

INFLUENCE OF FOOD PLANTS ON GROWTH AND DEVELOPMENT, AND
SUSCEPTIBILITY TO INSECTICIDES OF *SPODOPTERA LITTORALIS*
(BOISDUVAL)

by

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DEDICATION

This thesis is dedicated to my two daughters Nana Ackon and Nana Yaa Boatema. They have always been a source of encouragement in times of adversity.

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ABSTRACT

A series of experiments was conducted to evaluate the responses of *S. littoralis* (Boisd) to seven plants species. Probable factors responsible for the preference for some of the food plants were determined.

In multiple-choice preference tests, both 1st and 4th instar larvae preferred dicotyledonous to monocotyledonous plants. First instar larvae rejected millet. In two-choice tests using maize, millet and sorghum, the larvae always preferred maize.

Studies on growth and development of the larvae on the food plants were carried out. The larvae failed to develop to the pupal stage on millet. Growth was adversely influenced on maize and sorghum. Only 7% of the neonate larvae on sorghum and 32% of the larvae on maize developed to the adult stage.

Food digestion and utilisation studies indicated low food intake, digestion and utilisation of food by larvae on maize, millet and sorghum.

Analysis of the plants showed that differences in the larval performances on the plants could be due to both nutritional and allelochemical factors. There were more deterrent leaf extracts in the plants that reduced larval growth. Nitrogen, amino acids and the water content of the

plants were lower in the monocot than the dicot plants.

The total P-450 content and larval susceptibility to insecticides were influenced by the plants fed on by the larvae. Scanning of microsomal preparations showed that the variations in the peptide profiles of the microsomes were associated with the P-450 contents of the plants.

Piperonyl butoxide, barbital and β -naphthoflavone affected feeding of the last larval instar.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 *SPODOPTERA LITTORALIS*

1.1.1 Economic importance

The tropical and subtropical genus *Spodoptera* Guenée (Lepidoptera: Noctuidae) includes several economically important species in Africa, Asia and America (Todd and Poole, 1980; Delvare and Raspulus, 1994).

Spodoptera littoralis (Boisduval) is a polyphagous insect. It has long been known in economic entomological literature under the name *Prodenia litura* (Bishara, 1934; Brown and Dewhurst, 1965). Commonly, it is known as the Egyptian cotton leafworm.

It is the main pest of cotton in some cotton growing countries, especially Egypt, Israel and India. In East, West and North Africa, it often causes extensive damage to soybeans, vegetables and cowpeas. It could, therefore, threaten their cultivation in the future, if adequate control measures are not taken. When large numbers of the pest are present complete crop loss is possible (Singh and van Emden, 1979; Khalil, 1988). *S. littoralis* is also the single most important defoliator of cocoa in some West African countries (Entwistle, 1972).

In Ghana, it has been recorded on crops such as cocoa, tobacco, cabbage, cotton, eggplant, maize, jute, kenaf and cowpea (Forsythe, 1966; Angyeme-Sarpong, 1978; Duodu and Biney, 1981).

Considerable difficulties have been experienced in controlling *S. littoralis* especially in areas where chemical control is frequently used. This is because it has been observed to develop resistance in a relatively short time to the chemicals used (Broza et al., 1984; El-Sabae et al., 1993).

1.1.2 Geographic distribution

Spodoptera littoralis is widely distributed in the world. The northern limit of its distribution probably represents the extent of migrant activity only, while the actual distribution limits may be further south where the species is able to overwinter (Ingram, 1975; Miller, 1977). The literature provides little indication of specific factors limiting the northerly distribution, but mortality due to low winter temperatures may be responsible.

1.1.3 Food plants and seasonal activities

The food plant provides one or more of the four essential resources for the insect. These are feeding sites, mating sites, oviposition sites and/or refugia (Prokopy et al., 1984). It is uncommon to find plants that are immune to attack by virtually all insects. It is also uncommon to find insects that devour indiscriminately all plants in their geographical range.

Spodoptera littoralis attacks plants in 44 families containing at least 112 species of plants of varying

economic importance. However, only 8 families contain over 50 per cent of the plants infested. These families are Gramineae, Leguminosae, Solanaceae, Malvaceae, Cruciferae, Moraceae, Compositae and Chenopodiaceae (Moussa *et al.*, 1960; Arunin, 1978; Sarto and Monteys, 1988; Lal and Naji, 1990).

These plants may not be preferred equally for feeding and oviposition. It has been observed that on castor leaves, larvae of *S. littoralis* fed and developed normally, while on avocado, the leaves were toxic to the 1st and 2nd instars (Sneh and Gross, 1981).

Egg-mass infestation on cotton could reach a peak 3 times during the year. The largest population (first peak) was in early to mid-June. The other peaks were in mid to late July and August. These egg-masses were more abundant on young leaves on the upper parts of the plants than on older leaves on the lower parts (Afifi, 1990).

The larvae are nocturnal. They are active between 21.00h and 06.00h and mostly found on the upper third of the plant (Hosny *et al.*, 1982; Nasr *et al.*, 1984; Sanino *et al.*, 1987).

In light trap catches in Egypt, peak flight activity occurred between 20.00h and 00.00h in winter, 18.00h and 02.00h in the spring, and sunset and dawn in summer.

Irrespective of seasons, catches were highest between 20.00h and 22.00h (Nasr et al., 1984). In the entire year, 0.65% was captured in winter, 2.41% in spring. 36.59% in summer and 60.35% in autumn, in Egypt (Hosny et al., 1982; Matthews, 1989).

The insect pest has 8 generations in a year, four of which occur on berseem clover, *Trifolium alexandrium* (in winter and spring), 2-3 generations on cotton (in summer), and possibly one generation on corn (Hafez, 1972) or on vegetables (Ali, 1989). It is, therefore, able to survive throughout the winter months. The number caught in the traps was low in January and February and high in June and August. There was, however, no systematic seasonal change in the area of highest catch that could suggest seasonal migration. The pattern of nightly catches at most traps indicated that the build-up of local populations was the most important factor affecting the population dynamics of the pest. However, local redistribution by wind may also have affected the changes in the local populations (Nasr et al., 1984).

1.1.4 Nature of damage

Pest assessment studies show that crops in general vary greatly between sites and between years in their response to attack by similar numbers of insects. There could also be great variability in the reaction of individual plants of the same crop to pest attack. Damage

is, therefore, not always proportional to the size of the pest population.

There are no reports of detrimental effects of feeding of adults on crop plants. The insect is destructive only in the larval stage. The larvae are defoliators attacking crops in various stages of growth. In cotton and tobacco, the early instars of the larvae scrape the lower surfaces of the leaves while the older larvae eat the leaf blades-avoiding the main veins and thus reducing the leaves to skeletons. There is also a characteristic smell in a heavily infested field (Ripper and George, 1965; Sanino *et al.*, 1987).

Additionally, the larvae bore into young shoots and flower buds which then wither and die. During heavy infestations they may also attack young cotton bolls which then fail to develop. These attacks retard or stop further growth of the plant and thus reduce yield (Bishara, 1934; Sanino *et al.*, 1987; Matthews, 1989). In the early season, natural defoliation by *S. littoralis* could result in 50% loss of cotton yield (Russell *et al.*, 1993).

There are no indications of the introduction of pathogens into the plant organs that are bored into during larval feeding. However, in the laboratory, 28 species of fungi have been found in association with the various stages of the moth (Ismail and Abdel-Sater, 1993).

1.1.5 Parasitoids and predators

A large number of natural enemies of the larvae have been described (Gerling 1971; Hegazi et al., 1977; Ingram, 1981). There are, however, no reports of parasitism of egg masses or adults.

In Cyprus, *S. littoralis* is parasitised by 12 species of Hymenoptera and five species of Tachnidae (Ingram, 1981) and in Egypt, parasitoids belonging to five families have been recorded. Four of the families belong to the order Hymenoptera and one belongs to the order Diptera (Hegazy et al., 1977; Ibrahim, 1987). Some of the parasitoids observed in the field were the braconid *Zelet chlorophthalma*; *Chelonus inanitus* which regulated *S. littoralis* population by inducing a precocious onset of metamorphosis and developmental arrest in the precocious pre-pupa. Other parasitoids are *Strobliomyia orbata*, and *Microplitis rufiventris* a larval parasitoid which, in Egypt, produced 1-34 per cent parasitism of the larvae in the summer months (Ingram, 1981).

Ingram (1981) has observed that even though the apparent parasitism of *S. littoralis* in fields, in Cyprus, was 32%, effective control of the moth could not occur because most of the parasitoids themselves were polyphagous. Gerling (1971) and Matthews (1989) concluded that the extent of the activity of the parasitoids is insufficient to significantly control *S. littoralis* in

cotton fields in Egypt, especially at the time of peak flowering, which frequently coincided with periods of high rainfall.

Predators observed attacking and suppressing larval *Spodoptera littoralis* populations include spiders (Mansour, 1987), ladybird beetles, lace-wing and damsel bugs and birds (Ingram, 1975; Clercq et al., 1988).

1.1.6 Life cycle

Spodoptera littoralis has four growth stages, namely the egg, larval, pupal and adult stages.

The eggs are laid in masses mostly on the underside of leaves. Each egg mass is made up of layers of eggs which are covered with parental abdominal hairs. The number of eggs laid ranges from 500 to 3000 per female and the incubation period ranges from 3 to 7 days (Hosny et al., 1986; Sanino et al., 1987; Afifi, 1990).

The oviposition period ranges from 2 to 7 days (Ocete, 1984; Afifi, 1990). Some environmental conditions affect the number of eggs laid by the female. On cotton, the frequency of irrigation influences the number of eggs laid. Larger numbers of eggs are laid on plants that are more frequently irrigated (Bishara, 1934; Madkour and Hosny, 1973; Hosny et al., 1986).

There are 5 to 8 larval instars. The number of larval instars and the duration of the larval period depend on the food plant and the environmental conditions. The larval period ranges from 9 to 34 days (Bishara, 1934; Moussa *et al.*, 1960; Prasad and Bhattacharya, 1975). On hatching, the larvae are gregarious at first but disperse after the second instar.

Pupation normally takes place in cocoons in the soil. The pupal period ranges from 9 to 27 days Prasad and Bhattacharya, 1975).

The adults live for 7 to 20 days. The rate of oviposition and viability of the eggs are significantly dependent on the food offered to the adult moth (Ellis and Brimacombe, 1980).

The complete life cycle lasted 45 days at 28°C and 90 days at 22°C on tobacco (Sanino *et al.*, 1987), 39 days on sweet potato, 28 days on okra, 30 days on cotton and 26 days on berseem (Moussa *et al.*, 1960).

One of the factors which affects the life stages of the moth is light. Exposure to constant light, when the moth was reared on cotton, significantly reduced the number of eggs laid, the oviposition period and the adult lifespan. Darkness, even for a short period, stimulated the noctuid to lay more eggs and increased the adult lifespan.

1.2 Control of *S. littoralis*

Various methods ranging from chemical to cultural are being used to control *S. littoralis*. The emphasis, in recent times, has been on the combination of two or more methods, in an integrated pest management system. The choice and combination of the methods depend on the pest complex, the severity of the infestation and the cost of its implementation (Matthews, 1989).

1.2.1 Physical control

In Egypt, early season (May to July) control involves a very labour intensive method in which teams of small children go through the cotton fields once every 3 days to handpick the egg-masses, which are counted and then destroyed. (Hosny *et al.*, 1981). About 2 million children are employed during this period.

However, the rising cost of labour and the difficulty in recruiting and training the necessary number of children is making the technique expensive and difficult to maintain. Also, it has been observed that there is a strong negative relationship between the size of the egg - mass infestation and the efficiency of hand collection (Hosny *et al.*, 1986).

1.2.2 Cultural control

This method of control relies on placing restrictions on the cultivation of alternate food plants. Berseem clover

(*Trifolium alexandrium*) is cultivated as a winter crop in Egypt and is one of the preferred food plants of *S. littoralis*, which migrates from clover to cotton during the cotton season (Afifi, 1990).

To reduce the level of damage to the cotton plants, irrigation of clover after the 10th of May is prohibited. This method is used in recognition of the fact that of the total number of egg-masses deposited in cotton fields during late May and June, 90 percent are produced by adults emerging from pupae in the clover fields. Prohibition of irrigation of the clover fields after 10th May is also done in order to harden the soil and increase the mortality of the pupae in the soil (Bishara, 1934; Hosny et al., 1981; Hosny et al., 1982; Brader, 1984). Also, the intercropping of maize in cotton plants is being encouraged in some parts because some parasitoids associated with *S. littoralis* multiply faster on the maize plants and therefore, its cultivation could increase the number of parasitoids and the percentage of parasitism, thereby reducing the pest population (Shalaby et al., 1988).

1.2.3 Biological control

The development of resistance to a large number of insecticides by *S. littoralis* has resulted in the search for an effective biological control agent. The most widely used biological agent is *Bacillus thuringiensis*. Broza et al. (1984) used a spray liquid formulation of *B.*

thuringiensis var. *entomocidus* to successfully control the 1st and 2nd instars of *S. littoralis* but not the later stages. The treatment reduced the level of infestation and damage in cotton. Larval and pupal development of surviving moths were adversely affected. Adults derived from the treated larvae also showed a reduced lifespan, fecundity and egg hatchability (Queed et al., 1988).

The high cost of the commercial preparation and its poor persistence when applied in the field has limited its use. The poor persistence in some cases had been due to the inactivation of the spores by sunlight (Hosny et al., 1983; Broza et al., 1984; Salama et al., 1984).

The use of the nuclear polyhedrosis virus (NPV) in controlling *S. littoralis* on cotton has given inconclusive results. This has mainly been due to inactivation of the virus by ultra-violet radiation which, in Egypt, accounted for up to 88 percent loss in 4 days (Elnager and Nasr, 1980; Jones and McKinley, 1987; Jones et al., 1993). Other problems with the use of NPV, such as the physical loss caused by abrasion due to the action of wind and sand, inactivation by plant exudates as well as dilution of the deposit due to plant growth, could reduce its effectiveness further. Besides, a commercial form of the NPV is not yet available (Elleman and Entwistle, 1985; Santiago-Alvarez and Osuna, 1988).

Ghally et al. (1988) and Glazer et al. (1993) have reported that nematodes such as *Neoaplectana carpocapsae* have lethal effects on *S. littoralis*. However, other species of nematodes and bacteria have had variable effects in the control of the larvae. Overall, the use of the nematodes as a biological agent does not seem to be promising at this stage.

1.2.4 Use of pheromones

1.2.4.1 Mating disruption

The chemical components of pheromones emitted by *S. littoralis* have been identified, but their use as an effective mating disrupting technique has had limited success. It has been observed that a two day delay in mating, due to mating disruption by pheromones, reduced the larval population to between 10 and 20 percent in the subsequent generation (Ellis and Steele, 1982; Glazer et al., 1993; Kehat and Dunkelblum, 1993).

The lack of success with the use of the technique has been due to a number of factors. The pheromone blend of up to 4 components varies from place to place and this makes it difficult to synthesise a compound that could be used over a large area (Campion, 1984). Also, for effective mating disruption, large numbers of releasers need to be placed very close to each other (Kehat et al, 1986; Dunkelblum et al., 1987).

In the evaluation of the pheromone of *S. littoralis*, one difficulty has been the maintenance of the concentration needed when the moths were active. Shani and Klug (1980) suggested that over a period of time, degradation and isomerisation due to heat and sunlight could significantly reduce the effectiveness of the pheromone.

Campion (1984) suggested that mating disruption with pheromones is most likely to be successful with insects of restricted food plants and flight range. However, *S. littoralis* is a polyphagous insect with an indeterminate flight range. These features may be a disadvantage for the use of the mating disruption technique. Also, it has been observed that males respond to pheromone traps most actively when the number of virgin females has declined considerably (Kehat et al., 1986).

1.2.4.2 Mass trapping

As a pest control method, mass trapping has had very limited success. Large numbers of adults would have to be trapped to have a significant effect on the pest population. Thus trapping would most likely be successful at low pest populations (Matthews, 1989). Also, because *S. littoralis* is a polyphagous insect, traps would have to be deployed throughout the area under treatment and not confined to a specific crop (Campion, 1984).

In Egypt, 5 pheromone traps per hectare reduced the mean number of egg-masses by 77 percent compared with adjacent control areas (Campion, 1983). Trapping with a bait containing 2 mg (9z,11E)-9,11-tetradecadienyl acetate resulted in a reduction of 34 to 62 percent in the egg clusters and 20 to 30 percent in egg viability in the treated fields. The resultant reduction in insecticide treatment fields ranged from 21 to 45 percent over 4 years (Teich et al., 1985).

Campion (1984) has suggested, though, that the successes claimed for the mass trapping are sometimes the result of the activities of beneficial insects, since in the presence of the traps, insecticides are less likely to be used.

Ultra-violet light trap has been observed to reduce populations of *S. littoralis* by 37 per cent in the summer and 46 per cent in the autumn (Iss-Hak et al., 1981).

1.2.5 Chemical control

This is the most effective and widely used method of control. It is usually carried out when the plants are large and hand picking of the egg-masses is no longer efficient, or when the population of *S. littoralis* is large or when leaf holing is obvious and the larvae are large (later stages of development). In most areas, it is the main method of control (Abdallah, 1988).

Various chemicals have been used. These had included organophosphates, carbamates and organochlorines. Recently, however, there have been reports of the development of resistance in *S. littoralis* to some of these chemicals such as carbaryl, methomyl, thiodicarb and some pyrethroids (Abbassy et al., 1982; El-Guindy et al., 1983; Saad et al., 1986; Pinchard and Vassal, 1991).

The development of resistance, in some cases, has occurred after only a few seasons of use of insecticides (El-Sabae et al., 1993). In order to overcome the problem of resistance, mixtures of insecticides are now being applied. Outbreaks of white flies and aphids have been observed in some areas as a result of the use of these mixtures of insecticides (Matthews, 1993).

1.3 Insect response to xenobiotics

Insect herbivores encounter food plants that vary in nutrient and allelochemical content (Glendinning and Slansky, 1994). The levels of these chemicals in the plants could vary with plant species, plant age and environmental factors such as soil fertility (Slansky and Scriber, 1985).

Insects also vary in their response to the variations in the levels of nutrients and allelochemicals present in their food plants. Generally, insects respond in two major ways (Lindroth, 1991; Dussourd, 1992). These responses are:

1. Physiological and behavioural responses. These responses include 1: Modification of feeding behaviour, usually to reduce the amount of toxin ingested by the insect. Young larvae of *H. virescens* avoid feeding on gossypol producing glands in cotton foliage in the early instars but become non-selective in later larval stages because of the possession of detoxifying enzymes (Hedin et al., 1991 In: Rose et al., 1992). 2: Increasing the amounts of food ingested to compensate for a reduction in the nutrient content of the food plant. The larvae of *S. frugiperda* reared on artificial diets diluted with cellulose and water increased fresh weight of diet consumed over an undiluted diet (Wheeler and Slansky, 1991). The increase in weight of food ingested could be regarded as a compensatory response to compensate for the dilution of the diet (reduction in the nutrient content).

However, such an increase in food consumption could, in some situations, lead to an ingestion of a toxic dose of an allelochemical(s) present in the food, leading to deleterious effects on performance including death. When velvetbean (*Anticarsia gemmatilis*) caterpillars were fed diets with progressively diluted nutrient levels but containing the same concentration of caffeine, the larvae compensated for the reduced nutrient level by increasing food intake. Consequently, their rate of caffeine ingestion increased to a pharmacologically effective dose, interfering with food utilisation, slowing growth, reducing

subsequent feeding and lowering survival (Slansky and Wheeler, 1992).

2. Biochemical responses - Mainly by means of enzymatic degradation of potentially toxic compounds (and the subsequent excretion of the breakdown products) or by the exhibition of target site insensitivity (Berenbaum, 1986; Bernays, 1990; Fu-Shun *et al.*, 1990). Detoxication enzymes include mixed function oxidases, glutathione transferases and hydrolases. Yu (1986) has suggested that detoxication is a major post-ingestion mechanism that enables the utilisation of otherwise poisonous food plants by insects. Studies on enzyme induction indicate that induction by food plants (allelochemicals) results in insects detoxifying pesticides faster than non induced insects (Yu, 1986). Induction of the alfalfa looper (*Californica antographica*) and the cabbage looper (*Trichoplusia ni*) by allelochemicals contained in peppermint leaves resulted in increased tolerance for carbaryl and methomyl due to induction of P-450 (Agosin, 1985). "These results suggest that changes in the chemistry of the food plants (eg resistant variety) may influence the susceptibility of the insects to insecticides. Thus tolerance of the food plant allelochemicals and insecticides are related" (Rose *et al.*, 1992).

Growth, development and reproduction in insects are directly dependent on the quality and amount of plant

ingested. Ingestion of the plant would depend on its being found and accepted. Besides the food plant being available, acceptable and digestible, it must provide the insect with not only all the nutrients required but also the many other secondary chemicals that are necessary for other insect activities such as oviposition (Hagen *et al.*, 1984).

1.3.1 Response to plant nutrients

Most phytophagous insects obtain their nutrients from the food plants that they feed on. The nutritional status of the plant is therefore important in the growth of the insects. In the gypsy moth, *Lymantria dispar*, low protein and low mineral diets prolonged developmental time of the females and reduced pupal weights of males and females (Lindroth *et al.*, 1991)

The important compounds necessary for the growth of insects are carbohydrates, amino acids and proteins, lipids and vitamins. The most important ones in the interaction between plants and insects are proteins, carbohydrates and water.

1.3.1.1 Response to leaf proteins

Proteins are the main sources of amino acids and are required for the production of tissues and enzymes in insects. Protein degradation and absorption take place primarily in the midgut. An insect's activities could be influenced by variation in the protein content of the food

that it ingests. In *Aedes aegypti*, protease was increased by 26-fold after a meal of blood but there was only a 2-fold increase after a meal of syrup (Ishaaya, 1986). Some proteins, such as haemoglobin, could, however, inhibit the activity of digestive enzymes in some insects (Ishaaya and Casida, 1975).

Important though proteins are, it is the balance of amino acids that is particularly important to insects (Hagen *et al.*, 1984). Brodbeck and Strong (1987) have suggested that since nitrogen in plants could occur as alkaloids, nitrates, or non protein amino acids (which could not be used by the insect), the use of total nitrogen content in plants as an indication of the protein level could be inaccurate. The amino acid content of the plant would be a more appropriate indication of the protein level in the plant.

Amino acids are required in different amounts by insects for normal growth and development. These amino acids could be essential eg. alanine, or nonessential. Some other amino acids such as L-DOPA, are however, toxic to insects (Hagen *et al.*, 1984; Harborne, 1988). Generally, for normal insect growth, 10 amino acids are required by all insects. These are arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine and threonine (Broadbeck and Strong, 1987). These ten amino acids are sometimes referred to as

essential amino acids because they can only be obtained from the food of the insect. As long as tryptophan, methionine and histidine are adequate, Brodbeck and Strong (1987) suggest that levels of other amino acids will seldom be limiting. There are variations in the requirements for specific amino acids. The larvae of the silkworm (*Bombyx mori*) require proline and aspartic acid in addition to the 10 essential amino acids (Hagen et al., 1984).

1.3.1.2 Response to leaf carbohydrates

Carbohydrates include starch, cellulose and various mono-, di-, and trisacccharides. Carbohydrates are essential mainly as a source of energy and as feeding stimulants for insects (Ishaaya, 1986).

Various classes of sugars have various stimulatory effects on insects. Feeding activity is stimulated by sucrose in the colorado potato beetle (Hsiao and Fraenkel, 1968), and by sucrose and glucose in the tobacco hornworm (Yamamoto and Fraenkel, 1960). Schoonhoven (1990) has suggested that the stimulation of the insect feeding is due to the stimulation of certain chemoreceptors while Ishaaya and Meisner (1973) suggest that the induction of feeding by carbohydrates is due to the stimulation of digestive enzymes which increase the rate of digestion in insects.

Most sugars are stimulatory at the concentrations that they occur in plants (Chapman, 1974). However,

variations in sugar content of the plant also affects the responses of the insect feeding on it. The Chinese rose beetle *Adoretus sinicus* preferentially selects the leaves of its food plants that are high in carbohydrates (Arita et al., 1993).

1.3.1.3 Response to leaf water content

Dessication could be a problem for herbivorous insects, especially when the difference between the water content of the body of the insect and that of its food plant increases. Variation in the water content of the food plant of insects could have detrimental effect on the growth and development of the insect. For instance, larvae of the cecropia moth *Hyalophora cecropia* grew slowly and were less efficient at utilising nitrogen when fed excised leaves of wild cherry, which contained low plant leaf water (Weis and Berenbaum, 1989). Studies on 25 species of Lepidoptera and 4 species of Hymenoptera showed that the relative growth rates of the insects were positively correlated with the nitrogen and the water contents of the foliage (Slansky, 1992).

The examples given above clearly indicate that the nutrient contents of the food plant could play as crucial a role in the feeding and other behaviour of insects as allelochemicals. In *Daphnis nerii*, preference for young as compared to mature *Nerium oleander* leaves was due to changes in proteins, carbohydrates, lipids, amino acid,

nitrogen and water content. Developmental time was short and fecundity and longevity high when insects were fed on the preferred leaves (Murugan and George, 1992).

1.3.2 Response to plant allelochemicals

It has been hypothesised that food plants of insects may share secondary compounds of the same chemical classes even though the food plants themselves may not be close taxonomically (Harborne, 1988; Schoonhoven, 1990). Feeny (1976) has suggested that such observations may indicate that adaptation by an insect to particular secondary compounds in one food plant species confers preadaptation to utilisation of other plants containing similar compounds. Edwards and Wratten (1988), however, suggested that the most important factors are the insect species and the nutritional state of the food plant. This, they argue, is because it is unlikely that taxonomically separate plants would have similar secondary plant substances.

Secondary plant compounds include anti-feedants, attractants, phagostimulants, repellents and toxins. These have different effects on insects. *Spodoptera exempta* is deterred from feeding by azadirachtin but *S. littoralis* is relatively insensitive and would ingest it even though it is toxic (Bell, 1987).

