.97[ac]	1.74[b]	1.50[a]	3.76[ab]	2.69[a]	1.75[a]	2.71[a]	0.26			
SEM	0.00	0.00	0.01	0.01	0.01	0.01	0.22	0.31	0.18	0.56	0.77	1.62	2.51	-			
1.000	0.01[ab]	0.02[a]	0.05[a]	0.04[a]	0.04[a]	0.13[a]	0.42[b]	0.72[c]	1.25[a]	2.36[b]	3.27[a]	2.63[a]	3.44[a]	4.01	4.87		
SEM	0.00	0.00	0.01	0.01	0.01	0.04	0.07	0.14	0.24	0.41	0.59	1.40	1.57	2.20	-		

Table 39 The mean body weights (g) of larvae treated the third stadium with various concentrations of fenitrothion are presented with the standard error of the mean (SEM). Numbers in a column followed by a similar letter are not statistically different from each other using a Duncan's multiple range test.

Treatment (ppm)	Time (days)																
	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
0.000	0.001[a]	0.02[a]	0.04[a]	0.03[a]	0.04[a]	0.14[a]	0.71[a]	0.95[a]	2.10[ab]	4.32[a]	5.72[a]						
SEM	0.00	0.00	0.00	0.00	0.00	0.02	0.12	0.14	0.23	0.44	42						
0.001	0.01[a]	0.02[a]	0.05[ab]	0.03[ab]	0.04[a]	0.10[a]	0.43[a]	0.71[a]	1.13[c]	2.43[b]	3.09[a]	2.20[a]	1.88	1.81	4.85		
SEM	0.00	0.00	0.01	0.00	0.01	0.01	0.06	0.12	0.17	0.51	2.25	1.88	-	-	-		
0.010	0.01[a]	0.03[b]	0.07[b]	0.05[b]	0.07	0.24	1.05	1.91	2.59[a]	3.68[ab]	0.63	0.79	0.69	1.38	1.85	2.01	4.70
SEM	0.00	0.00	0.01	0.01	0.01	0.05	0.19	0.37	0.54	1.32	0.28	0.06	0.05	-	-	-	-
0.100	0.01[a]	0.02[a]	0.05[ab]	0.04[ab]	0.04[a]	0.15[a]	0.65[a]	1.13[a]	1.49[bc]	3.73[ab]	2.69[a]	1.75[a]	2.71	0.26			
SEM	0.00	0.00	0.01	0.01	0.01	0.03	0.11	0.18	0.20	0.60	0.77	1.62	2.51	-			
1.000	0.01	0.03[b]	0.05[ab]	0.04[ab]	0.04[a]	0.12[a]	0.39[a]	0.64[a]	1.26[b]	2.70[ab]	3.24[a]	3.73[a]	6.06	4.37	5.98		
SEM	0.00	0.00	0.01	0.01	0.01	0.02	0.08	0.14	0.30	0.51	0.13	0.99	1.44	-	-		