It is now accepted that feeding preferences and other insect responses are regulated by a balance between

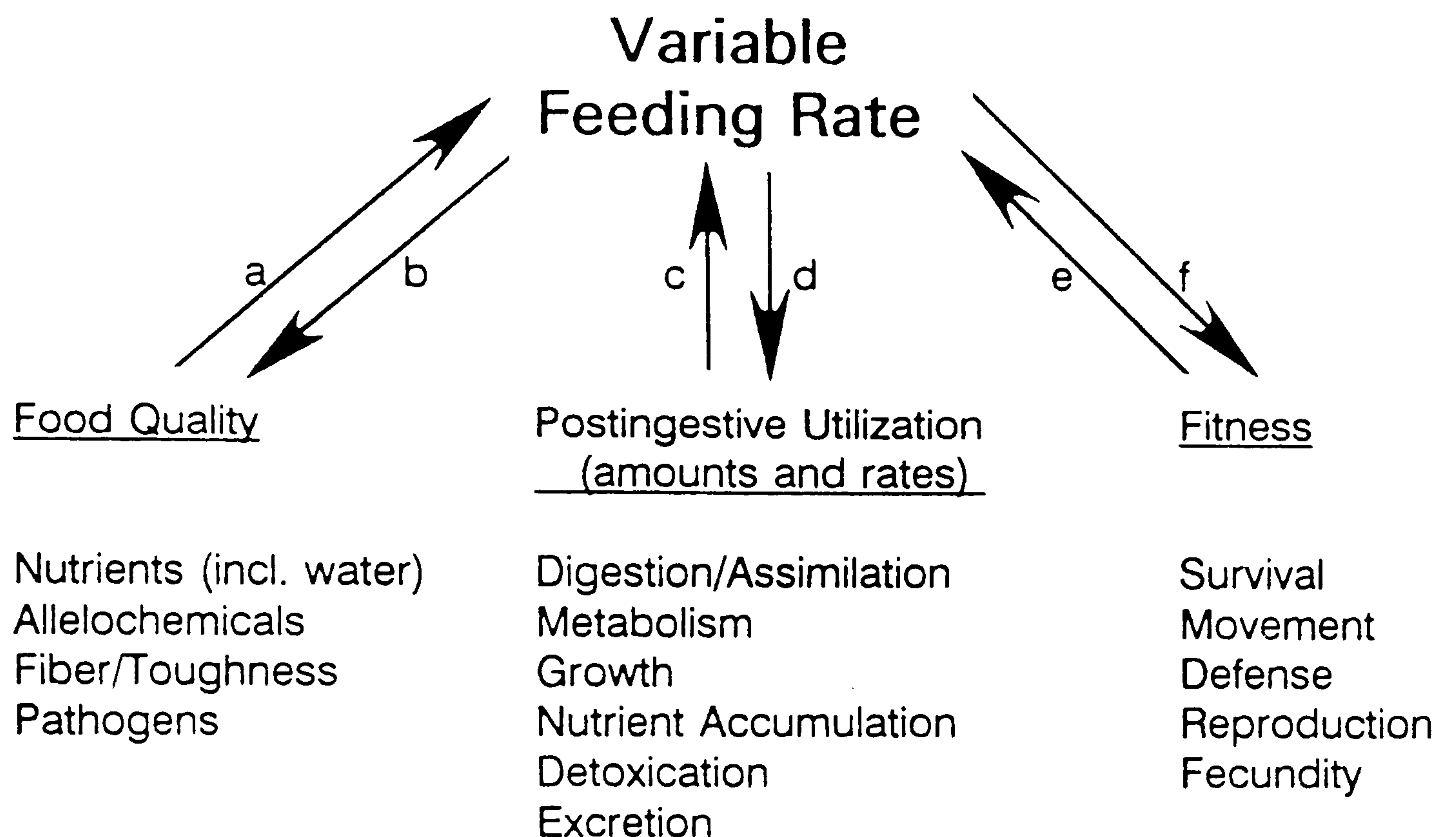
stimulants and deterrents that occur in their food plants and not due to either acting alone (Schoonhoven, 1990). For example, sinigrin acts as a feeding stimulant in *Ceutorhynchus constrictus* only when other plant constituents are present (Nielsen *et al.*, 1989).

The importance of studies of xenobiotics is that elucidation of ways in which they affect insects could be of considerable economic importance. For instance, knowing that a secondary compound disrupts a metabolic pathway present in insects or interferes with an enzyme system present in one insect but not in another insect could aid in the development of control programmes and overcoming the problem of insecticide resistance. Figure 1.1 summarises the interaction between the food plant and the insect.

1.3.3 Interaction between food plants and insecticides

Several studies have shown that food plants influence insects' responses to insecticides. The differences in the responses have been attributed to the food plant's influence on the enzymes that detoxify the insecticides (Omer *et al.*, 1993).

The interaction between food plants (particularly their constituents) and enzyme induction needs to be understood with respect to the use of insect-resistant genotypes and their effect on susceptibility of insects to insecticide (Rose *et al.*, 1992). Host plant resistance



Interactions between feeding rate and food quality, postingestive utilization, and fitness. (a) Food quality can affect feeding rate, such as when a compensatory increase in feeding occurs in response to nutrient dilution. (b) Feeding may affect food quality, such as through a feeding damage-induced increase in allelochemical concentration. (c) Postingestive food utilization can affect feeding rate, such as due to gut stretch-receptor and hemolymph-composition feedback involved in the regulation of feeding. (d) Feeding rate can affect postingestive food utilization, such as when insufficient food intake results in slowed growth. (e) Fitness components may affect feeding rate, such as when feeding increases in reproductive females. (f) Feeding affects fitness, such as due to greater exposure to natural enemies during feeding.

Figure 1.1. Interaction between food plant and the insect.

(Source: Slansky, 1992).

could contribute to the increase in the susceptibility of insects to insecticides. Theoretically, however, insects surviving on resistant food plants could become resistant to some insecticides due to the induction of enzymes by allelochemicals in the foliage or adaptation to the presence of allelochemicals (van Emden, 1991; Rose *et al.*, 1992). Such a development could have serious consequences for crop protection.

The interaction between food plants and insecticides may be advantageous or disadvantageous depending on their pest management effect. Insects on resistant food plants usually have smaller body weights than those on susceptible ones. Thus the same percentage kill of insects on susceptible and resistant plants could be obtained by reduced doses of insecticide on the latter (van Emden, 1991). He has also suggested that though the lower dose required could be due to differences in body weight, the actual effects are due to physiological sensitivity to the insecticide which is related to the stresses that the insects undergo from feeding on the resistant food plant.

The use of insecticides on crop plants may also result in reduction of food intake (Alford, 1991). Larval food consumption of spruce budworm, *Choristoneura fumiferana*, was lower on an insecticide treated artificial diet (Alford, 1991). Phloem feeding by *Nilaparvata lugens* was severely inhibited when nitromethylene heterocycle was

applied at a sublethal dose on rice (Losel and Goodman, 1993). These observations could explain why in some situations insecticides applied to the plant surface may not be effective. The insects may not ingest enough food to absorb lethal doses of the insecticide.

The use of plant resistant varieties to reduce crop losses due to *S. littoralis* may be difficult to achieve because of the insect's polyphagous nature. It would, therefore, in the immediate term, be more useful to identify crop plants that are likely to reduce the reproductive potential of the insect and then to incorporate them in an integrated control program.

1.4. General Objectives

Spodoptera littoralis feeds on a wide range of food plants. This would suggest the utilisation of several mechanisms, which could be metabolically expensive, in order to establish itself on the food plants. Although the effect of food plants on the biology of the insect has been studied (Hosroy and Kotby, 1960; Bhatt and Bhattacharya, 1976), some key aspects of the relationship of the food plant with the pest have not received adequate attention. These areas include:

1. Few studies have dealt with larval food digestion and utilisation on a range of food plants (Duodu and Sam, 1990).

2. The economic threshold for the commencement of chemical control in some countries is usually based on the number of egg masses (Hosny et al., 1986), yet there is hardly (to my knowledge) any study on the effect of food plants on the egg size within an egg mass or the effect of food plants on the size of the egg mass.

3. The application of chemical insecticides has led to the development of resistant strains of *S. littoralis* (El Sabae et al., 1993). It is possible too, that, some larvae may pick up sub-lethal doses of chemicals. There are few studies on the effect of sub-lethal doses of chemical insecticides on larval feeding.

4. Studies on the biology of the pest show that there are some food plants that it does not feed on. However, with respect to an important taxonomic group, the Gramineae, their suitability or otherwise is not firmly established. It has been observed on rice and maize (Moussa et al., 1960; Lal and Naji, 1990). However, studies have also shown that the larvae fail to accept several species of the Gramineae (Moussa et al., 1960; Prasad and Bhattacharya, 1975). It is important to establish the suitability of the Gramineae for larval development because of the economic importance of its members. Other polyphagous insects have shown such preferences for larval feeding. The tufted apple bud moth, *Platynota idaeusalis*, feeds on most orchard crops, but in Pennsylvania, it feeds only on apple and

surveys have indicated that different resistance patterns occur on different food plants (McPheron and Carlini, 1992). *Spodoptera frugiperda* is a major pest of, and shows preference for graminaceous plants (Ng, et al., 1993; Jamjanya and Quisenberry, 1988). The literature indicates that *S. littoralis* is adversely affected by members of the Gramineae. Is it possible that there is a factor(s) common to the Gramineae that is responsible for the deleterious effects observed on these plants.

The overall objective of the research presented in the thesis was to investigate the interaction of *S. littoralis* with seven food plants and to determine the possible factors in the preference for the plants. These involved:

1. The investigation of whether preference for dicot over monocot existed in the insect.
2. The investigation of the effects of the food plants on the biology of the insect in a no-choice situation.
3. The determination of the possible nutritional and biochemical basis for the preference shown for either the monocots or dicot plants used for the study on the preference.
4. To determine the effects of the food plants on larval susceptibility to topically applied insecticides.

CHAPTER TWO

INFLUENCE OF FOOD PLANTS ON THE LIFE CYCLE OF *SPODOPTERA LITTORALIS*

2.1 INTRODUCTION

Food plant selection is based on a sequence of responses by the insect. These responses, according to Saxena (1969), include:

1. Food plant recognition and orientation: positive or negative responses that could result in the attraction or the repellence of the insect.
2. Feeding: initiation and maintenance of feeding is influenced by the nutrient status and the presence or absence of feeding inhibitors.
3. Metabolism of ingested food.
4. Growth, survival and oviposition.

The sequence of responses need not occur in the steps outlined above. Orientation could be followed by oviposition as with adult female Lepidoptera. The acceptance or rejection of a food plant by an insect, therefore, could occur when any of the sequence of responses needed for any activity, e.g. feeding, could be interrupted by a chemical cue.

Detailed knowledge of these responses of the insects could be utilised in the development of control strategies.

The most important response is feeding, because, insect growth, survival and oviposition would depend on it. The feeding activity of phytophagous insects is governed by

the balance of feeding deterrents and stimulants present in the food plants (Chapman, 1974; Ishaaya, 1986). In Lepidoptera larvae, the detection of these chemicals is achieved through chemoreceptors located mainly in the antennae and mouth parts (Schoonhoven, 1990). These chemicals determine the suitability of the food plants.

The suitability of the food plant is one of the important factors in any insect-food plant interaction. It refers to factors of the food plant (plant surface characteristics, nutritional factors and the balance between feeding stimulants and deterrents) that affect intrinsic performance such as survival growth and reproduction of the insect using the plant (Singer, 1986; Scriber and Slansky, 1992).

These chemical characteristics of the plant would affect the insect's physiology and behaviour. Beck (1965) categorised all such chemicals as:

1. Attractants i.e. stimuli that result in an insect's orientation and movement towards the source.
2. Arrestants, i.e. stimuli that result in the cessation of locomotion when the insect comes into contact with the plant.
3. Feeding incitants, i.e. stimuli that evoke a biting or piercing reaction.
4. Feeding stimulants - these promote continuous feeding.
5. Feeding repellents - cause the insect to move away from

the source.

The suitability of the plant could be reflected in the degree of its acceptance by the insect. The degree of acceptance of a food plant could be measured as growth, development, survival and reproduction of the insect feeding on it (Prasad and Bhattacharya, 1975). Thus adverse effects of food plants would result in the reduction of the population of an the insect. The reduction in the overall population of an insect is an important factor in any control strategy (Brown, 1970).

Because *S. littoralis* develops resistance very quickly to chemical insecticides (El-Sabae et al., 1993), other methods of control are being investigated. For most farmers in the third world, cultural control (crop rotation or mixed cropping) would have to be considered because of the low cost of its implementation. Since *S. littoralis* is a polyphagous insect, the choice of crop plants in such a control programme would have to be done with extreme care.

Ripper and George (1965) have observed that, unlike in Egypt, *S. littoralis* is not a major pest in the Sudan because crop plants that are cultivated during the cotton off season are not suitable for the development of the pest. Also, increase in the levels of damage to cotton by *S. littoralis* in Morocco was observed to be a direct result of crop rotation and the introduction of new crops into

fields adjoining cotton (Brader, 1984).

To understand better the interaction between *S. littoralis* and its food plants, more studies would need to be carried out on its responses to food plants.

2.1.1 Background of study

Variations in the development of polyphagous insects have been recorded on a wide range of plants belonging to various taxonomic groups. These studies have indicated that in spite of their polyphagous nature, there are some plants that they do not feed on (Woodhead and Bernays, 1982; Jamjanya and Quisenberry, 1988; Ng et al., 1993).

When several food plants were tested, *S. littoralis* preferred 16 food plants for egg laying only, 45 for feeding and 12 for both feeding and oviposition (Moussa et al., 1960; Hosroy and Kotby, 1960; Prasad and Bhattacharya, 1975).

Few larval food digestion and utilisation studies on *S. littoralis* have been reported (Duodu and Biney, 1981; Darwish et al., 1987). Such data on food digestion and utilisation are essential in identifying trap crops or resistant plant species for genetic engineering research. Information gained from such leaf consumption studies could be used in establishing economic injury levels as well as indicating the potential effects of defoliation on yield of

food plants (Garner and Lynch, 1981).

Studies on the ovipositional behaviour of the pest have reported on the number of eggs laid (Hosroy and Kotby, 1960; Moussa et al., 1960; Salama et al., 1971; Hosny et al., 1986). The effects of food plants on the sizes and numbers of the eggs have not been reported on.

2.1.2 Experimental objectives

These experiments were to test the hypothesis that though *S. littoralis* is a polyphagous insect it does show some preference for some food plants. Therefore, the objective of these experiments is to determine the suitability of seven food plants for the growth and development of *S. littoralis*. Specifically, the experiments were designed to determine:

1. Whether the rearing of *S. littoralis* on a semi-artificial diet would have some detrimental effect on the growth and development of the larvae.
2. Whether early instar larvae show preference for food plants in choice tests as compared to late instars.
3. The influence of food plants on food digestion and utilisation efficiencies using the methods of Waldbauer (1964).

2.2 GENERAL MATERIALS AND METHODS

2.2.1 Rearing of *S. littoralis*

2.2.1.1 Rearing on a semi-artificial diet

Spodoptera littoralis was initially reared on a semi-artificial diet to determine whether such rearing could have adverse effects on their biology.

Eggs of *S. littoralis* (Canary Island strain) were obtained from the Natural Resources Institute (NRI), Kent. Subsequently, the insect culture was maintained on a kidney bean-based semi-artificial diet (Dimetry, 1976). The components of the diet are shown in Table 2.1.

The kidney bean diet was chosen on the basis of lower larval mortality and higher oviposition, after evaluating the results of the development of the moth on two semi-artificial diets (Appendix 1). In the other semi-artificial diet, the kidney beans were substituted with maize seeds.

2.2.1.2 Preparation of semi-artificial diet

The diet was prepared according to the method of Dimetry (1976). The appropriate amounts of the milled kidney beans, brewers' yeast, ascorbic acid and methyl p-hydroxybenzoate were weighed into a bowl and 300 g of water was then added. The ingredients were then thoroughly mixed. Separately, the agar was dissolved in 300 g of water, in a separate beaker, and the agar-water mixture then brought to the boil. On cooling to about 50°C, the agar

Table 2.1. Composition of the kidney bean - based semi-artificial diet.

Ingredient	Quantities (g)	% total weight
Milled kidney beans	236	26.46
Dried brewers yeast	37	4.15
Ascorbic acid	3	0.34
Methyl p-hydroxybenzoate	2	0.22
Agar	14	1.57
Water	600	67.26

was then added to the ingredients in the bowl and mixed with a hand held blender. About 30 cm³ of the diet was dispensed into each of 30 280-cm³ rearing jars. The diet, in the jars, was stored in a deep freezer until needed for use. When needed, the diet was warmed at room temperature and the surface 'scarified' prior to the introduction of the larvae.

2.2.1.3 Larval rearing on semi-artificial diet

About 50 newly hatched *S. littoralis* larvae were placed on the diet in the jars. Some tissue paper (to absorb moisture) was placed at the mouth of the jar. The tissue paper was held in place with a loosely fitting lid. The jars were placed on their sides (and with the bottom end of the jar facing the light source) in an incubator at

25° C, 60-70% RH and a 16 h:8 h photoperiod. After 4 days, the larvae were transferred onto fresh diet. Subsequently, the diet was changed every 48 h. As the larvae grew in size, a smaller number was placed in each jar, such that at the time of pupation, there were about 10 larvae in each jar.

Table 2.2 shows the biological parameters used in all the studies on growth and development of the insect.

2.2.1.4. Measurement of head capsule widths of larvae reared on semi-artificial diet

Since larval feeding and susceptibility to insecticides varies with instars, it is important to determine accurately instars of the larvae to be used in any experiment. Dyar (1890) first recognised the relationship between larval instars and head capsule widths for the larvae of the Lepidoptera. Since then, larval head capsule width has been used to determine larval instars (Morita and Tojo, 1985; Grossniklaus *et al.*, 1994; Kloft *et al.*, 1994). Head capsule widths were used in the determination of the larval instars in the present study.

Eggs laid by a single pair of male and female adults were used for the study. On hatching, the larvae were either reared individually or in groups of ten on the diet. The larvae were examined daily for signs of moulting. On moulting, the larvae were killed by freezing and

Table 2.2. Parameters used in the study of the development of *S. littoralis* on the semi-artificial diet and on the various food plants.

1. Larval mortality at 5, 7, 9, 11, 13, and 15 days after hatching and until 50% pupation.
 2. Larval period - from hatching to pupation of 50 percent of the surviving larvae.
 3. Larval weight at 9, 11, 13 and 15 days after hatching and until 50% pupation.
 4. Pupal weight - taken 24 h after pupation.
 5. Pupal period - period between the formation of the pupae and adult emergence.
 6. Pupal survival (mortality) - calculated from the number of adults that emerged.
 7. Weight of adults - taken 24 h after emergence.
 8. Sex ratio and adult longevity.
 9. Growth index - calculated by dividing the % pupal formation (No of pupae/No of larvae X 100) by the mean larval period (Sekhon and Sajjan, 1987).
 10. The ultimate survival - the percentage of neonate larvae surviving to the adult stage on each food plant.
 11. The percentage loss in weight at pupation is given by:
(Weight of larvae/Weight of pupa) X 100 (Karowe and Schoonhoven, 1992).
-

immersed in 10% sodium hydroxide solution for 3 h. Measurements of the head capsule widths were taken with a stereo microscope fitted with an ocular micrometer. The head capsule widths of the first instar larvae were

measured soon after hatching. Also, 10 larvae, from those reared in groups and of the same age, were randomly selected each day and their head capsule widths measured.

The growth ratio for each instar was obtained by dividing the head capsule width of that instar by the head capsule width of the preceeding instar. The first instar larvae therefore does not have a growth ratio.

2.2.2 Rearing of larvae on food plants

The experiments were conducted to determine the suitability of seven plant species for the growth and development of *S. littoralis*. The food plants used in the study are listed in Table 2.3.

The plants were grown, from seeds, in trays in a greenhouse at 25°C and then transferred to a growth room at 25°C and 18 h:6 h light:darkness photoperiod and 60-70% RH. The seedlings were then maintained in the growth room until needed. The seedlings of the cereal food plants (Gramineae) were used at the 4 leaf stage while the other food plants were used at the 6 leaf stage.

To study the development of the larvae on the food plants, neonate larvae were placed on excised leaves in jars (plate 2.1). To determine whether the use of excised leaves adversely affected the biology of *S. littoralis*, preliminary tests were carried out to observe whether there

Table 2.3 Food plants used in the study of the biology of *S. littoralis*.

Family	Food plant	Source	Variety
Leguminosae	Broad Bean	U.K.	Imperial Windsor White
	Soybean	Mexico	Stan Rosa
	Cowpea	U.K.	California Black Eye
Gramineae	Maize	Ghana	Dobidi
	Millet	U.K.	HSM/025
	Sorghum	Ghana	Naga Red/Manga Nara
Cruciferae	Chinese Cabbage	U.K.	Wang Bok

were significant differences in the larval responses when the larvae were reared on potted plants as well as on excised leaves.

To maintain the freshness (turgidity) of the leaves in the jars, moist filter paper was placed on the sides of the jars, prior to the introduction of the leaves and the larvae. The jars, loosely covered with lids, were then placed in an incubator at 25°C, at 60-70% RH and 16 h:8 h light:darkness photoperiod. The leaves were changed every 48 h until pupation. There were 15 larvae per replicate and 4 replications for each plant species.

2.2.3 Rearing of pupae

24 h after pupation, the pupae were removed, sexed, weighed, and then placed individually in glass tubes (10 cm³) containing about 3 cm³ vermiculite. The tubes, covered with perforated plastic lids, were then placed in an incubator until the adults emerged.

2.2.4. Rearing of adults

24 h after emergence, each adult emerging on each food plant was weighed and the total number of males and females, in each replicate, was recorded.

Each pair of male and female adults was placed in a 1 L Kilner jar. The inner sides of the jars were lined with paper to act as a substrate for egg laying. The adults were fed on 20 percent (w/v) honeyed water. The honeyed water was placed in glass vials and pieces of cotton wick were used to plug the mouth (thus soaking up the honeyed water and enabling the adults to suck from it). The honeyed water was changed every 48 h. Male adults that died were replaced. For the studies on longevity, males and females, randomly selected from emerging adults on each food plant, were placed separately and individually in 1 L kilner jars and fed 20% honeyed water until they died.

2.2.5. Oviposition

For the studies on oviposition, the eggs laid by the female of a pair of adults were collected daily (until the

female died) by cutting out the areas of the paper lining containing the egg mass. For all studies on egg hatch and egg size, the first 10 egg masses laid by the female (on each of the food plants) were used. The eggs of *S. littoralis* are laid in masses and glued to the substrate and covered with abdominal hair. Therefore, to determine the number of eggs laid by the female adults, the eggs had to be separated.

The egg masses laid by the female were therefore immersed in a 20% (v/v) washing liquid detergent solution. This treatment removed the adhesive material binding the eggs together (separating them into single eggs) and made it easy to count the individual eggs. The detergent solution, with the eggs, was filtered with a Whatman^R No.1 filter paper, and the eggs washed 5 times with distilled water and air dried. The diameters of the eggs were measured with a stereo microscope fitted with an ocular micrometer.

To determine the percentage of egg hatch, 10 egg masses on each food plant were used. Each egg mass was treated as a replicate. Each egg mass was placed in a covered 10 cm³ jar until the eggs hatched. On hatching, the larvae that emerged were counted. The egg mass was then examined under the microscope for unhatched eggs. These unhatched eggs were peeled off with a pair of forceps and then counted.

2.2.6. Calculation of larval and pupal period index (L-P index) and pupal weight index (P-index)

To compare the performance of *S. littoralis* on the food plants used in the study, the larval and pupal period index (L-P index) and the pupal weight index (P-index) were calculated, for each food plant, using the methods of Prasad and Bhattacharya (1975). Because the insect culture was maintained on the semi-artificial diet, the diet was taken as the standard. The calculations were made as follows:

$$\text{L-P index} = \frac{\begin{array}{cc} \text{Mean larval period} & \text{Mean pupal period} \\ \text{on diet} & \text{on diet} \end{array} +}{\begin{array}{cc} \text{Mean larval period} & \text{Mean pupal period} \\ \text{on food plant} & \text{on food plant} \end{array} +}$$

$$\text{P-index} = \frac{\text{Mean pupal weight (mg) on food plant}}{\text{Mean pupal weight (mg) on diet}}$$

The artificial diet, taken as the standard, was assigned a value of one. Therefore, if in any one comparison, the calculated index was more than one, then the food plant was assumed to be superior. If a food plant was inferior then it had an index less than one.

The assumption is that on a 'superior' food plant, the immature growth stages of an insect would have shorter

durations and heavier weights than insects on 'inferior' food plants (Prasad and Bhattacharya, 1975). Even though the terms 'superior' and 'inferior' were not defined, it could be assumed that they referred to the nutritional status of the plants. No reference was made to the possibility of deleterious effects of plant allelochemicals. Even though the allelochemicals could have deleterious effects on the insects, for the purposes of calculating the indices, the assumption would be that 'superior' food plants are the ones that supply the insects nutritional and allelochemical needs.

2.2.7 Preference, food consumption and utilisation by larvae

2.2.7.1 First and fourth instar larval preference of leaves

Preferences of 1st and 4th instar larvae were determined on the leaves of all the food plants used in the previous tests. Sections of excised leaves (covering an area of 5 cm X 3 cm) of each of the food plants were arranged, randomly, on the periphery of a large circular moistened filter paper. The moist filter paper was placed at the bottom of a large plastic container (Sealfresh^R, 13 L capacity) prior to the introduction of the leaves. Using a moistened paint brush, 150 larvae of each instar were placed at the centre of the filter paper and at equal distances from each of the leaf sections. The container was then covered with a lid and placed in an incubator at

25°C, 60-70% RH and 24 h darkness (to prevent the light from influencing larval preference during the experimental period). Each crop plant leaf section was taken as a replicate and there were 3 replicates.

After 24 h, the leaf sections were examined under a hand held magnifying glass. The number of larvae on or underneath each leaf section of each food plant was recorded. Larvae not found on the leaf sections, i.e. walking on the sides of the container, were not counted.

In a two-choice experiment, 4th instar larvae only were given a choice between any two of maize, millet and sorghum in any one test. Tests consisted of 3 pairs of maize vs. millet; maize vs. sorghum; sorghum vs. millet. The tests were conducted in 25 cm diameter plastic petri dishes. The leaf pieces were arranged in a circular ABABAB arrangement, where AB represented the leaf sections (1 cm x 1 cm) of the food plant in each pairing. 100 larvae were introduced into each petri dish. The experimental conditions were the same as in the multiple choice tests.

2.2.7.2. Calculation of preference indices

Preference refers to "deviations from random behaviour" where 'random' refers to the situation in which variations in insect behaviour are not related to variation among the plants encountered. Thus preference can be measured as the relative likelihood of accepting plants that

are encountered" (Singer, 1986).

Preference (expressed as a preference index, PR) for the food plants tested was calculated by the method of Mikolajczak and Reed (1987). The calculation was made as follows:

$$PR = O_L/E_L$$

Where:

O_L = Observed number of larvae feeding on testplant in each replicate

E_L = Expected number of larvae feeding on test plant in each replicate assuming equal preference for all plants

$E_L = T_L/P_N$ in each replicate.

Where :

T_L = Total number of larvae feeding on replicate

P_N = Number of test plants in choice test

E_{PR} : The Expected Preference (Ratio) index (assuming equal preference for the test plants) is calculated by: E_L/T_L

An acceptable food plant is one in which the PR index $> E_{PR}$ and an unacceptable food plant is one in which PR index $< E_{PR}$.

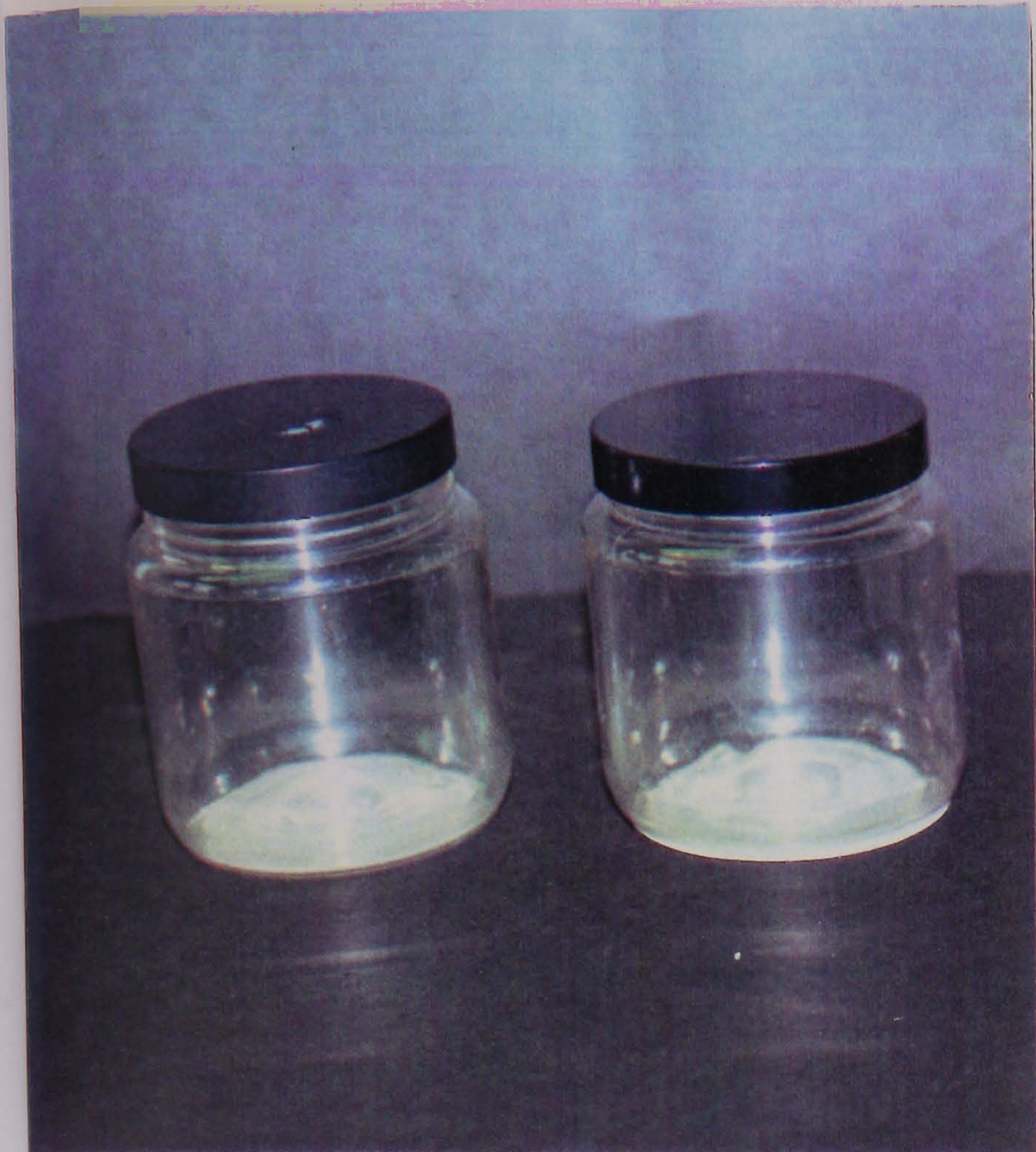
2.2.7.3 4th and 6th instar larval food consumption, digestion and utilisation

Larval food consumption and food utilisation were determined over the duration of the 4th instar and also

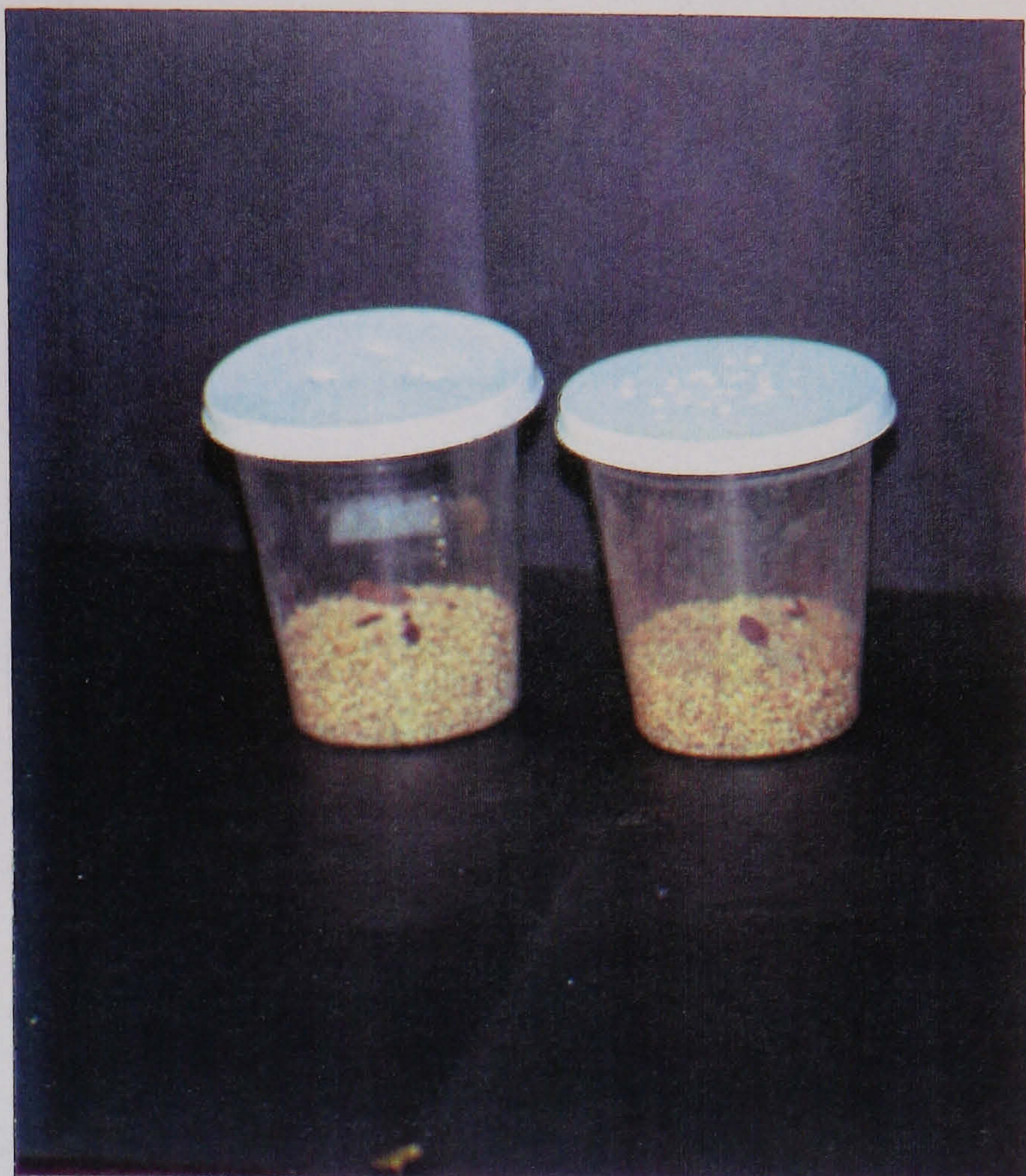
over the duration of the 6th instar, in no-choice tests. The calculations were made on a dry weight basis and using the methods of Waldbauer (1964). The calculations were made on dry weight basis because the main nutrient components, i.e. carbohydrates, protein, and lipid comprise a major proportion of the dry mass of plants (Slansky, 1992).

Maize, millet, sorghum, broad bean, soybean, cowpea and cabbage were used. The larvae were reared on the semi-artificial diet and on reaching the appropriate instar, were randomly selected, starved for 12 h, weighed and placed individually in 10 cm diameter petri dishes. Moistened filter paper was placed at the bottom of each petri dish prior to the introduction of the larvae and the food plants. Each larva was given a weighed amount of foliage from the second pair of leaves of each food plant. The maize, millet and sorghum were used at the 4 leaf stage. The other food plants were used at the 6 leaf stage.

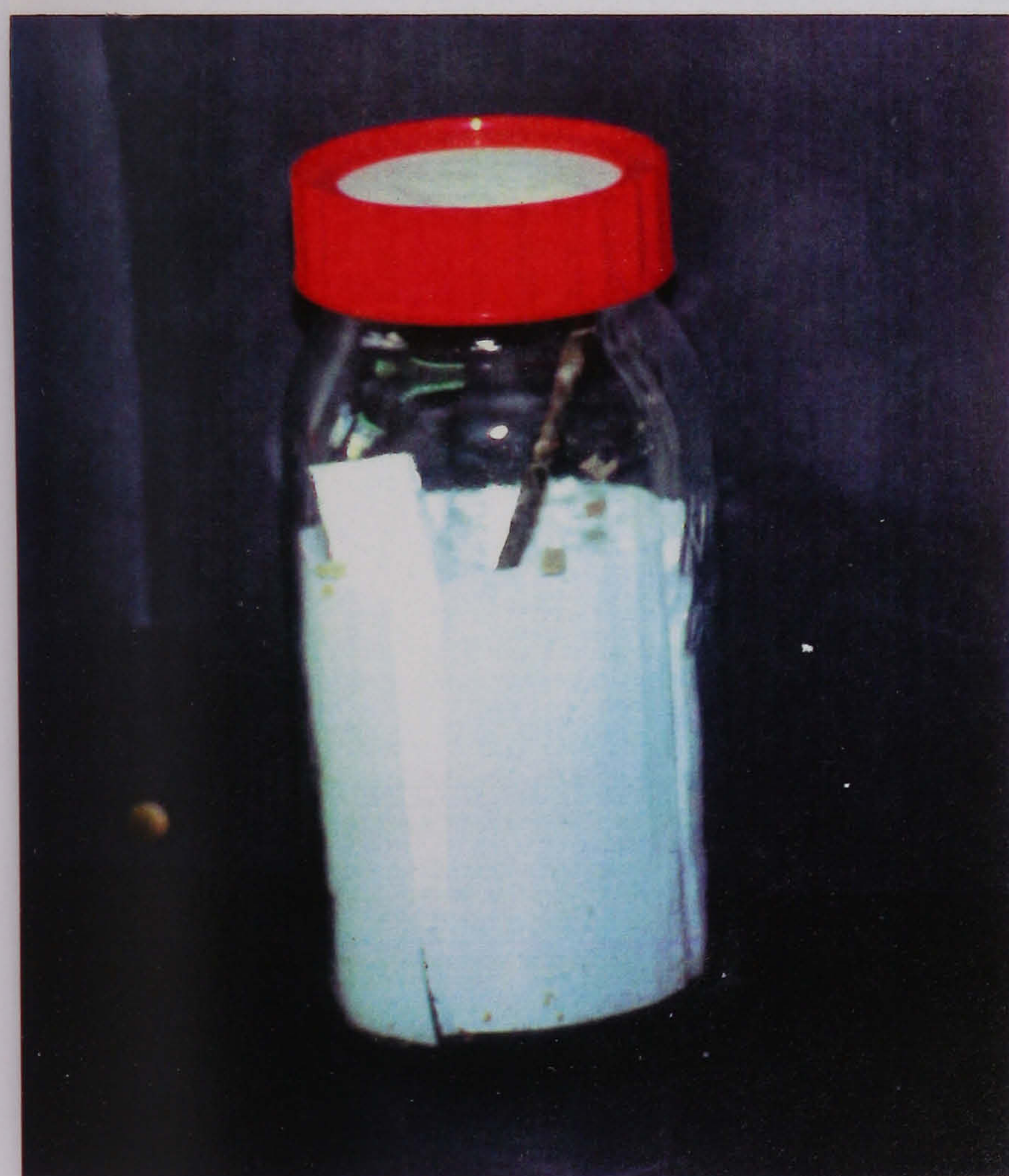
Waldbauer (1964) showed that the symmetrical separation of a leaf into right and left halves by splitting it longitudinally along the midrib provides samples that are representative of the whole leaf. In this study, therefore, each leaf to be fed to the larva was cut along the mid rib into two symmetrical portions. One half was weighed and fed to the larva and the other half (the sample) was weighed and then dried to a constant weight. The fresh and the dry weight of the sample were used to



a



b

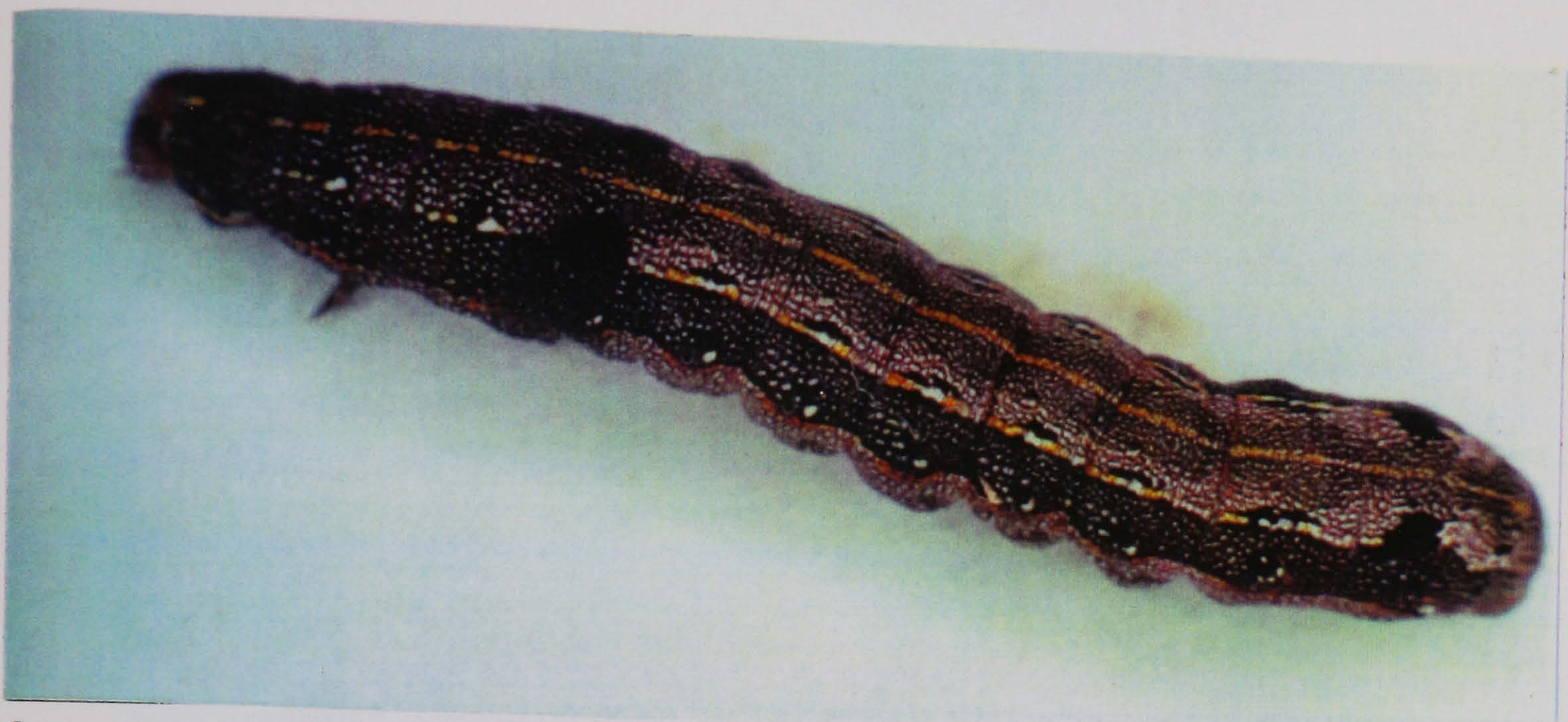


c

Plate 2.1. Rearing jars for larvae (a) , pupae (b) and adults (c) .



a



b

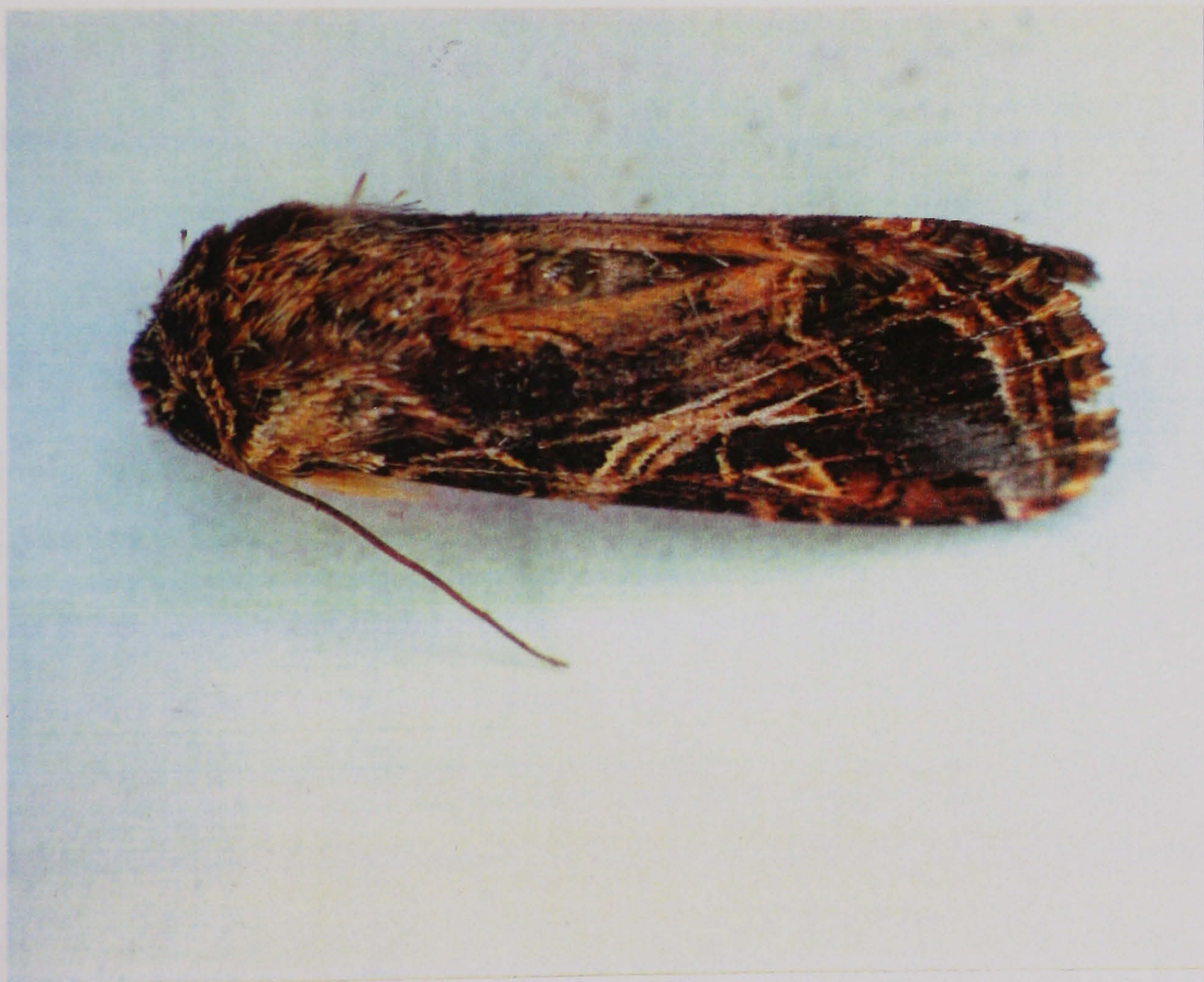


c

Plate 2.2 Eggs (a), 6th instar larva (b) and pupa of *S. littoralis*.



a



b

Plate 2.3. Female (a) and male (b) of *S. littoralis*

calculate a fresh weight/dry weight ratio. The initial dry weight of the introduced food, for each larva, could not be determined directly. It was, therefore, estimated by multiplying the dry weight/fresh weight ratio of the sample by the fresh weight of the foliage given to the larvae.

The uneaten food as well as the faecal pellets, for each larva on each food plant, were removed daily, separated and dried in an oven at 60°C for 48 h. They were then separately weighed. Growth of the larvae was measured by recording, daily, the larval fresh weight.

The initial dry weight of the larvae used in the tests was estimated from the dry weight of a sample of 50 freshly moulted and starved (for 12 h) larvae which had been dried to a constant weight at 60°C for 48 h. The final dry weights of the larvae fed on the food plants were obtained by drying the larvae feeding on each of the food plants, at the end of the experimental period, at 60°C for 48 h (Jamjanya and Quisenberry, 1988). There were 15 larvae/replication and 3 replications.

In a no-choice experiment, the 4th instar larvae, starved for 12 h, were given weighed amounts of the first pair of leaves of 4 leaf stage and 6 leaf stage maize, sorghum, and millet for 24 h. After 24 h, the leftover food and faecal pellets were dried and weighed. The amount of food ingested was then calculated. There were 10 larvae

per replicate and 3 replicates.

2.2.7.4. Calculations of food consumption and utilisation indices

Calculations of nutritional indices were made following the method of Waldbauer (1964):

Approximate digestibility (ratio between absorbed food and ingested food), AD:

$$AD = \frac{\text{Dry weight of food ingested} - \text{Dry weight of faeces}}{\text{Dry weight of food ingested}} \times 100$$

Efficiency of conversion of ingested food to body matter (ratio of weight gain and ingested food - it indicates overall nutritive value), ECI:

$$ECI = \frac{\text{Dry weight gained by larva}}{\text{Dry weight of food ingested}} \times 100$$

Efficiency of conversion of digested food to body matter (ratio of assimilated food (wt gain) and absorbed food), ECD:

$$ECD = \frac{\text{Dry weight gained by larva}}{\text{Dry weight of food ingested} - \text{Dry weight of faeces}} \times 100$$

Consumption index (rate of intake relative to the mean weight of the insect), CI :

$$CI = \frac{\text{Weight of food ingested}}{\text{Duration of feeding period} \times \text{Mean weight during feeding period}}$$

2.2.8. Consumption of food by 0-10 day old larvae on three plants.

The cumulative amount of food ingested was determined in 0-10 day old larvae on maize, broad bean and cabbage, representing each of the taxonomic groups used in the previous experiments.

Neonate larvae were offered weighed amounts of the food plants. After every 24 h, the leftover food was removed, oven dried and weighed. Fresh and weighed foliage was offered to the larvae daily. Feeding was terminated after 10 days. Experimental conditions were the same as in 2.2.7.3.

All means were separated by the Student-Newman-Keuls test (SNK) (Zar, 1974).

2.3. RESULTS AND DISCUSSION

2.3.1. Larval instars on semi-artificial diet

The accurate determination of instars of insects is

necessary in all studies on ecology and control of insects. This is commonly achieved in lepidopterous larvae by the measurement of head capsule widths. Dyar (1890) showed that the head capsule of caterpillars grows in geometrical progression, increasing at each moult by a ratio which is constant for a given species. For the Lepidoptera, he suggested a growth ratio of 1.4.

There were 6 larval instars on the semi-artificial diet, with a mean growth ratio of 1.5 (Table 2.4). The number of instars agrees with the finding of McKinley et al. (1984), who also reported six larval instars, based on measurement of head capsule widths, of *S. littoralis* when reared on artificial diet. However, the head capsule widths observed in the two studies differ. The differences observed in the two studies may be due to the differences in biotypes of *S. littoralis* used.

The mean growth ratios for the various instars obtained in this study are higher than the 1.4 suggested by Dyar (1890). Gillot (1980) and Smith (1984) noted that Dyar's law, which required 1. a growth ratio of 1.4 and 2. duration of larval instars to be constant, is frequently inapplicable because several factors could affect the growth rate and the headcapsule widths of the larvae. These factors include crowding and starvation of some instars (Morita and Tojo, 1985) and larval parasitism (Choi and Ryoo, 1994; Grossniklaus et al., 1994). In the present

study, the durations of the instars were not the same.

Table 2.4. Mean head capsule widths (mm) of larvae reared on semi-artificial diet.

Instar	Head capsule width (mm)	Mean Growth Ratio
	mean \pm SE	
I	0.34 \pm 0.01	
II	0.52 \pm 0.02	1.53
III	0.82 \pm 0.08	1.58
IV	1.26 \pm 0.25	1.54
V	2.01 \pm 0.28	1.60
VI	3.00 \pm 0.35	1.49

n = 40 for each instar.

Figure 2.1 shows the relationship between the larval instars and the log transformation of the head capsule width and the approximately linear relationship obtained, which conforms fairly reasonably with Dyar's law. In the present study, larval head capsule widths were used in the determination of larval instars.

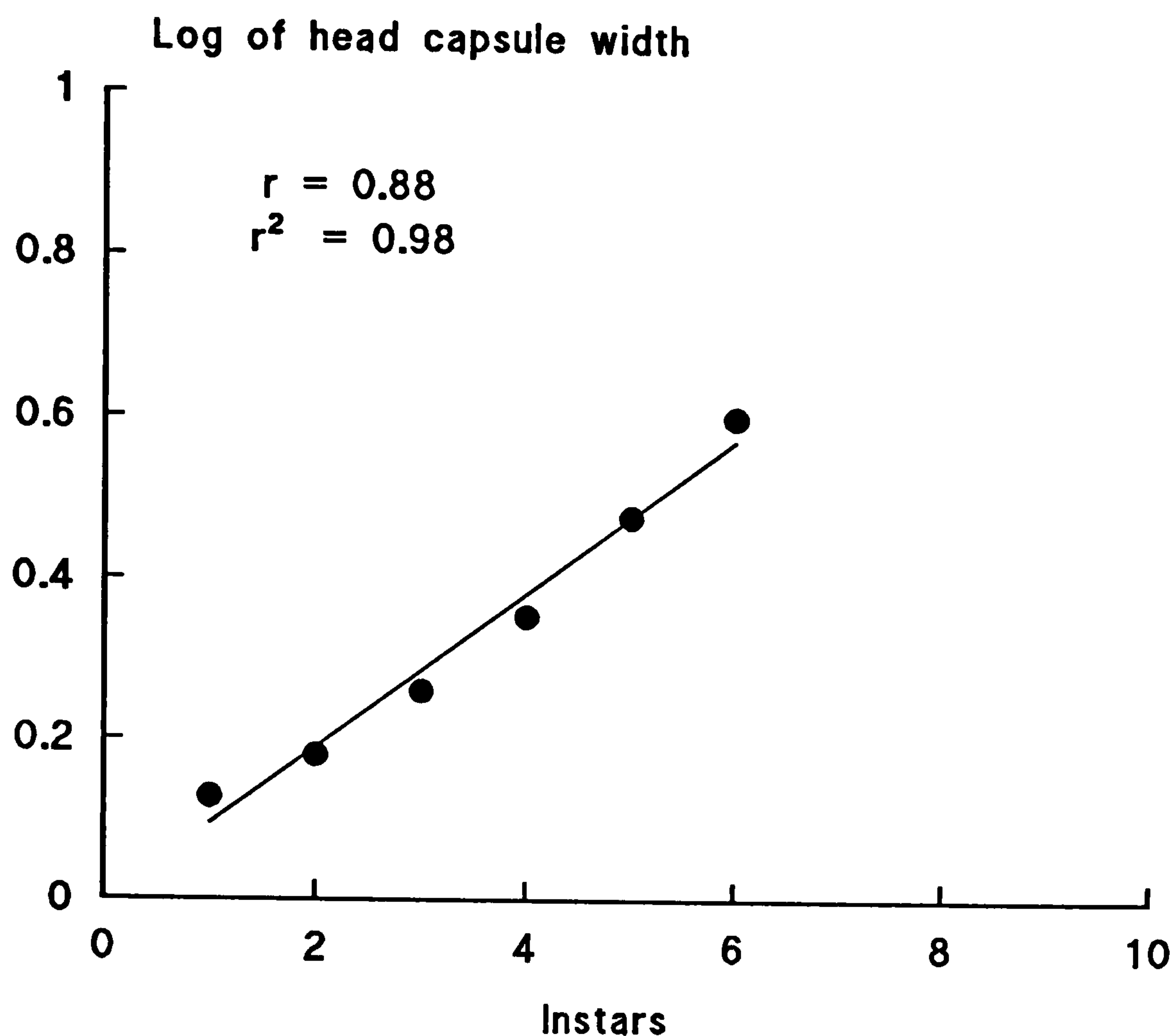


Figure 2.1. Relationship between larval instars and log of head capsule width on semi-artificial diet.

2.3.2 Larval instars on food plants

There were six larval instars on the cowpea, soybean, cabbage and broad bean. On the maize and sorghum, there were seven larval instars (Appendix 3). The extra moult on the maize and sorghum may have been due to the effects of the food plants.

Extra larval instars have been observed to occur in *S. littoralis* under adverse conditions (McKinley et al., 1984)

and had been observed to have an extra instar on cotton and *Urena* (Duodu and Biney, 1981).

In other Lepidoptera, supernumery ecdysis has been reported in *S. litura* due to larval crowding and starvation and this resulted in larvae having smaller head capsule widths (Morita and Tojo, 1985). Bilapate et al. (1985) also reported variation of larval instars of *Helicoverpa armigera* on cotton.

The extra instar spent on these food plants may enable the larvae to acquire enough resources for the growth and development of the subsequent growth stages. In *Mythimna convectus*, supernumary moults on the same artificial diet resulted in heavier pupae (Smith, 1984) and in *Spodoptera litura*, extra moults enabled the larvae to recover from a reduction in larval size due to food shortage (Morita and Tojo, 1985).

2.3.3. Larval weight gain on food plants

Larval development from 9 days after hatching to 50% pupation was observed on seven food plants belonging to three families of plants.

There were variations in the development of the larvae on these food plants (Fig. 2.2). The larvae reared on the millet were unable to complete their development to the pupal stage. On the other food plants, there were

differences in larval development. At 50% pupation, the heaviest larvae were on the cabbage and the lightest larvae were on the sorghum.

Differences in larval development on food plants have also been observed by Duodu and Sam (1990) and Dimetry (1972) on *S. littoralis* as well as in other Lepidoptera (Garner and Lynch, 1981; Sekhon and Sajjan, 1987; Ng et al., 1993). These differences were attributed to the effects of the plant species on which the insects had been reared.

In the entire larval period, the larvae on the maize and sorghum (which had extra instars) weighed less than the larvae on the other food plants (this may be due to poor adaptation on these food plants). It could be inferred that the extra instar on these food plants did not result in an increase in an overall larval weight gain. Smith (1984) had reported that in *Mythimna convecta*, 7 instar larvae had lived longer and produced heavier pupae than 6 instar larvae. In the present study, the seven instar larvae weighed less than the 6 instar larvae on the other food plants. It is probable that there were more feeding deterrents in the maize and millet used in this study.

However, the survival of the larvae on these varieties of maize (and possibly sorghum) contrasts with the observations by Moussa et al. (1960) and Prasad and

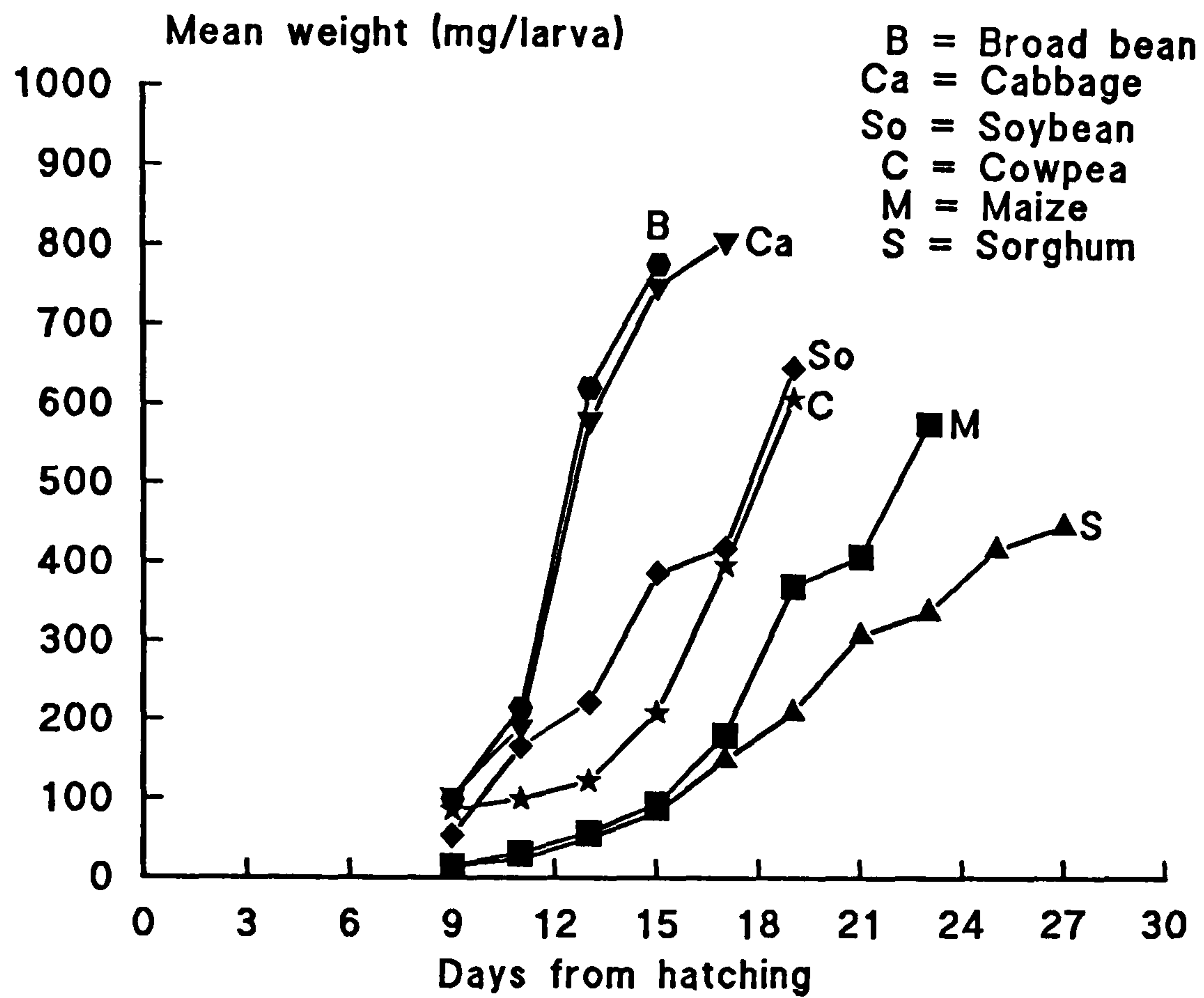


Figure. 2.2. Mean weight (mg) of surviving larvae on food plants.

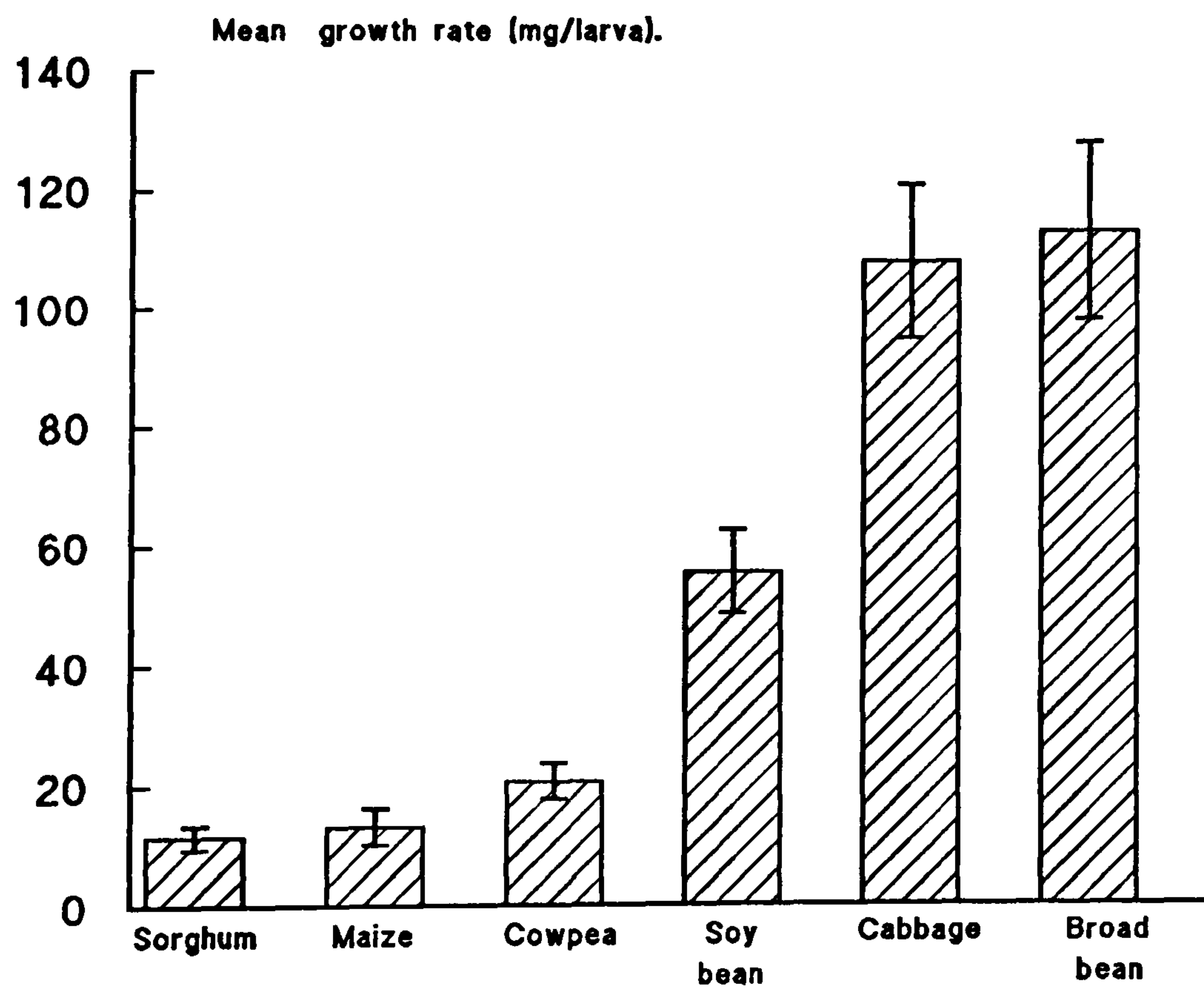


Figure 2.3. Growth rate (mg/larva) of larvae from 9–15 days on food plants.
 Bars represent Standard Errors

Bhattacharya (1975) who reported that the larvae of *S. littoralis* had failed to survive on maize seedlings for more than 24 h.

The prolonged larval periods on the maize and sorghum, if observed under field conditions, could result in increased exposure to natural enemies (Vet et al., 1990) and asynchrony with food plant phenology (Thomas, 1989) and mates (Lederhouse et al., 1982). Thus their cultivation in a cropping system could offer a potential means for the control of *S. littoralis*.

The larval growth rate (weight gain/day) between days 9 and 15 (Fig. 2.3) also indicates that the larvae on the cereal food plants did not perform well.

2.3.4. larval mortality on food plants

One of the most important limiting factors in the increase of any insect population is the mortality in the immature stages in the development of the insect (Dahms, 1972).

On all the food plants tested, there was a decrease in the number of surviving larvae with the age of the larvae (Fig. 2.4) There are 3 distinct periods from 0-5 days, 5-10 days, and 10 days-pupation of 50% of larvae. These periods would correspond to the early larval mortality (egg to 3rd instar), mid larval mortality (3rd to 4th instar)

and late larval mortality (4th instar to pupation).

On millet, the high mortality period falls within 5-11 days, while on maize and sorghum, the critical period falls within 11 days to the time of pupation.

Since larval mortality would have an effect on the overall damage to the vegetative stage of plant growth, the pattern of larval mortality on the cereal food plants would suggest that there would be a reduction in damage to the plant with age of the larvae. The reduction in plant damage will be due to the reduction in the population of larvae on these food plants.

These high mortalities may have been due to either low larval food intake and poor quality of the food plants (Pereyra, 1994) or to the effects of probable toxic factors present in the food plants (Chapman, 1974). The larvae on the maize and sorghum, with high larval mortalities, also had lower larval fresh weight.

Since larval mortalities on the cereal food plants, particularly on millet, occurred mainly from day 5 onwards, it could be inferred that mortalities were probably due to the chronic effects of toxic principles within the plants, low larval food intake or poor quality of food plants. In preliminary experiments, the larvae had also failed to establish themselves on rye grass and seedlings of rice

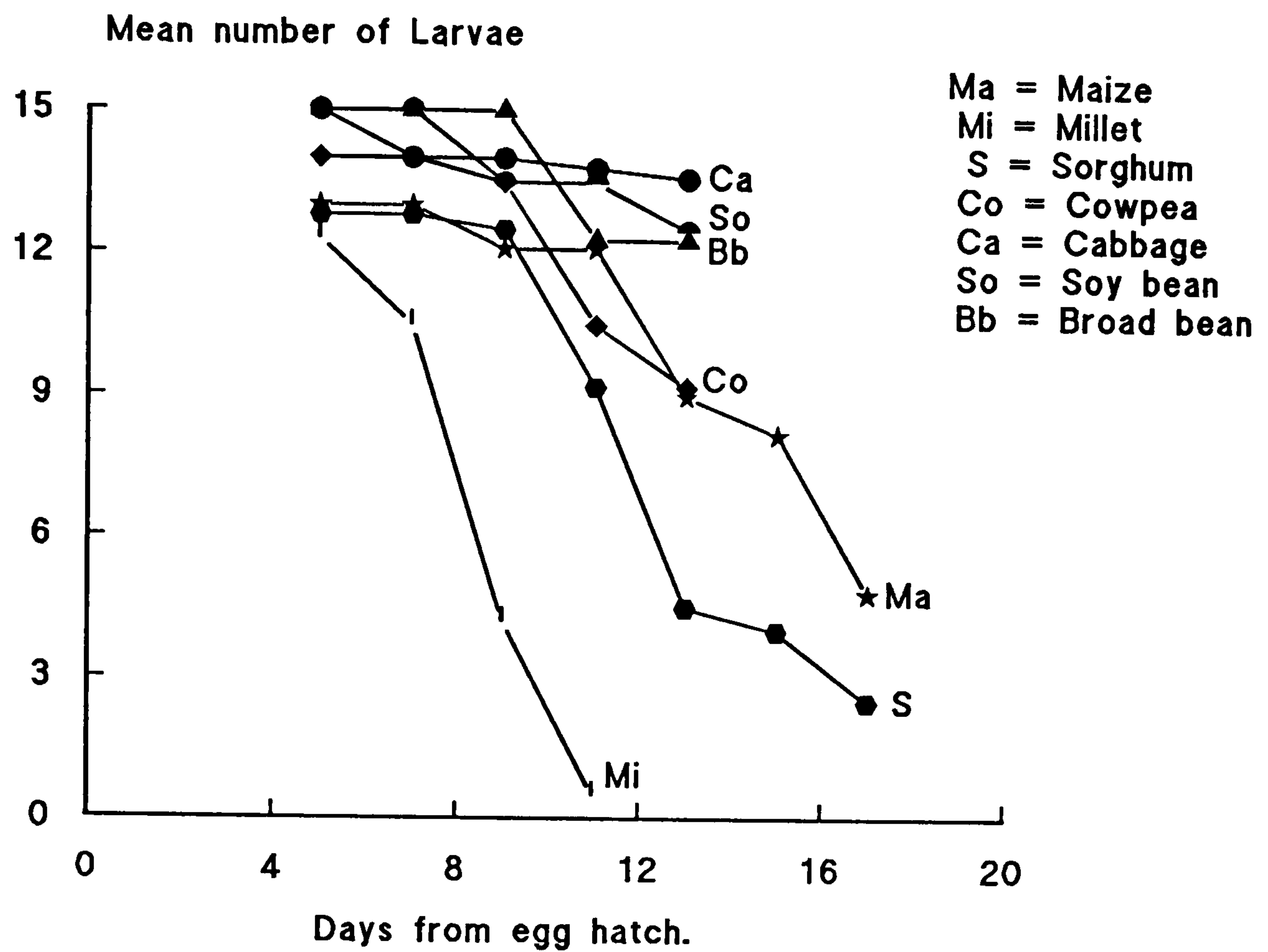


Figure 2.4. Mean number of surviving larvae on food plants.

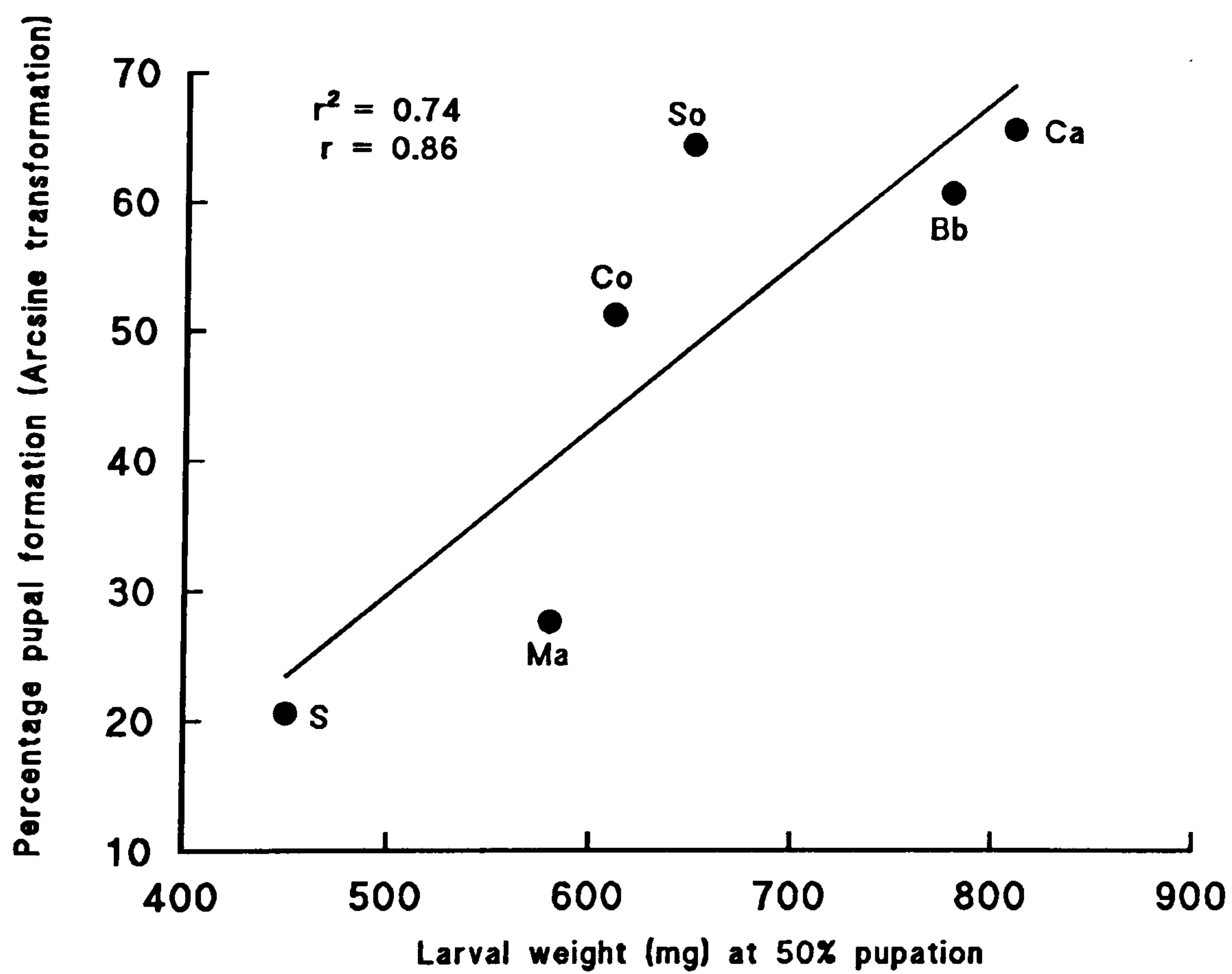


Figure 2.5. Relationship between larval weight at pupation. and percentage pupal formation.

and wheat. It would appear, therefore, that cereal food plants would not be natural food plants for *S. littoralis*.

2.3.5. Larval periods on food plants

Table 2.5 shows that larvae on the broad bean had a significantly shorter larval period than larvae on the maize and sorghum, indicating differences in the rates of larval development and the number of instars observed in the earlier part of the study.

Differences in food plant quality have been reported to affect the larval periods of *S. littoralis* on various food plants (Abdel-Fattah *et al.*, 1977; Duodu and Sam, 1990; Afifi, 1990). Larvae on the maize and sorghum, with low weights and a higher number of larval instars, tended to have longer larval periods.

2.3.6. Pupal formation on food plants

The percentage pupal formation reflects the larval mortalities. There were significant differences in the numbers of pupae that formed on the food plants (Table 2.6). Pupal deformities were observed only on soybean (4%), broad bean (5%) and cabbage (8%). The deformities may have been caused by chemical factors present in the food plants. There was a relationship between larval weight at 50% pupation and pupal formation (Fig. 2.5). This observation indicates that larval mortalities are influenced by larval weight.

Table 2.5 Mean larval period (mixed sexes) of *S. littoralis* on food plants.

Food plant	Duration (days)
	Mean \pm SE
Broad bean	15.0 \pm 0.1 a
Cabbage	17.1 \pm 1.0 ab
Cowpea	18.5 \pm 0.1 ab
Soybean	19.1 \pm 0.2 b
Maize	24.4 \pm 1.4 c
Sorghum	27.6 \pm 2.2 c

Means followed by the same letter are not significantly different at 5% probability level (SNK).

Table 2.6. Mean pupal formation on food plants.

Food plant	Pupal formation (%)
Sorghum	12.52 a
Maize	21.66 a
Cowpea	61.06 b
Broad bean	76.67 c
Soybean	81.66 c
Cabbage	83.51 c

Means in a column followed by the same letter are not significantly different at 5% probability level (SNK).

2.3.7. Pupal weights on food plants

There were significant differences in the pupal weights on the food plants (Table 2.7). Food plants producing heavy larvae also produced heavy pupae. Fig. 2.6 shows the relationship between pupal weight and pupal formation.

Table 2.7. Mean pupal weights (mg) of *S. littoralis* on food plants.

Food plants	Males	Females
	Mean \pm SE	Mean \pm SE
Sorghum	140.5 \pm 16.6 a	156.2 \pm 8.5 a
Maize	165.7 \pm 4.9 a	166.5 \pm 7.8 a
Cowpea	207.2 \pm 6.9 b	236.6 \pm 4.7 b
Soybean	269.1 \pm 8.9 c	274.5 \pm 8.1 bc
Cabbage	302.2 \pm 9.9 c	336.9 \pm 7.2 c
Broad bean	307.2 \pm 8.8 c	343.8 \pm 9.2 c

Means in a column followed by the same letter are not significantly different at 5% probability level (SNK).

2.3.8. Pupal periods on food plants

Significant differences were observed in the pupal periods on the food plants (Table 2.8). The differences between the male and female pupal periods were not significant. The pupal period (mixed sexes) on the maize was the longest even though the larvae on the sorghum had a longer larval period.

Table 2.8. Mean pupal period (days) of *S. littoralis* on food plants.

Food plants	Males	Females
	Mean \pm SE	Mean \pm SE
Broad bean	10.3 \pm 1.1 a	9.7 \pm 0.8 a
Cabbage	10.9 \pm 1.4 a	10.5 \pm 0.9 a
Soybean	11.2 \pm 1.9 a	10.1 \pm 1.5 a
Cowpea	11.6 \pm 1.6 ab	10.2 \pm 0.9 a
Sorghum	13.4 \pm 1.0 b	11.2 \pm 1.4 b
Maize	17.3 \pm 1.8 c	15.0 \pm 2.7 c

Means in a column followed by the same letter are not significantly different at 5% probability level (SNK).

2.3.9. Adult emergence and sex ratio

The adults emerged mainly at night. Females were observed to emerge earlier than males.

There were significant differences in the percentage adult emergence on the food plants (Table 2.9). The highest percentage emergence was on the soybean and the lowest percentage emergence was on the maize. Deformities of emerging adults were observed on cabbage (10%), broad bean (4%), cowpea (4%) and maize (0.5%).

Table 2.9. Adult emergence and sex ratio

Food plant	Adult emergence percentage \pm SE	Sex ratio Male : Female
Maize	53.8 \pm 4.72 a	1 : 1.1
Sorghum	55.5 \pm 6.83 a	1 : 1.1
Cowpea	70.2 \pm 7.31 b	1 : 0.9
Cabbage	77.2 \pm 8.41 bc	1 : 1.2
Broad bean	81.0 \pm 5.41 bc	1 : 1.4
Soybean	84.4 \pm 9.08 c	1 : 1.1

Means in a column followed by the same letter are not significantly different at 5% probability level (SNK).

Figure 2.7 shows the relationship between mean pupal weight and percentage adult emergence, indicating that adult emergence was higher on plants with heavier pupae.

Overall, the percentage of adults emerging from the pupae was higher than the percentage of pupae emerging from the larvae, particularly on the maize and sorghum. This observation implies a lower pupal mortality than larval mortality. These differences may mean that the food plants used in this study are better suited for pupal and adult development than for larval development. Only on cabbage was the percentage pupal formation higher than the percentage adult emergence. Only on cabbage and broad bean

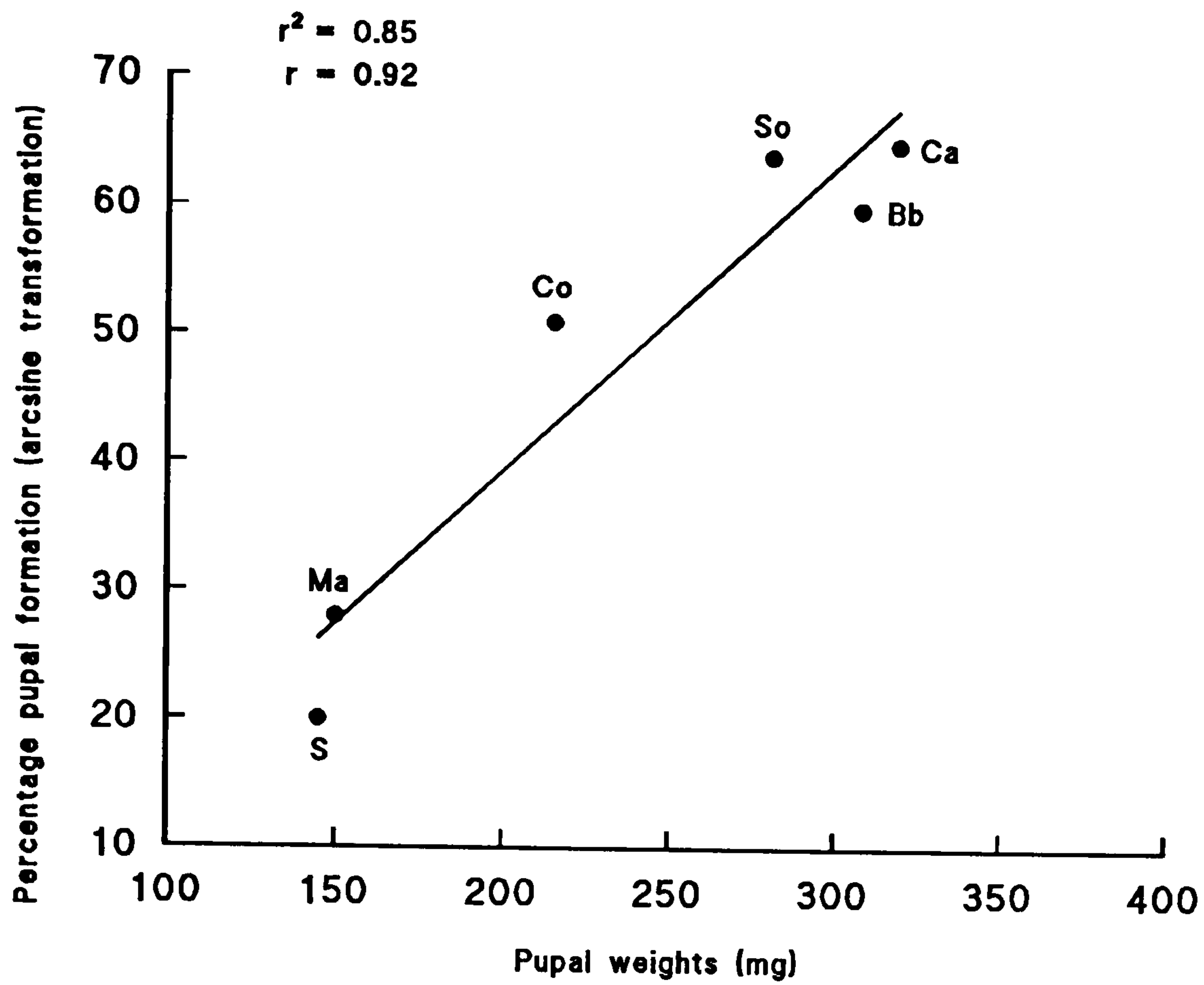


Figure 2.6. Relationship between mean pupal weight and pupal formation

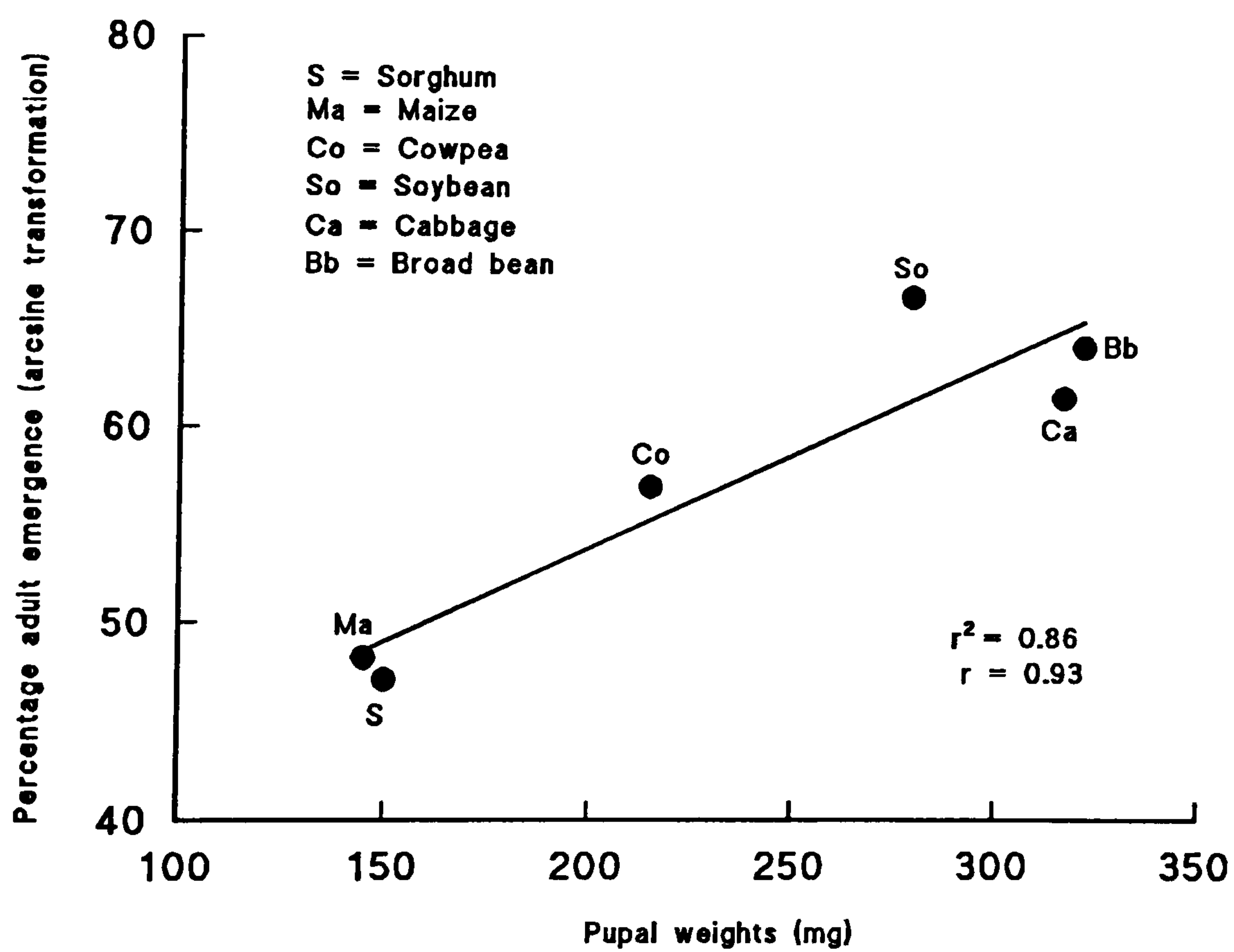


Figure 2.7. Relationship between mean pupal weights and percentage adult emergence

were both pupal and adult deformities observed. When paired, such deformed adults were unable to produce fertile eggs, probably because the deformed wings made mating difficult.

It is possible that the deformities observed in this study may have been due to the presence of some chemical factors or an imbalance of nutrients such as proteins, carbohydrates, lipids, water and vitamins in those plants. Pupal deformities occurred in *S. eridania* raised on a diet containing *Mucuna* seed. The seed contains L-Dopa, which interfered with cuticle formation (Rehr et al., 1973). Dahlmam and Rosenthal (1975) have reported that an allelochemical, L-canavanine, present in the diet of *Manduca sexta*, resulted in pupal distortions and wing malformations in the adult. A deficiency in ascorbic acid in the diet of *M. sexta* and *S. littoralis* resulted in deformities in the pupae (Navon et al., 1985).

Generally, proportionately more females than males were produced. On cabbage and broad bean, higher proportions of female adults were produced. Unusual male to female ratios (female biased) have also been observed in some other insect species such as the African rice gall midge, *Orseola oryzivora* (Bouchard et al., 1992) and in the gypsy moth *Lymantria dispar* (Carter et al., 1992).

Differential larval and pupal mortalities have been

suggested for the abnormal male to female ratios observed in some insects (Brimacombe, 1980; Hurst and Majerus, 1993). Shukla and Tripathi (1993) suggested that the texture of the leaf surface and possibly allomones of the food plants may affect the sex ratios in aphids. Graig et al. (1992) observed that the sex ratio in *Euura iasiolepsis* varied with plant quality and growth. It was male biased in slow growing plants and female biased in fast growing plants. It was concluded that changes in sex ratio were an adaptive response to variation in plant quality.

Larvae on the broad bean and cabbage, which had heavier pupae and adults, also had proportionately more females and deformed adults. The higher number of females may be a adaptive feature to compensate for the deformed adults (this would ensure that adequate number of eggs are laid to enable the maintainance of the insect population.

2.3.10 Adult weights on food plants

There were significant differences in the weights of emerging adults on the food plants (Table 2.10). The females were heavier than the males on all the food plants tested. Adults emerging on the cabbage were significantly heavier than those emerging on the other food plants. The lightest adults were obtained on the sorghum and maize.

2.3.11. Adult longevity on food plants

There were no significant differences in the adult

Table 2.10. Mean adult weights (mg) of *S. littoralis* on food plants.

Food plants	Males	Females
	Mean \pm SE	Mean \pm SE
Sorghum	89.2 \pm 4.3 a	102.3 \pm 5.8 a
Maize	95.5 \pm 5.1 a	114.0 \pm 7.1 a
Cowpea	115.1 \pm 8.3 b	133.6 \pm 6.4 b
Soybean	138.5 \pm 8.9 c	168.6 \pm 8.0 c
Broad bean	154.9 \pm 10.1 c	209.8 \pm 6.1 d
Cabbage	183.5 \pm 11.9 d	214.3 \pm 9.3 d

Means in a column followed by the same letter are not significantly different at 5% probability level (SNK).

Table 2.11. Mean adult longevity (days) (mixed sexes) of *S. littoralis* on food plants.

Food plant	Mean \pm SE
Sorghum	5.2 \pm 0.6 a
Maize	7.9 \pm 0.9 a
Cabbage	9.9 \pm 0.8 ab
Cowpea	10.0 \pm 1.1 b
Broad bean	10.2 \pm 1.8 b
Soybean	10.5 \pm 1.3 b

Means followed by the same letter are not significantly different at 5% probability level (SNK).

longevity on the cabbage, broad bean, soy bean and cowpea. (Table 2.11). Also there were no differences between the longevity of adults on the maize and sorghum.

2.3.12. Ultimate survival (number of neonate larvae surviving to the adult stage) and growth indices.

Table 2.12 shows the growth indices and the number of adults emerging from the neonate larvae that were used to infest each food plant at the start of the experiment.

Table 2.12. Ultimate survival (percentage of larvae surviving to adult stage) and growth index of *S. littoralis* on food plants.

Food plant	Emergence (%)	Growth index
	Mean \pm SE	
Sorghum	7.8 \pm 0.8 a	0.5 a
Maize	32.8 \pm 3.1 b	0.9 a
Cowpea	38.1 \pm 4.4 b	3.3 b
Broad bean	65.3 \pm 5.7 c	4.5 b
Soybean	72.9 \pm 7.4 c	4.2 b
Cabbage	73.6 \pm 6.8 c	4.3 b

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Only 7% of larvae on sorghum and 32% of larvae on maize survived to the adult stage. In a preliminary experiment, the larve had failed to develop to pupation on ryegrass, wheat and rice as well as several varieties of maize, millet and sorghum. Bassili and Basturus (1983) observed 44.5% and 43-56% mortalities of larvae and pupae of *S. littoralis* on maize plants.

The high larval mortalities observed in these experiments may explain why Ahmed (1978) observed that even though the larvae of *S. littoralis* attack maize seedlings, they never reach damaging levels (they never survive in large numbers on the maize plants).

The reasons for the differences in the cummulative mortalities are not very clear. The plant characteristics are more likely to be the cause of the differences in mortalities.

Variations in the growth indices were observed. Larvae with low growth indices also had low percentage survival and low rates of development on the food plants. The low growth indices indicate poor adaptation on the maize and sorghum. The growth indices obtained in this study on soybean and cowpea are lower than reported by Prasad and Bhattacharya (1975) and Bhatt and Bhattacharya (1976), due probably to differences in sources of insects, food plants and environmental conditions.

2.3.13. Larval-pupal period index (L-P index) and pupal weight index (P-index)

Comparison of the performances on the food plants with that on the artificial diet for larval and pupal development showed that only cabbage and broad bean had indices greater than one and, therefore, were superior to the kidney bean artificial diet for growth and development of *S. littoralis* (Table 2.13). The other food plants were all inferior to the artificial diet.

Since nutritionally superior plants would support heavier pupae and produce larvae with shorter larval periods, broad bean and cabbage could be considered to be nutritionally superior to the other food plants. The P-indices were lower than the L-P indices. This could mean that generally, the food plants were superior for larval development as against pupal development.

2.3.14. Weight loss at pupation and at adult emergence

Karowe and Schoonhoven (1992) have suggested that differences in weight loss at pupation and at adult emergence would give an indication of the efficiency with which pupation and adult emergence occur.

The percentage of weight at pupation as a percentage of larval weight at pupation is shown in Table 2.14. Pupation on the maize and sorghum occurred with a smaller weight loss than pupation on the other food plants.

Table 2.13. Mean L-P index and P-index on food plants.

Food plants	Larval pupal index (L - P INDEX)	Pupal index (P - INDEX)
Sorghum	0.71 a	0.46 a
Maize	0.81 a	0.51 a
Cowpea	0.99 b	0.66 a
Soy bean	0.99 b	0.67 a
Broad bean	1.10 bc	1.00 b
Cabbage	1.17 c	1.06 b

Means followed by the same letter are not significantly different at 5% probability level (SNK).

Table 2.14. Mean weight of pupae as a percentage of larval weight at pupation.

Food plant	Mean weight (%) (Mean \pm SE)
Soybean	57.1 \pm 4.2 a
Cabbage	60.9 \pm 4.9 a
Broad bean	64.1 \pm 4.9 ab
Cowpea	64.8 \pm 4.7 ab
Sorghum	67.8 \pm 5.2 ab
Maize	74.1 \pm 7.9 b

Means followed by the same letter are not significantly different at 5% probability level (SNK).

There were no significant differences in the weight loss on any of the plants (between the pupa and the adult) of the emerging male adult. However, there were significant differences in the loss in weight at adult female emergence (Table 2.15). The differences in weight between the males and the females may be an adaptive measure to ensure that the females had the resources necessary for oviposition.

Table 2.15 Weight of adult as a percentage of pupal weight at emergence of male and female adults.

Food plant	Mean weight (%)	
	(Mean \pm SE)	
	Male	Female
Broad bean	48.5 \pm 3.9 a	60.6 \pm 1.2 a
Cabbage	48.8 \pm 9.2 a	62.3 \pm 2.2 a
Soybean	51.6 \pm 1.8 a	57.1 \pm 2.6 a
Cowpea	55.5 \pm 3.8 a	56.4 \pm 5.4 a
Maize	59.9 \pm 2.3 a	63.5 \pm 3.4 a
Sorghum	60.1 \pm 7.5 a	73.9 \pm 6.2 b

Means followed by the same letter are not significantly different at 5% probability level (SNK).

The pupae on the maize and sorghum had a lower per cent weight loss than the ones on the other food plants. The

lower percentage loss in weight at pupation and adult emergence on maize and sorghum could not compensate for the low larval and pupal weights obtained on these food plants.

2.3.15. Oviposition

The oviposition period and the number of eggs laid were affected by the food plant (Table 2.16).

Table 2.16. Oviposition period and mean number of eggs laid by adult female.

Food plant	Oviposition period	Eggs laid Mean \pm SE
Sorghum	2.4 \pm 0.3 a	141.5 \pm 18.1 a
Maize	3.6 \pm 0.7 ab	279.2 \pm 29.3 b
Cowpea	4.2 \pm 0.3 ab	452.7 \pm 48.5 c
Broad bean	5.8 \pm 0.5 b	558.4 \pm 53.3 d
Soybean	5.9 \pm 0.5 b	609.5 \pm 41.1 de
Cabbage	6.7 \pm 0.5 b	664.3 \pm 74.4 e

Means in a column followed by a common letter are not significantly different at 5% probability level (SNK).

The numbers of eggs laid by the female observed in the study were lower than reported by Moussa *et al.* (1960), Sanino *et al.* (1987), Duodu and Sam, (1990) and Anderson *et*

al. (1995). Differences in the strain of insect or in the varieties of food plants used in the studies could account for the differences.

2.3.16. Fecundity index

Table 2.17 shows that females emerging on the cowpea laid most eggs per day and the females emerging on the maize, the least. The low fecundity on the maize and sorghum could have been due to the quality of the food plants. Matsumura (1976) has suggested that chronic effects of toxic principles could be manifested in an organism in the reduction of fecundity and egg viability.

2.3.17. Mean diameter of eggs laid on food plants

Table 2.18 shows the variation in the mean diameter of the eggs of *S. littoralis*.

2.3.18. Mean number of eggs/egg mass

Table 2.19 shows the mean number of eggs per clutch and the percentage hatch of the eggs.

Table 2.17 Fecundity index (Number of eggs laid by female per day during oviposition period).

Food plant	Mean fecundity index
Maize	77.5 a
Sorghum	79.8 a
Broad bean	96.2 b
Cabbage	99.1 b
Soybean	103.1 bc
Cowpea	107.3 c

Means followed by the same letter are not significantly different at 5% probability level (SNK).

Table 2.18. Mean diameter of eggs of *S. littoralis* on food plants.

Food plants	Diameter (mm) Mean \pm SE
Sorghum	0.24 \pm 0.03 a
Maize	0.26 \pm 0.03 a
Cowpea	0.27 \pm 0.04 a
Soybean	0.29 \pm 0.04 a
Broad bean	0.35 \pm 0.03 b
Cabbage	0.36 \pm 0.04 b

Means followed by the same letter are not significantly different at 5% probability level (SNK).

Table 2.19. Mean number of eggs/clutch and the percentage hatch of eggs by *S. littoralis* on food plants.

Food plant	Eggs/clutch	Percentage hatch
	Mean ± SE	Mean ± SE
Sorghum	25.4 ± 2.2 a	72.0 ± 8.2 a
Maize	73.1 ± 9.5 b	75.3 ± 8.7 a
Broad bean	97.6 ± 10.0 bc	92.5 ± 10.4 a
Cabbage	100.7 ± 11.1 bc	88.3 ± 9.5 a
Soy bean	113.0 ± 14.6 bc	97.8 ± 10.2 a
Cowpea	127.8 ± 14.8 c	93.1 ± 10.9 a

Means in the same column followed by the same letter are not significantly different at 5% probability level (SNK).

Studies of reproduction in Lepidoptera have shown that there is a decrease in the size of eggs with maternal age. However, the effects of egg size on offspring performance have not been fully established (Wiklund and Pearson, 1983; Karlson and Wiklund, 1984; Wickman et al., 1990). If the hypothesis that the decrease in egg size with maternal age is due to a gradual depletion of protein and lipids derived from larval feeding (Wiklund and Karlsson, 1984; Boggs, 1986) is assumed, then the differences in the sizes of the eggs observed in this study were due to the differences in the nutritional values and the amounts of the food plants eaten.

The relationship between clutch size and egg size was not fully established in the study. Females on cabbage and broad bean, which produced bigger eggs, had relatively smaller clutch sizes. However, females on the maize and sorghum, which produced smaller eggs, also had smaller clutch sizes. Marshall (1990) did hypothesize that multiple reproductive strategies within a species, resulting from differences in reproductive effort expended, may explain why expected trade-offs in reproductive parameters e.g. egg size versus egg number, may not be observed. The egg size could give an indication of the size of the mouth parts of the first instar larvae which, in turn, could indicate the feeding potential of the larvae (Wiklund and Karlson, 1984).

Considering the overall performance of the immature stages on the food plants, it does appear that food plants had significant effects on the reproductive ability of the *S. littoralis*.

Larval nutrition could have played a role in oviposition, in that the heavier larvae produced bigger and greater number of eggs. The extent of the role played by larval nutrition would be difficult to estimate in the present study because females that were starved produced some eggs which, however, were mainly infertile. Thus, adult feeding seems to be important in egg laying in *S. littoralis*. It was fed on honeyed water which was low in

nitrogen.

Engelmann (1970) and Crowe (1995) have suggested that nutrients obtained in the adult stage were to maintain body weight without depleting resources for oviposition. Similar relationships of such fitness associated performance and insect body size have been reported in other insect species (Isenhour *et al.*, 1989; Wickman and Karlson, 1989; Banno, 1990).

The importance of egg clutch size lies in the fact that it usually determines the size of larval aggregation, growth and survival of the early instars (Gregiore, 1988; Lawrence, 1990; Stamp and Bowers, 1990). In the present study, such a relationship was not very clear. Even though the insects on cowpea had a high clutch size, only 32% on the larvae survived to the adult stage. The clutch sizes on maize and broad bean were not statistically different, but on maize, only 38% of larvae developed into adults.

2.3.19 Larval preference for 7 food plants

Herbivorous insects frequently encounter food plants that vary in nutritional and allelochemical contents. Food plants, therefore, have to be chosen before they can be utilised by the insect. The choice of a particular food plant or the part of the plant to be used is based on visual and chemical cues (Waldbauer and Friedman, 1991; Slansky, 1992).

These chemicals are perceived by a complement of chemosensory organs such as antennae and mouth parts (de Boer, 1993). Schoonhoven (1990) suggested that general intoxication or lack of specific nutrients is associated with certain food types, which affect food choice.

The first and fourth instar larvae were given a choice of seven food plants. Table 2.20 shows the variation in the number of 1st instar larvae that were counted on the food plants after 24 h. The fourth instar larvae accepted all the foliage offered (Table 2.21). The most preferred food plant for both instars was broad bean.

Beckers et al. (1994) have suggested that there might be an early learning period for food odour in insects such as ants. The trend in the choice of food plants was the same in both instars and might suggest that such an early learning period may not exist in *S. littoralis*.

2.3.19.1 Two choice tests

Although in most insect species, adult females are usually directly responsible for the selection of food for the immature stages, it has been reported that immature stages could select and balance their own diets in order to obtain better performance (Zucoloto, 1990).

Growth and development of *S. littoralis* were inhibited on maize, millet and sorghum (2.3.3). The choice tests were

Table 2.20 Mean number of 1st instar larvae feeding on foliage after 24 h.

Food plants	Number (Mean \pm SE)	PR (Mean \pm SE)
Millet	5.5 \pm 1.7 a	0.29 \pm 0.03
sorghum	9.6 \pm 1.9 a	0.53 \pm 0.04
Maize	17.5 \pm 2.2 a	1.00 \pm 0.03
Soy bean	19.5 \pm 2.2 ab	1.12 \pm 0.07
Cowpea	20.5 \pm 1.9 b	1.18 \pm 0.11
Cabbage	23.5 \pm 2.6 bc	1.36 \pm 0.15
Broad bean	28.3 \pm 3.1 c	1.64 \pm 0.19

Means followed by a common letter are not significantly different (P=0.05) (SNK).

E_{PR} 0.43

Table 2.21. Mean number of 4th instar larvae feeding on foliage after 24 h.

Food plant	Number (Mean \pm SE)	PR (Mean \pm SE)
Millet	8.3 \pm 1.5 a	0.47 \pm 0.02
Sorghum	11.3 \pm 1.3 a	0.68 \pm 0.05
Maize	12.3 \pm 1.6 a	0.76 \pm 0.09
Cowpea	16.6 \pm 1.9 b	1.02 \pm 0.12
Soy bean	19.5 \pm 2.1 bc	1.21 \pm 0.15
Cabbage	21.9 \pm 2.8 cd	1.37 \pm 0.18
Broad bean	25.0 \pm 3.1 d	1.55 \pm 0.22

Means followed by a common letter are not significantly different (P=0.05) (SNK).

E_{PR} = 0.36

carried out only on maize, millet and sorghum.

Fig. 2.8 shows that the 1st instar larvae preferred maize to the other two food plants in any two choice test with either sorghum or millet. Fig. 2.9 shows that in two choice tests among maize, millet and sorghum, maize was always preferred to either sorghum or millet by 4th instar larvae.

When the second pair of leaves of both the 4 leaf stage and the 6 leaf stage of each food plant were offered to the 4th instar larvae, the preference for the 4 leaf stage over the 6 leaf stage was not significant (Fig. 2.10A). When the second pair of leaves of both the 4 leaf stage and the 8 leaf stage of each food plant were offered to the 4th instar larvae, the leaves of the 4 leaf stage were preferred ($P < 0.05$) to the leaves of the 8 leaf stage (Fig. 2.10B).

The observations generally indicate a preference for younger leaves by the 4th instar larvae. Murugan and George (1992) have suggested that the relative preference for young leaves by insects could be attributed to differences in protein, carbohydrate and amino acid content of the leaves. Damage to such younger leaves could have, potentially, far greater effect on the integrity of the plant, because younger leaves have higher photosynthetic rates than older leaves and would therefore contribute more

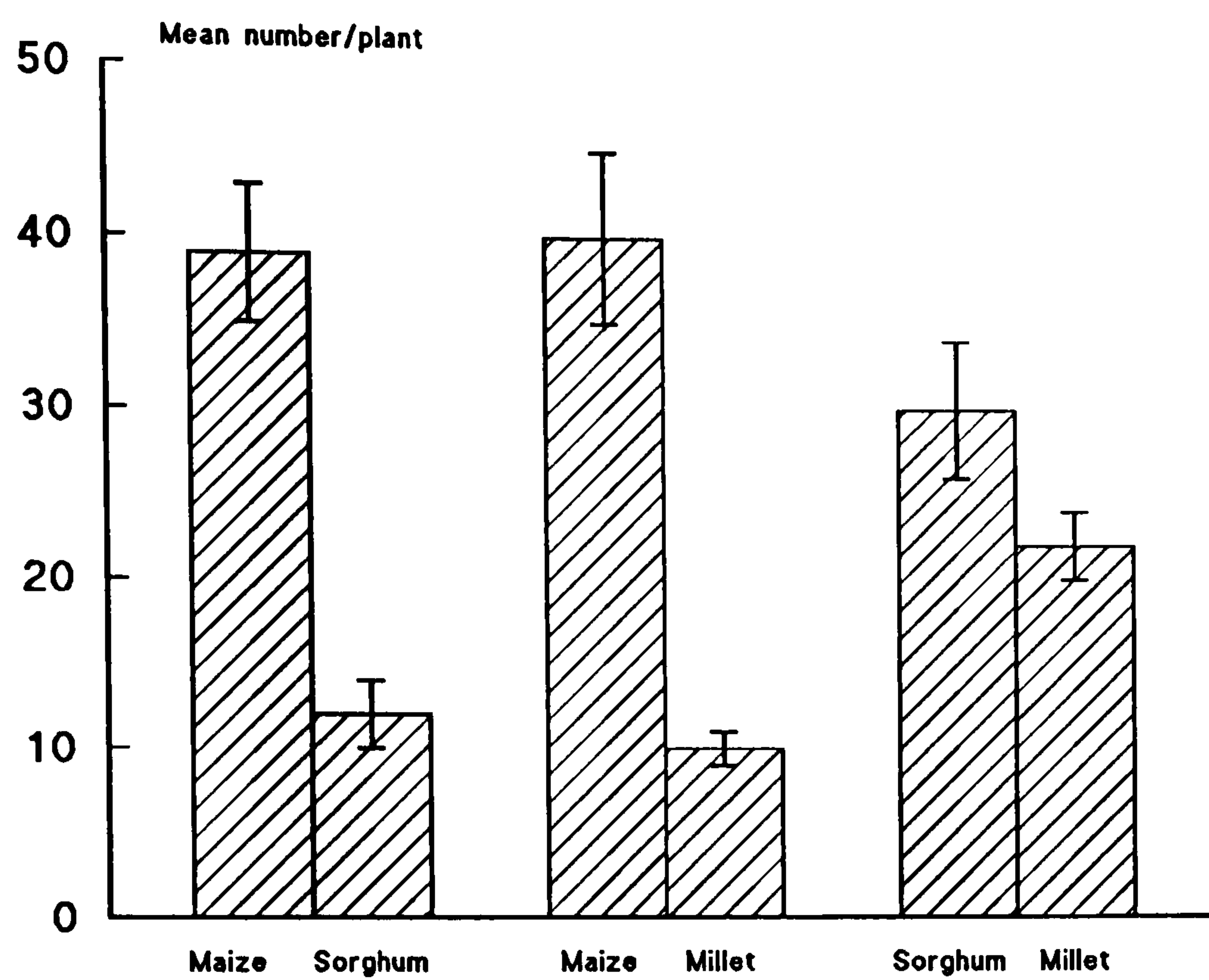


Figure 2.8. Preference of first instar larvae for maize, millet and sorghum in a two choice test. Bars represent Standard Errors.

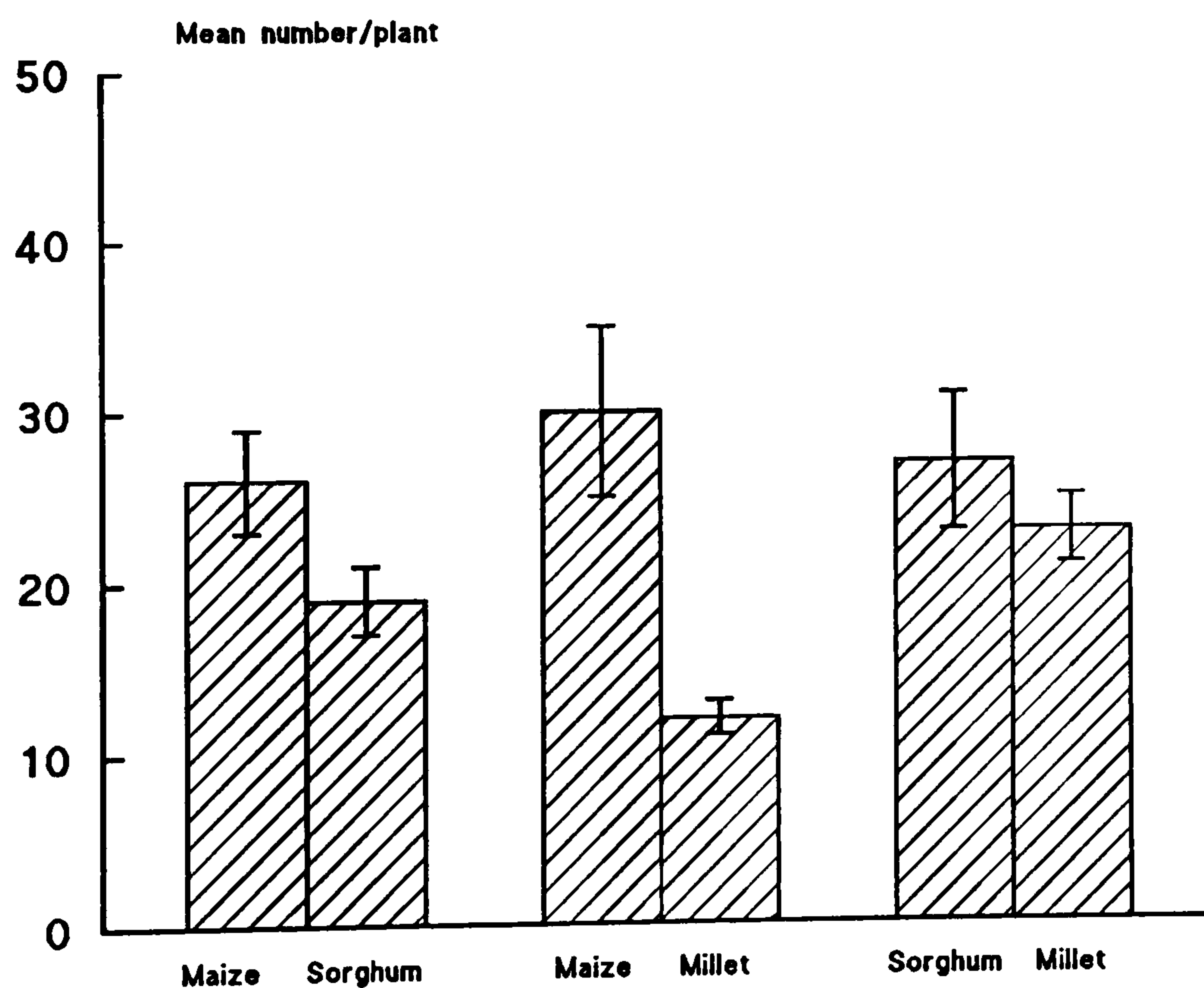
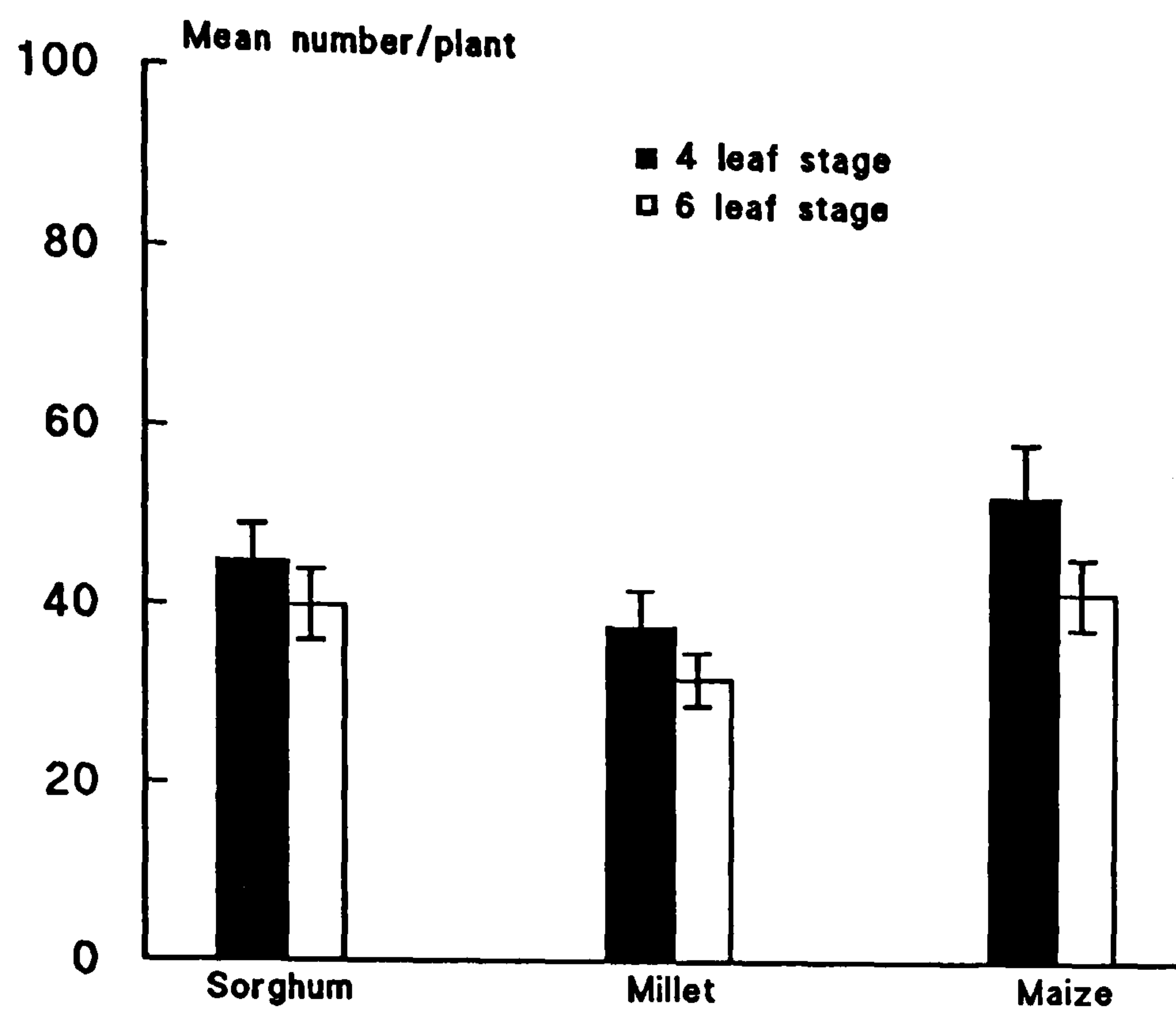
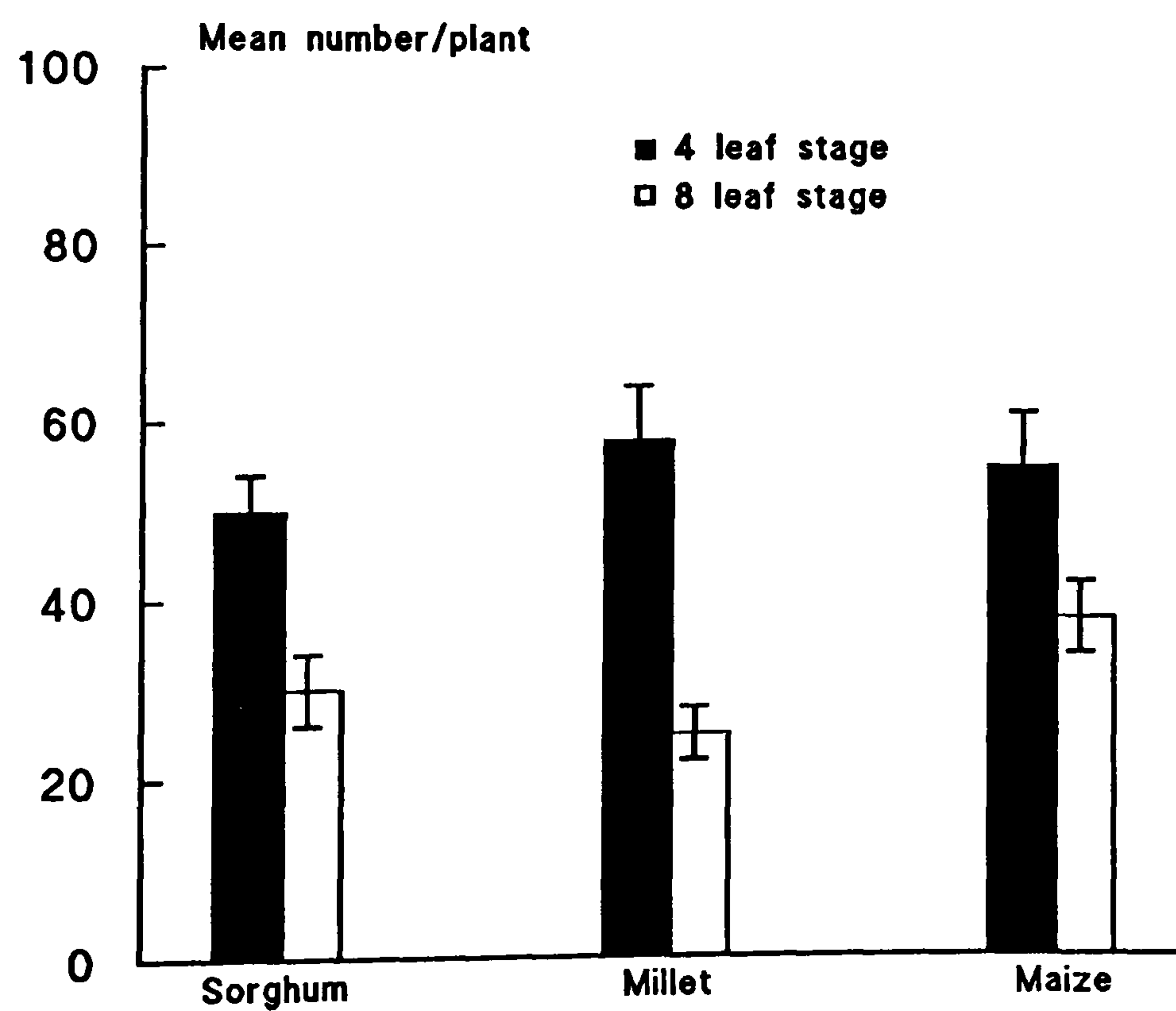


Figure 2.9. Preference of fourth instar larvae for maize, millet and sorghum in a two choice test. Bars represent Standard Errors.



A



B

Figure 2.10. Preference of 4th instar larvae for 4 leaf and 6 leaf stage (A) and 4 leaf and 8 leaf (B) stages in two choice tests. Bars represent Standard Errors.

photosynthate to the plants. It is probable that differences in the chemical profiles of the food plants could have resulted in the different numbers of the larvae observed on the food plants.

Since the larvae were reared on the artificial diet prior to the start of the experiments, the larval preferences observed in the study could not have been influenced by prior experience on any of the food plants used for the study. The preferences were, therefore, due to the plant characteristics.

2.3.20. Larval food consumption, digestion and utilisation

No larval mortalities were observed on any of the food plants except in the 4th instar larvae on maize (15%).

2.3.20.1. Larval food consumption

According to Singer (1986), if different plant species are not consumed in proportion to their relative abundance, then the insect shows food preference.

Fig. 2.11 shows the daily weight of the 4th instar larvae and Fig. 2.12 shows the daily weight of the 6th instar larvae on the food plants.

Mean cumulative dry weight of foliage ingested varied for each of the food plants. Larval food consumption was low on the maize, millet and sorghum, both in the 4th

instar (Fig. 2.13) and in the 6th instar (Fig 2.14).

The amount of food consumed by a larva depends on its rate of feeding and the length of time during which feeding occurs (Slansky, 1992). More food was consumed in the 6th instar than in the 4th. The sixth instar was of a longer duration than the fourth. The variations in the amounts of food ingested could be due to the differences in the nutritional and physiological needs of the various instars.

Similar variations in food ingestion has been reported by Afifi and Attia (1990) in *S. littoralis* and by Garner and Lynch (1981) in *S. frugiperda*. In all the observations, 60 to 80% of the total amount of food ingested in the larval stages was ingested in the 4th-6th instars. In *S. frugiperda*, 62.5% of the total amount of food ingested in the larvae was consumed by the 6th instar. Slansky (1992) reported that the changes in the larval food intake in the various instars were due to changes in the feeding rhythm in instars. For instance, increase in feeding in the last larval instar of *M. sexta* was due to a greater amount of time spent feeding (Bowden, 1988). This is an adaptive behaviour to ensure that adequate nutrients are obtained for the development of the subsequent growth stages.

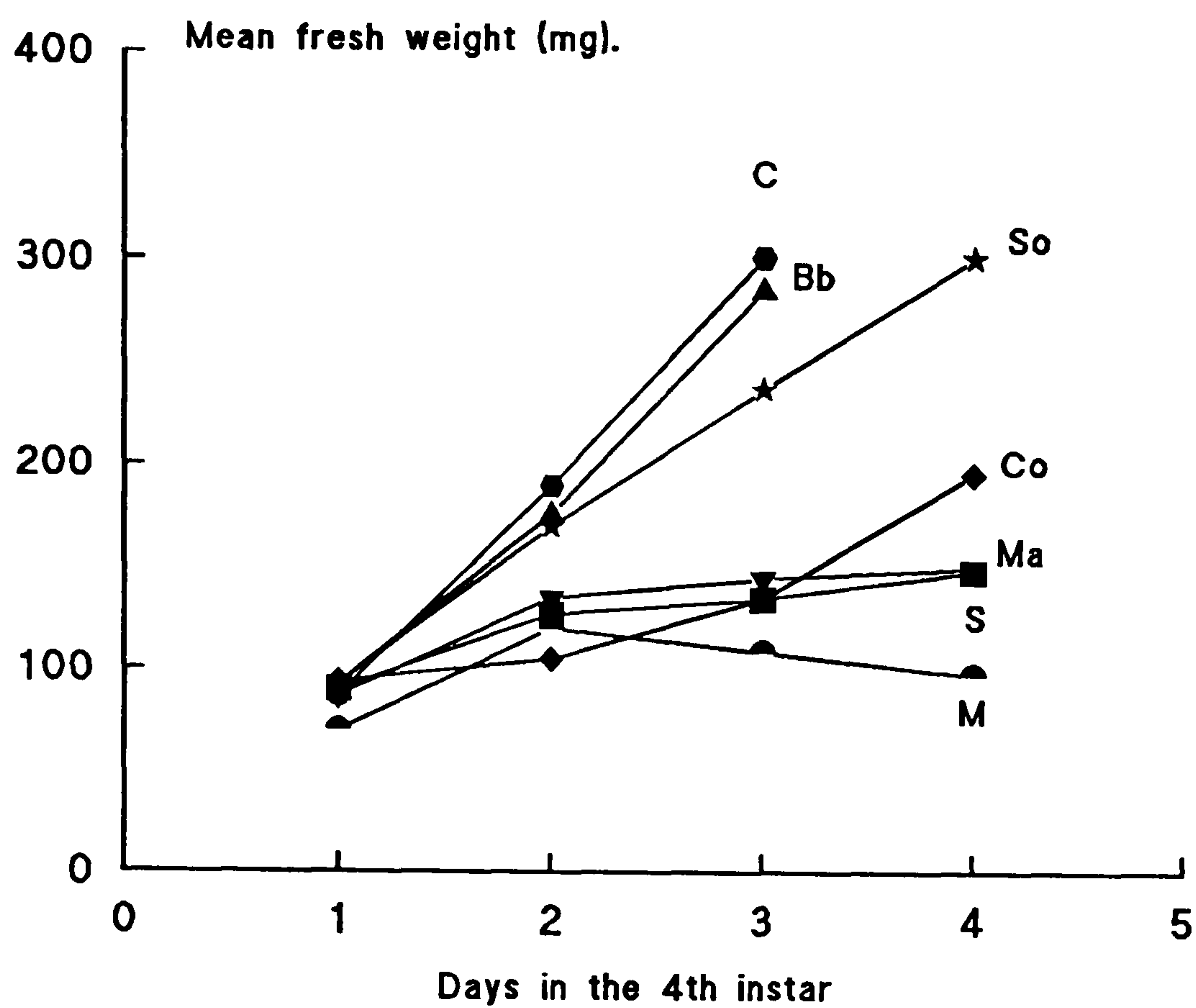


Figure 2.11. Daily 4th instar larval fresh weight (mg) on food plants.

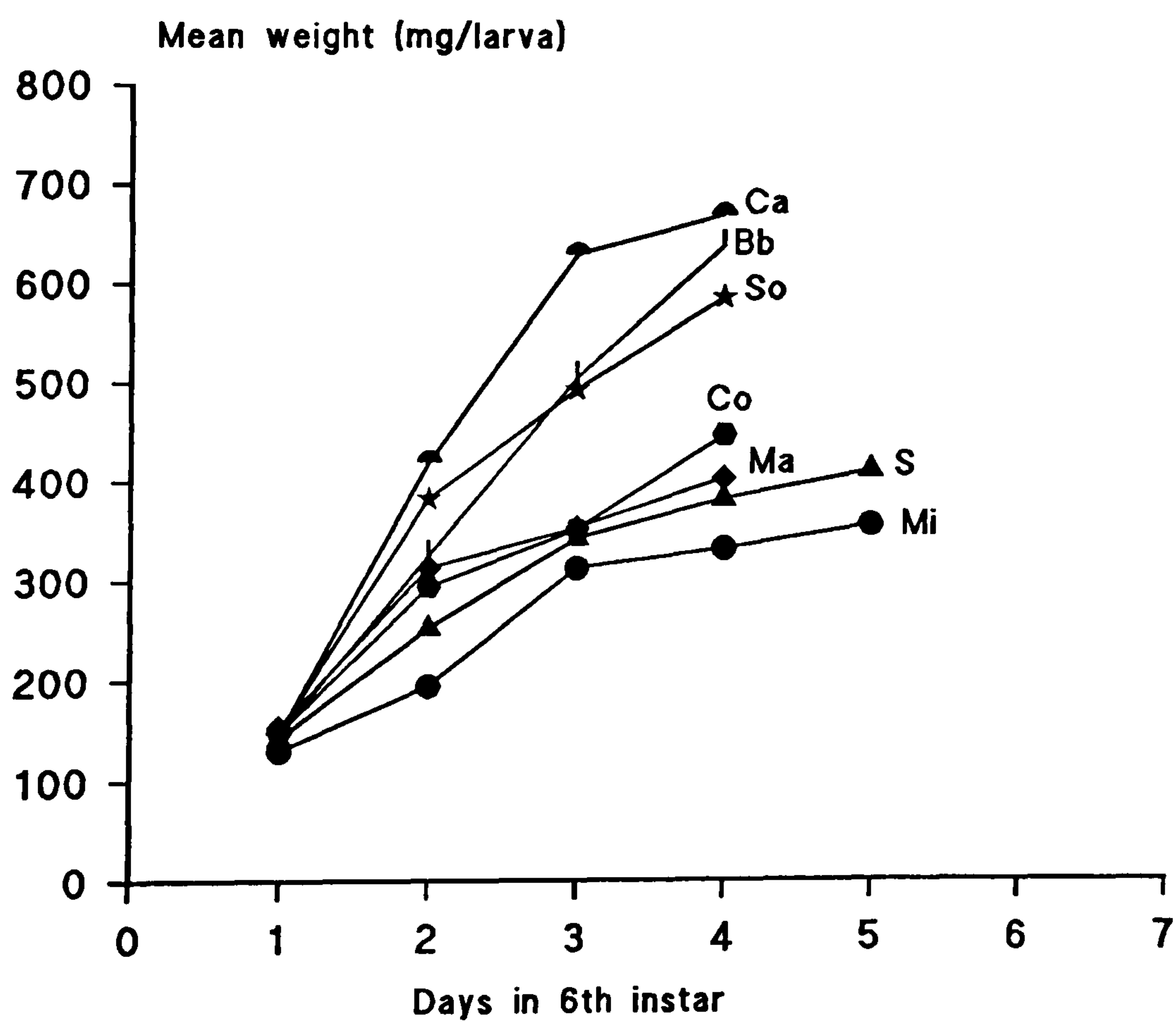


Figure 2.12. Daily 6th instar larval fresh weight (mg) on food plants

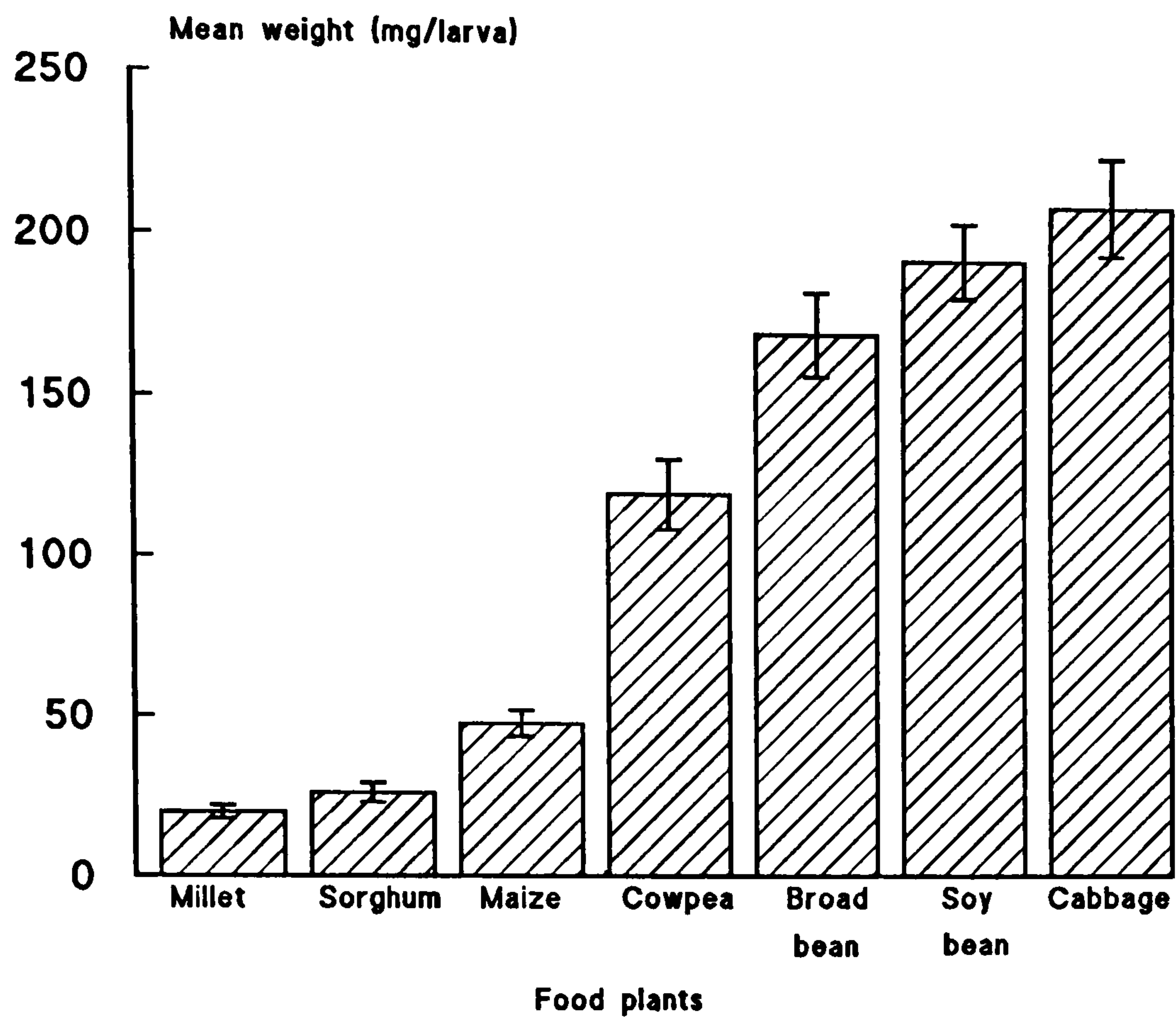


Figure 2.13. Mean dry weight (mg) of foliage ingested by 4th instar larvae. Bars represent Standard Errors.

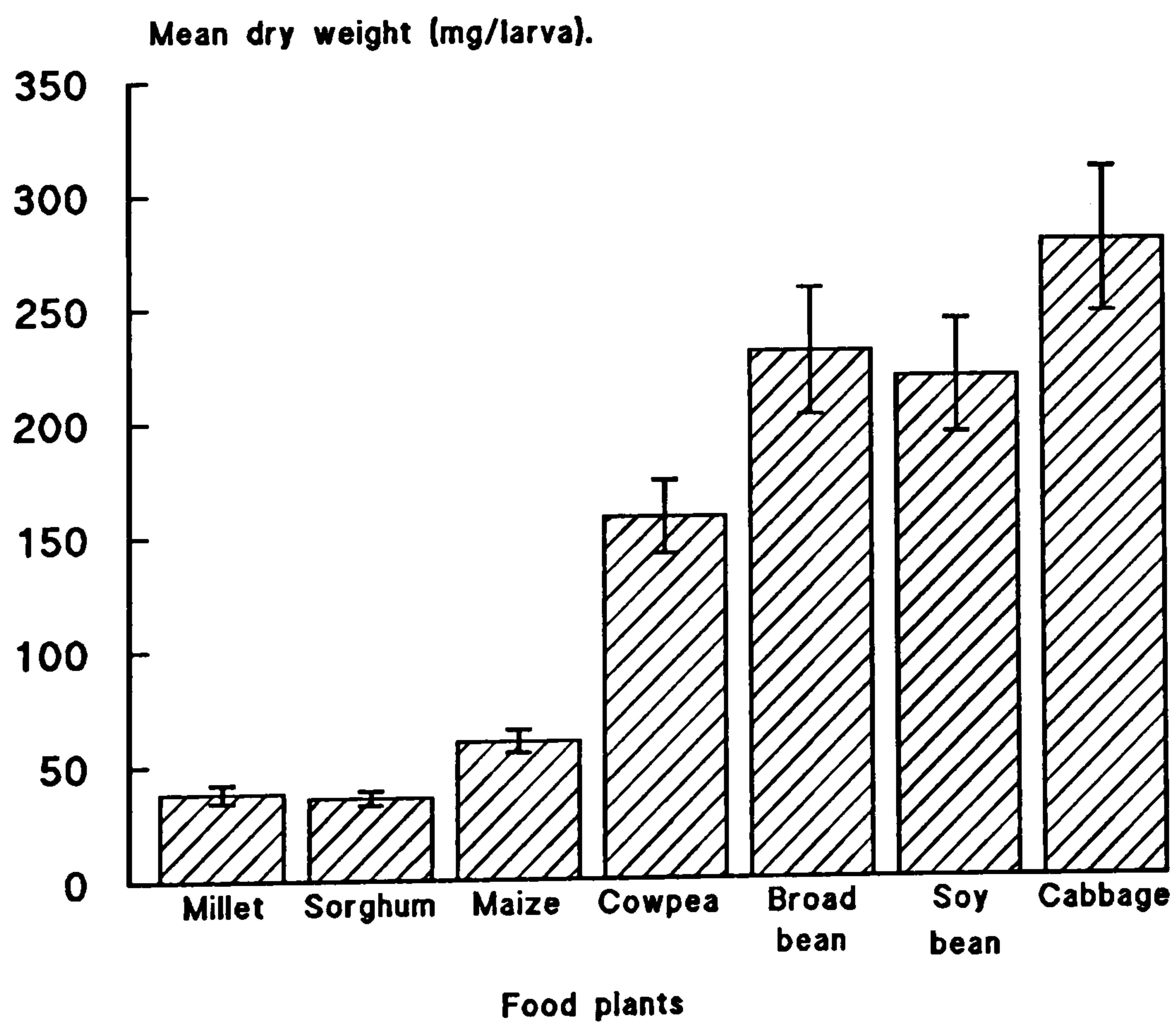


Figure 2.14. Mean dry weight (mg) of foliage ingested by 6th instar larvae. Bars represent Standard Errors.

2.3.20.2. Dry weight of food plants

The mean percentage dry matter content of the leaves varied with the food plants (Table 2.22).

Table 2.22. Mean percentage of dry matter content of all leaves of the plant.

Food plants	Dry matter content (%)	
	(Mean \pm SE)	
Cabbage	9.4	\pm 0.3 a
Cowpea	10.1	\pm 0.5 a
Soybean	10.3	\pm 0.3 a
Broad bean	12.4	\pm 0.6 b
Millet	18.3	\pm 0.7 c
Sorghum	21.4	\pm 0.9 d
Maize	23.1	\pm 1.2 d

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Low food intake occurred on maize, millet and sorghum, which had water contents of between 77 and 82%. It is unlikely that only the water content of the food plants played a major role in the amounts of food ingested. According to Slansky (1992), leaves could be classified as

low water foliage if the water content is between 50 and 60% and high water foliage if the content is over 80%. It does appear that the water content of the food plants was adequate for the larvae, since none of the plants was in the low water category.

A certain amount of dietary water is important and foliage water is an important factor that influences the relative growth rates of insects (Slansky and Scriber, 1985; Slansky, 1992). This is because larvae obtain the bulk of their water requirement from their food (Martin and van't Hof, 1988).

In the Indianmeal moth, *Plodia interpunctella*, the suitability of an artificial diet for development was correlated with diet moisture content (Johnson *et al.*, 1992) and oviposition in *Rachipusia nu* was adversely affected by low foliage water content of soybean (Pereyra (1994).

In the present study, leaf consumption was higher in the food plants with lower dry weight. The larval growth rate was higher on these same food plants. Slansky (1992) has observed that high water leaves often have a greater nutrient level than leaves with lower water content. It could, therefore, be suggested that the differences in the larval food intake were due to the nutrient status of the food plants.

2.3.20.3. Larval weight gain

Fig. 2.15 shows the mean dry weight gain of the 4th instar larvae and Figure 2.16 shows the larval dry weight gain of the 6th instar larvae on the food plants. Generally, the weight gains on the maize, millet and sorghum were lower than on the other food plants. Fig. 2.17 and Fig. 2.18 show the variation in the faecal pellets on the food plants.

When offered a choice between the 4 and the 6 leaf stages of the food plants, more of the 6 leaf stage was eaten on the maize (Fig. 2.19) However, on all the food plants, more faecal pellets were produced on the 6 leaf stage (Fig. 2.20). This resulted in higher weight gain on the 4 leaf plants (Fig. 2.21).

2.3.20.4 Nutritional indices

The approximate digestibility measures the portion of consumed food that passes through the gut wall and into the haemolymph and is thus available for metabolism and growth.

There were significant differences in the approximate digestibility for the 4th instar larvae (Table 2.23) and for the 6th instar (Table 2.24). The AD was generally lower for the 6th than for the 4th instar.

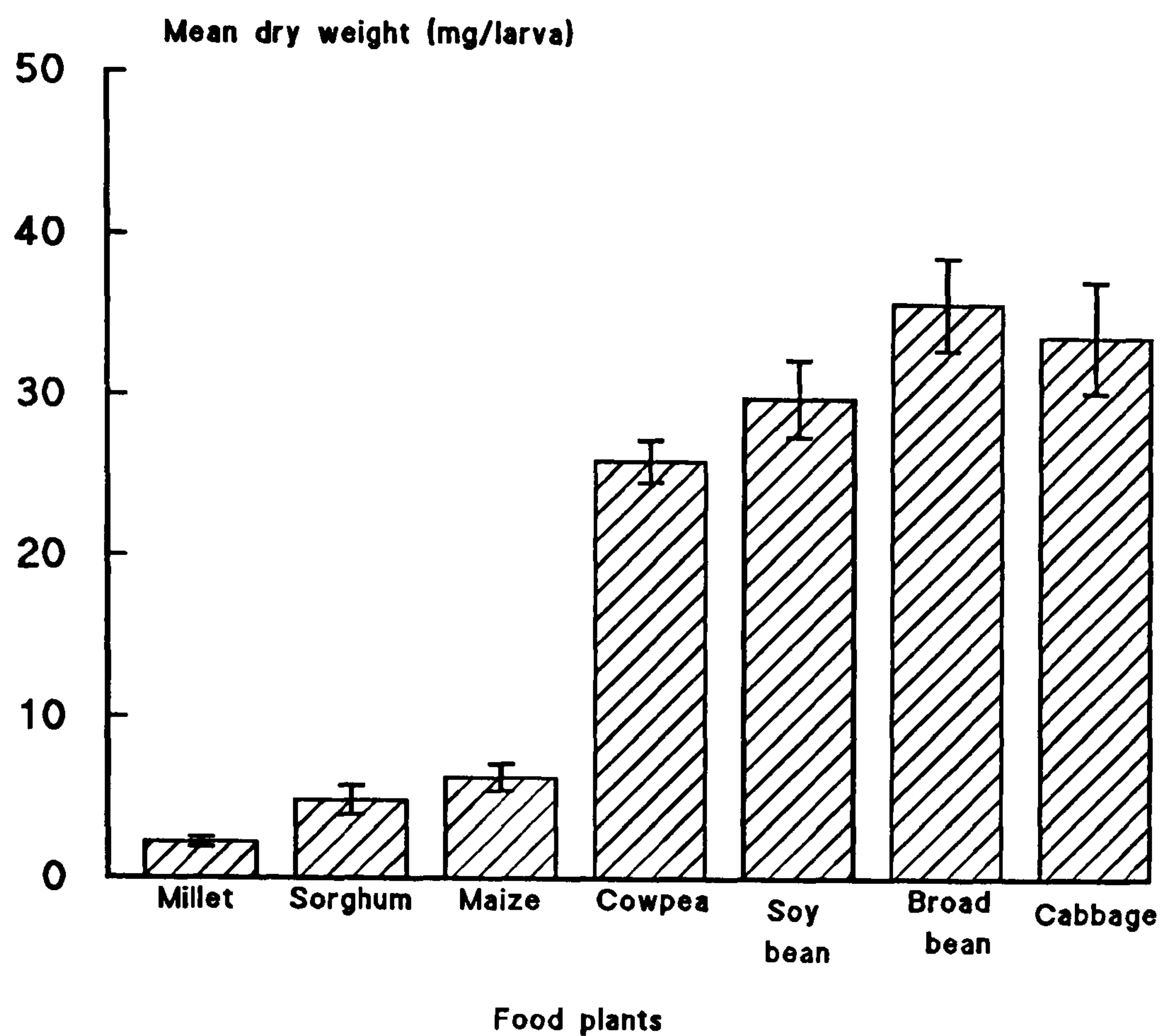


Figure. 2.15. Mean gain in weight (mg) of 4th instar larvae on food plants. Bars represent Standard Errors.

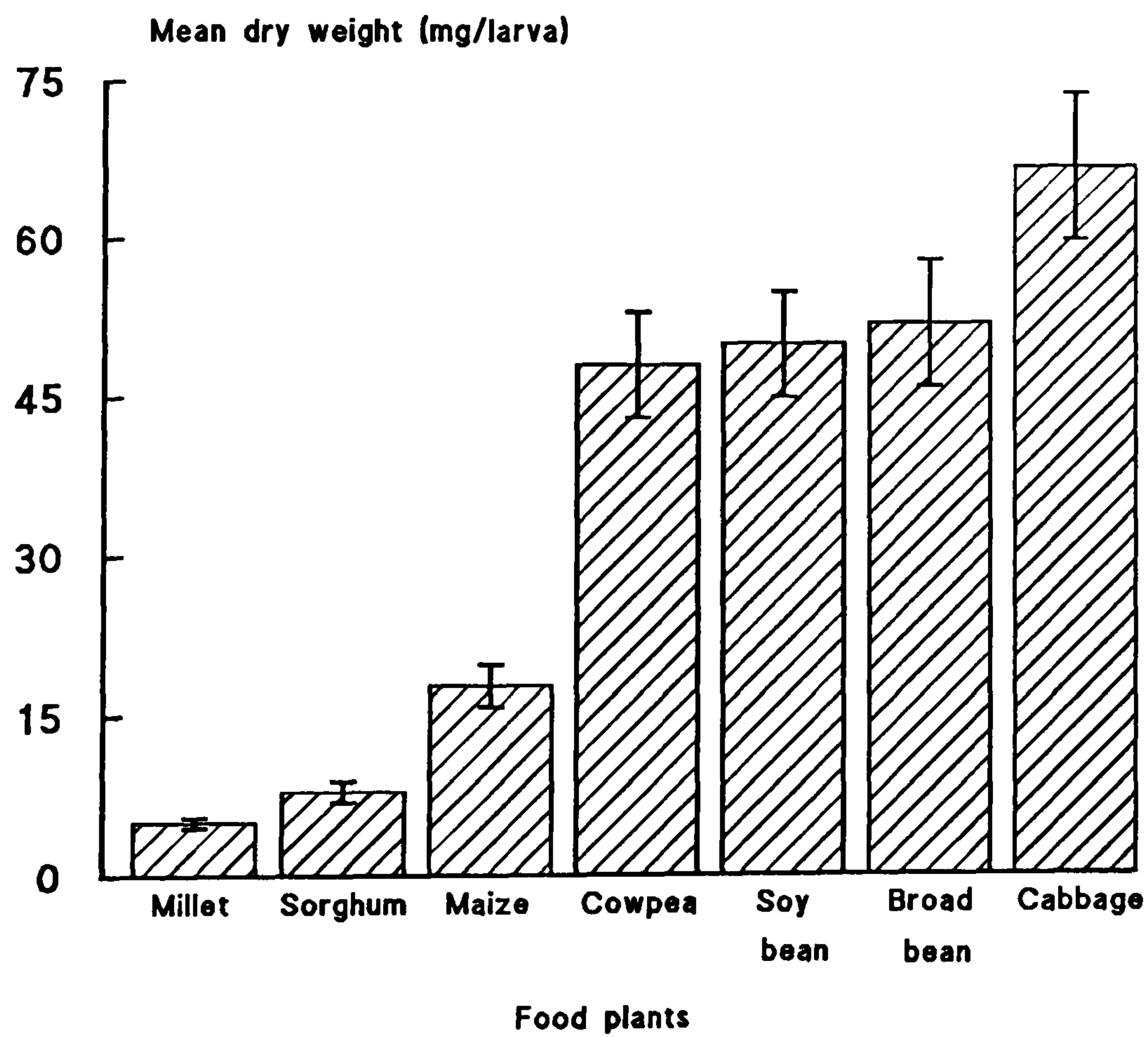


Figure 2.16. Mean gain in weight (mg) of 6th instar larvae on food plants. Bars represent Standard Errors.

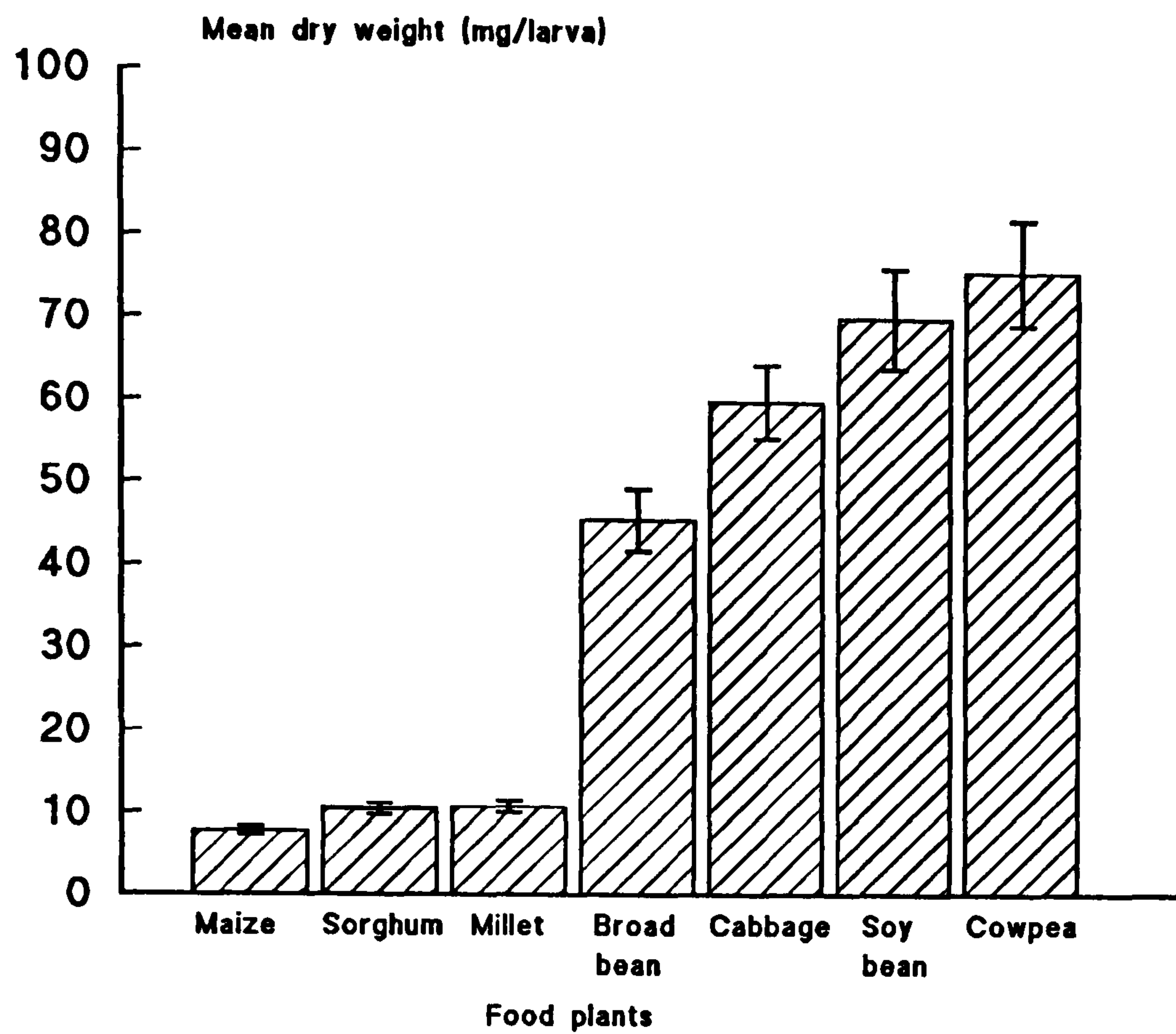


Figure 2.17. Mean dry weight (mg) of faecal pellets of 4th instar larvae.
Bars represent Standard Errors.

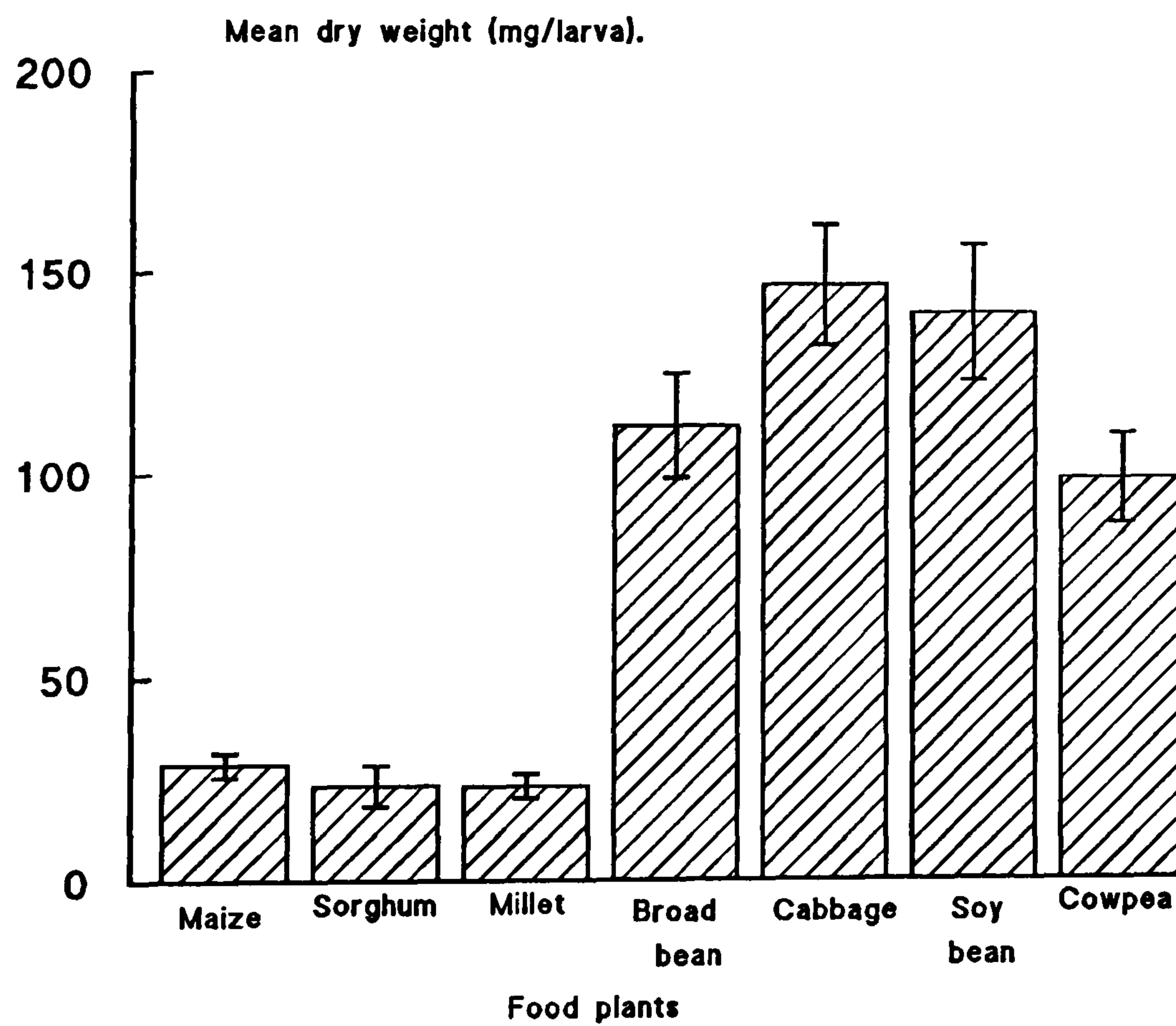


Figure 2.18. Mean dry weight (mg) of faecal pellets of 6th instar larvae.
Bars represent Standard Errors.

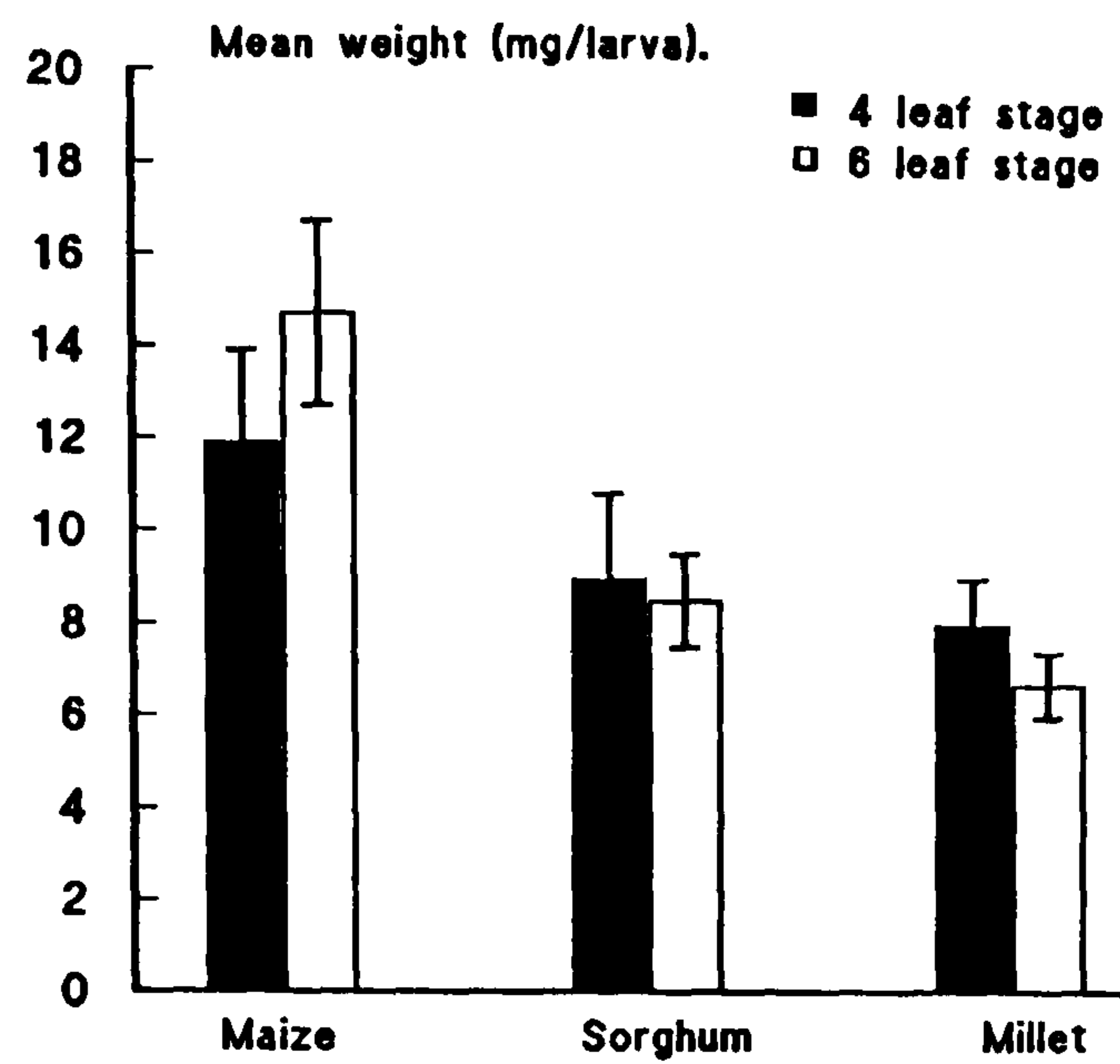


Figure 2.19. Weight (mg) of foliage ingested by 4th instar larvae on food plants at two stages of growth. Bars represent Standard Errors.

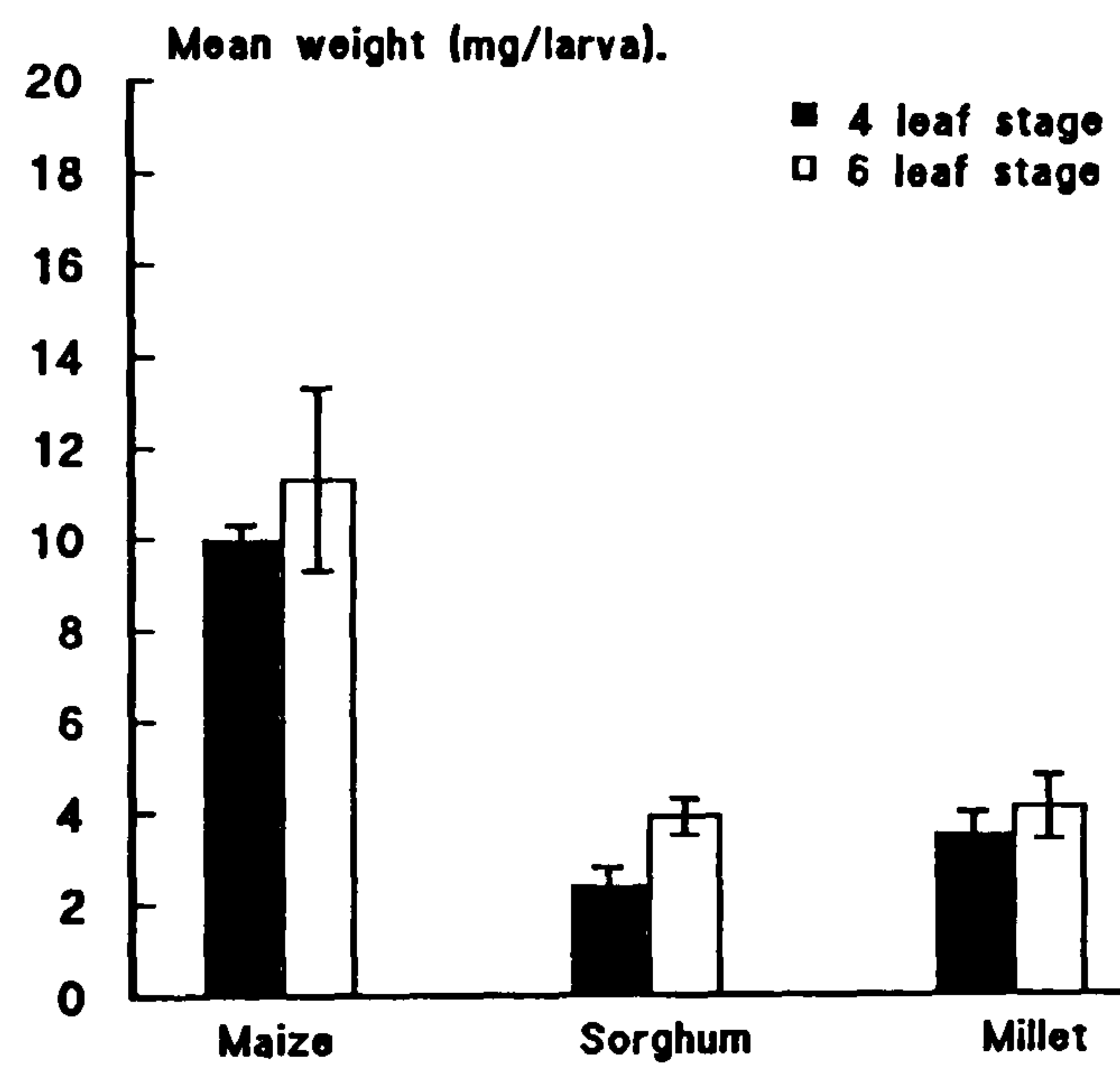


Figure 2.20. Weight (mg) of faecal pellets produced on food plants at two growth stages. Bars represent Standard Errors.

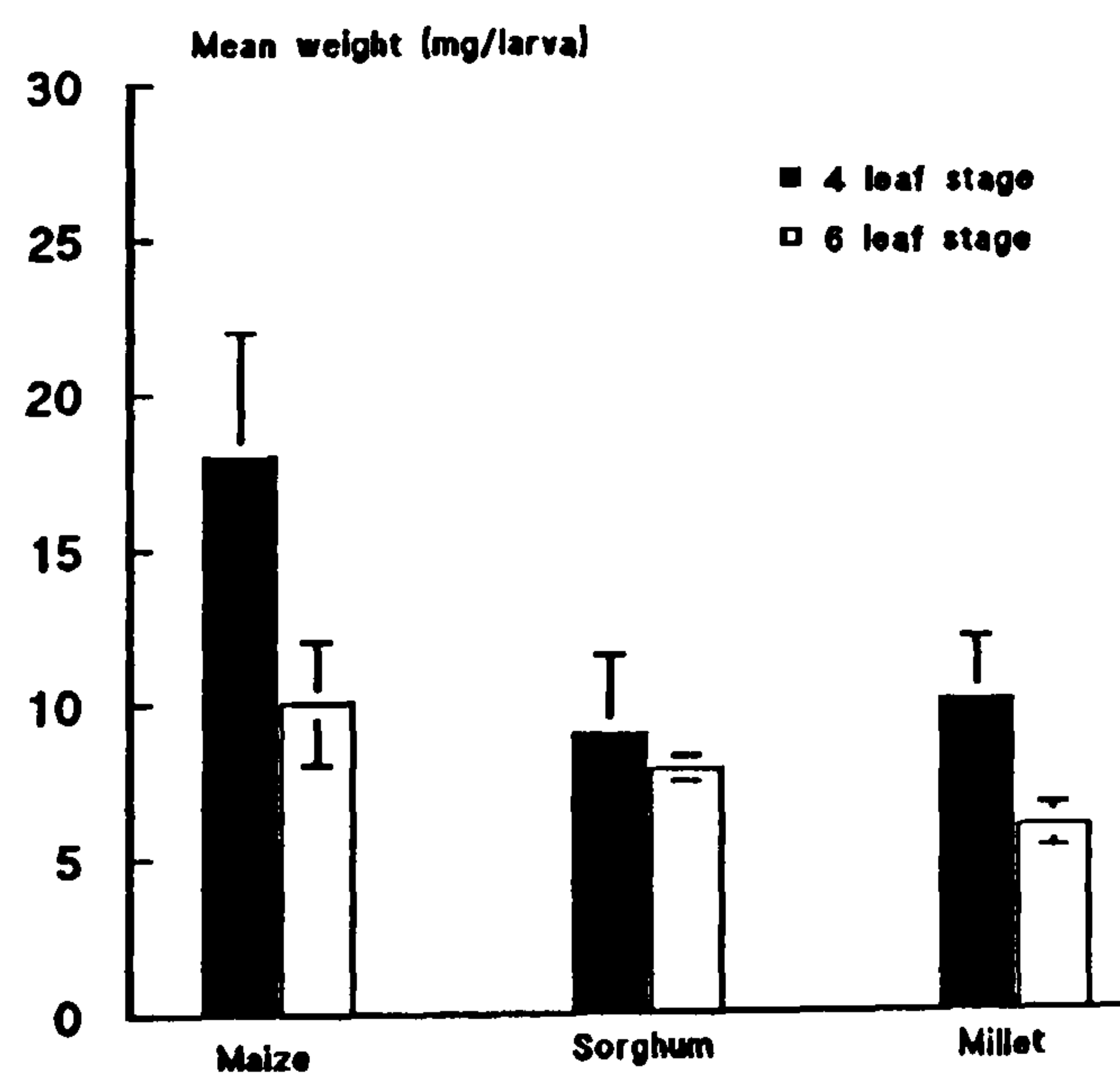


Figure 2.21. Gain in weight (mg) by 4th instar larvae on food plants at two growth stages. Bars represent Standard Errors.

Table 2.23 Mean approximate digestibility (AD) for 4th instar larvae on food plants.

Food plant	AD (Mean \pm SE)
Millet	40.5 \pm 1.5 a
Sorghum	49.8 \pm 2.6 b
Maize	57.8 \pm 2.3 bc
Cowpea	63.0 \pm 4.5 cd
Cabbage	68.8 \pm 5.2 de
Broad Bean	71.1 \pm 4.4 de
Soybean	73.2 \pm 5.1 e

Means in a column followed by the same letter are not significantly different at 5% probability level (SNK).

Table 2.24. Mean approximate digestibility for 6th instar larvae on food plants.

Food plants	AD (Mean \pm SE)
Millet	29.4 \pm 2.1 a
Sorghum	32.0 \pm 3.7 a
Maize	32.3 \pm 3.5 a
Cowpea	35.6 \pm 3.9 a
Cabbage	41.9 \pm 4.3 ab
Broad bean	51.0 \pm 4.8 b
Soybean	64.2 \pm 5.9 c

Means followed by the same letter are not significantly different at the 5% probaility level (SNK).

The efficiency of conversion of ingested food to body matter (ECI) varied with the food plant in the 4th instar (Table 2.25) and the 6th instar (Table 2.26).

The efficiency of conversion of digested food to body matter (ECD) measures the proportion of assimilated food that is converted to body mass. It varied in the 4th instar (Table 2.27) and in the 6th instar (Table 2.28). The indices in the 6th instar were higher than in the 4th instar.

The mean dry weight weight consumption index (CI) - the total weight consumed per unit of larval dry weight per day - varied with the food plants on the 4th instar (Table 2.29) and on the 6th instar (Table 2.30). The CI of the 4th instar was higher than the CI of the 6th instar because of the greater amount of food ingested by the 6th instar larvae. Another probable reason is that in the 6th instar the higher ECD would ensure that weight gain in the 6th instar would be higher than in the 4th.

Table 2.25. Mean efficiency of conversion of ingested food to body matter of 4th instar larvae on food plants.

Food plant	ECI (Mean \pm SE)
Millet	14.7 \pm 1.5 a
Sorghum	19.5 \pm 1.1 b
Soybean	21.8 \pm 1.3 bc
Maize	23.3 \pm 1.6 c
Broad bean	24.5 \pm 2.1 c
Cowpea	25.0 \pm 1.9 c
Cabbage	25.5 \pm 0.8 c

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Table 2.26. Mean efficiency of conversion of ingested food to body matter of 6th instar larvae on food plants.

Food plant	ECI (Mean \pm SE)
Millet	13.3 \pm 1.4 a
Sorghum	20.7 \pm 1.9 b
Soybean	20.8 \pm 2.2 b
Broad bean	22.6 \pm 2.7 b
Cabbage	24.1 \pm 2.8 b
Maize	29.6 \pm 3.4 c
Cowpea	30.1 \pm 3.5 c

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Table 2.27. Mean efficiency of conversion of digested food to body matter of 4th instar larvae on food plants.

Food plant	ECD (Mean \pm SE)
Sorghum	28.0 \pm 2.0 a
Millet	32.0 \pm 2.8 b
Maize	34.0 \pm 2.1 b
Soybean	34.3 \pm 3.2 b
Cabbage	34.7 \pm 1.8 b
Cowpea	40.1 \pm 2.1 c
Broad bean	58.0 \pm 3.5 d

Means followed by a common letter are not significantly different at the 5% probability level (SNK).

Table 2.28. Mean efficiency of conversion of digested food to body matter of 6th instar larvae on food plants.

Food plants	ECD (Mean \pm SE)
Millet	35.1 \pm 3.8 a
Sorghum	38.2 \pm 4.3 a
Cabbage	46.0 \pm 5.5 b
Broad bean	54.3 \pm 6.1 c
Maize	57.1 \pm 6.9 c
Cowpea	64.4 \pm 7.3 cd
Soybean	68.2 \pm 7.6 d

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Table 2.29. Mean consumption index (CI) of 4 instar larvae on food plants.

Food plant	CI (Mean \pm SE)
Millet	0.28 \pm 0.02 a
Sorghum	0.32 \pm 0.05 a
Maize	0.58 \pm 0.09 b
Cowpea	1.01 \pm 0.11 c
Broad bean	1.42 \pm 0.15 d
Soybean	1.44 \pm 0.13 d
Cabbage	1.59 \pm 0.19 d

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Table 2.30. Mean consumption index (CI) of 6th instar larvae on food plants.

Food plants	CI (Mean \pm SE)
Millet	0.06 \pm 0.01 a
Sorghum	0.06 \pm 0.01 a
Maize	0.08 \pm 0.02 a
Cowpea	0.21 \pm 0.02 b
Broad bean	0.30 \pm 0.04 c
Soybean	0.42 \pm 0.05 d
Cabbage	0.44 \pm 0.07 d

Means followed by a common letter are not significantly different at the 5% probability level (SNK).

Even though larval food consumption over the entire larval period was not determined, the food consumption and utilisation by the 4th and 6th instar larvae indicate larval food preferences. Scriber and Slansky (1981) have observed that most of the total food consumption and growth usually occurred during the penultimate and final instars and therefore, performance values calculated for these instars tend to be representative for the entire larval stage.

The low weight gain of the larvae on the maize and sorghum in comparison with the weight gain on the other food plants was probably due to the low food intake and efficiencies with which the digested materials were converted to body tissues on the various food plants.

There was a compensatory response in the 6th instar. Even though the AD was lower in the 6th instar, the ECD was higher and the amount of food ingested was also higher resulting in the overall higher weight gain.

In the few published reports on food intake in larvae that had provided food intake and nutritional indices in each instar, 6th instar *S. littoralis* had a higher ECI and ECD than the 4th instar on soybean. On cotton, ECI and ECD were lower in the 4th instar. The AD was lower in the 4th instar than the 6th on both crops (Afifi and Mesbah, 1990; Afifi, 1990; Afifi and Attia, 1990). Only the ECD was

age related, increasing with age of the larvae. The other indices did not follow a discernible trend.

In the present study, the AD was lower in the 6th instar, suggesting a decline in AD with age. The decline in AD could be related to the eating behaviour of the 6th instar larvae. The older *S. littoralis* larvae eat the entire leaf including the veins, whereas the younger larvae avoid the veins. The decline in AD with larval age in other insect species is often associated with less selective feeding by later instars resulting in the consumption of a higher proportion of indigestible fibre (Kogan and Cope, 1974).

The ECD values were higher than the ECI on all food plants. These results are similar to those obtained by Soo Hoo and Fraenkel (1966), with *Spodoptera eridania*, Jamjanya and Quisenberry (1988) on the fall armyworm, and in *S. littoralis* on cotton and soybean (Afifi and Attia, 1990) but differ from those obtained by Duodu and Biney (1981) on *S. littoralis* on cotton, kenaf and cabbage and Kohler et al. (1987) with grasshoppers.

The low ECI, ECD, and CI of the larvae on the maize, millet and sorghum would reflect low adaptation on these food plants (Slansky and Scriber, 1985; Zucoloto, 1990) since low ECI and ECD reflect high energy cost (most food being used for metabolic activities).

2.3.21. Cumulative food intake of 0-10 day old larvae on 3 food plants

Figure 2.22 shows the cumulative food intake of larvae (from 0-10 days after hatching - **DAH**) fed on maize, broad bean and cabbage. From 0 to 7 days, more cabbage was eaten than other food plants. However, between 7 and 10 days, more broad bean was ingested. The amount of food ingested in the 10 day period was lower than the amount of food ingested by the larvae in the 4th instar on these food plants (2.3.19). This agrees with observations on other species that the greater part of the food ingested in the larval period is ingested from the 3rd to the 6th instar (Garner and Lynch, 1981 and Ng et al., 1993 on *S. frugiperda*; Afifi and Attia, 1990 on *S. littoralis*).

It is unlikely that the quality of each food plant would have changed in the course of the experiment. Therefore, the variation in the weight gain on the food plants, with respect to the amount of food ingested, would have been due to the changes in the physiology of the larvae.

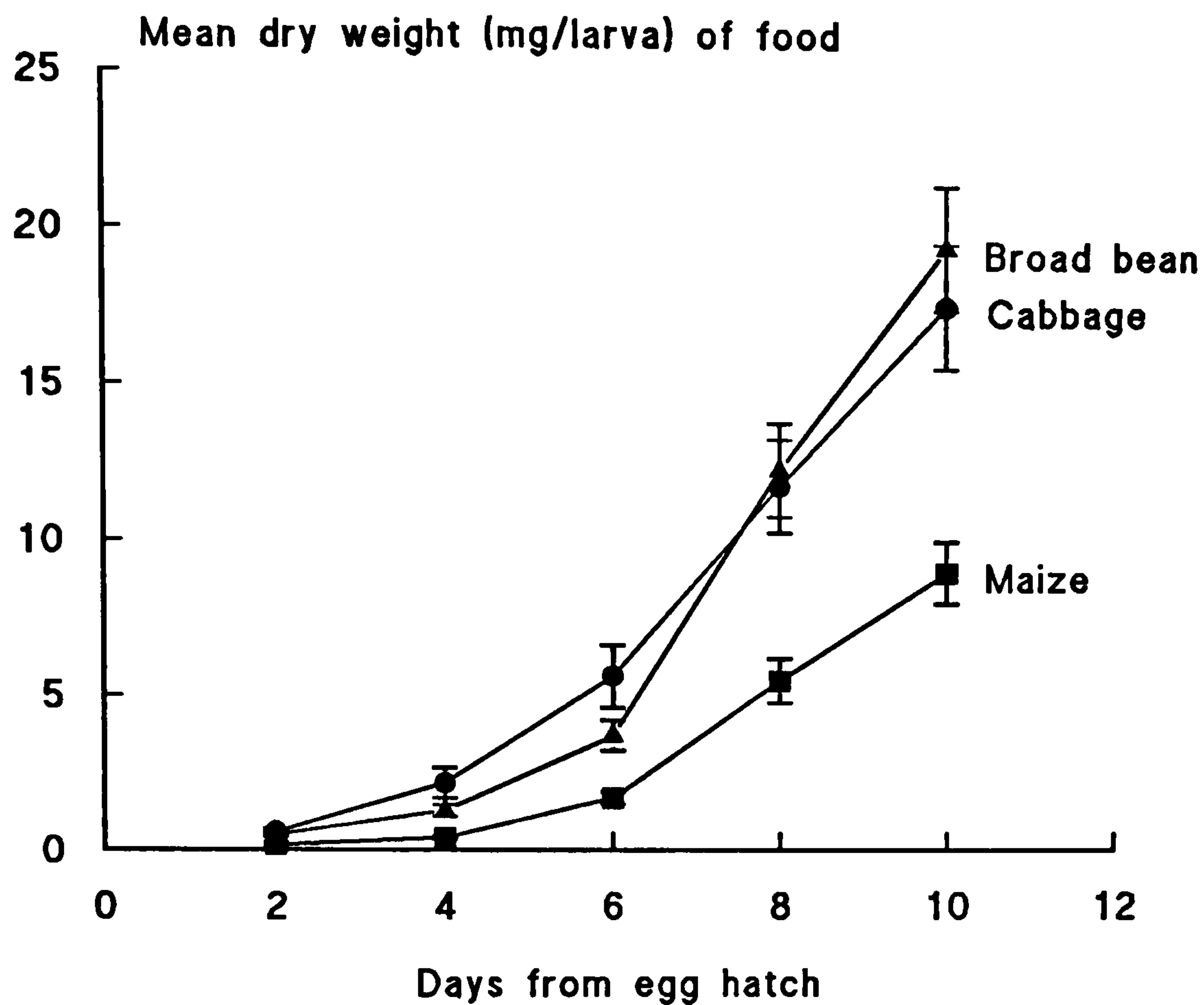


Figure 2.22. Cumulative food intake (mg) of larvae on three food plants. Bars represent Standard Errors.

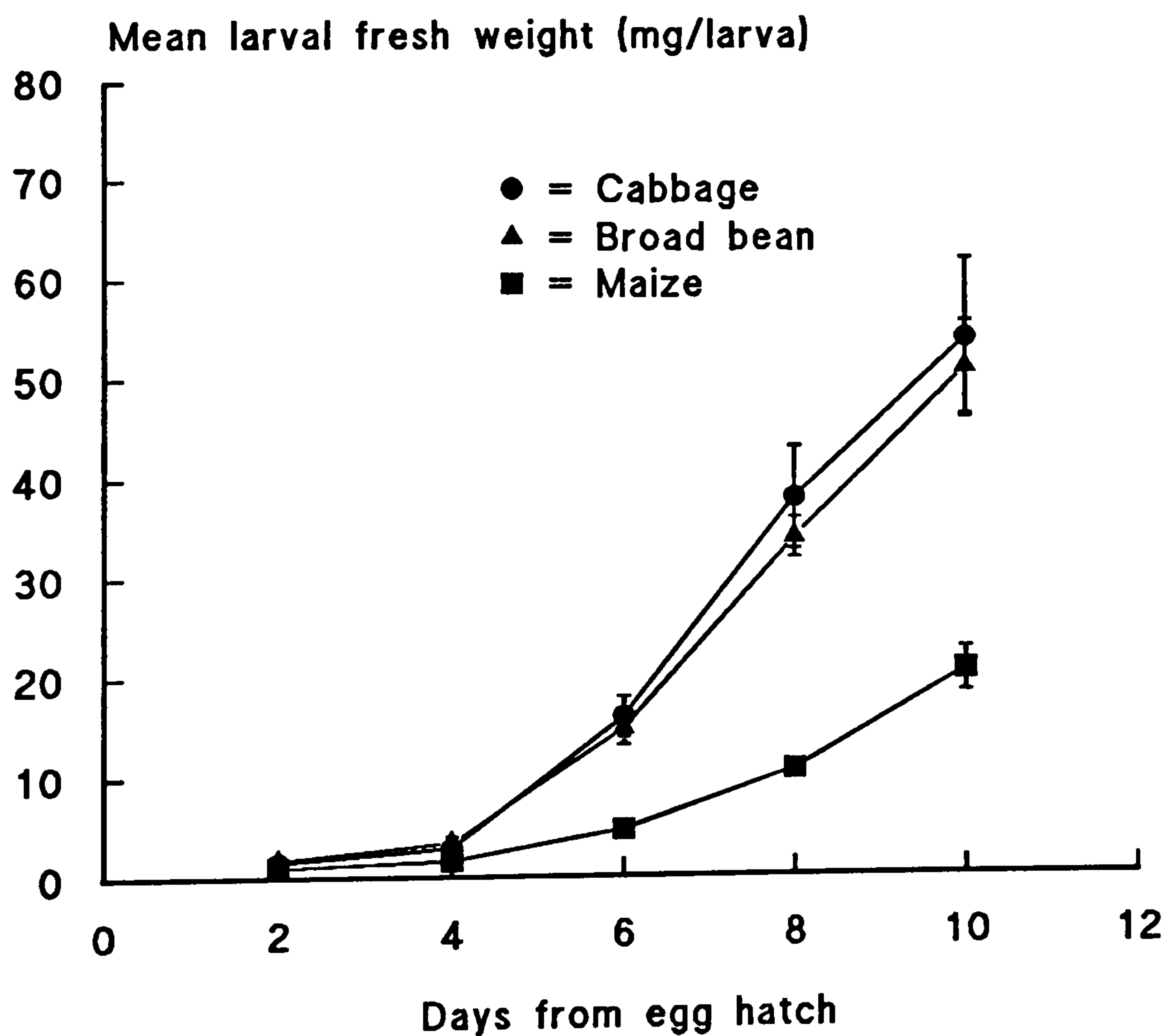


Figure 2.23. Larval fresh weight (mg) on three food plants. Bars represent Standard Errors.

2.4. CONCLUSIONS

1. The results of the study of the influence of food plants on *S. littoralis* indicates that even though the pest is a polyphagous insect, maize, millet and sorghum would adversely affect its development when it is reared on them.
2. The results obtained in the study on the larval food digestion indicates that the variation in the development of *S. littoralis* observed on the food plants was probably due to differences in the amounts of food ingested and also in the utilisation of the food ingested.
3. In terms of overall performance of the insects, the food plants could be ranked as cabbage > broad bean > soybean > cowpea > maize > sorghum.

CHAPTER THREE

ASSESSMENT OF CHEMICAL FACTORS AFFECTING LARVAL FEEDING ON SOME FOOD PLANTS

3.1 INTRODUCTION

Insect-plant interactions are to a large extent based on chemical cues. Accordingly, the chemical composition of plants is of great significance in their acceptance or rejection by insects (Harbone, 1988; Wheeler and Slansky, 1992). The main chemoreceptors responsible for accepting or rejecting a plant for food are located on the maxillary palpi (Schoonhoven, 1990).

Many of the chemical compounds present in the plant are of general occurrence. Nutrients such as amino acids, carbohydrates, proteins, lipids and vitamins are found in varying concentrations in all plants (Douglas, 1993; Lanza *et al.*, 1993). However, certain other plant chemical compounds, allelochemicals, occur only in certain plant species or are restricted to certain plant taxa (Koul and Isma, 1992). These allelochemicals could serve as kairomones which evoke feeding responses in insects or may act as allomones having a repelling or deterring effect on insects, thereby protecting plants from ingestion (Champagne *et al.*, 1992).

Food plant selection by insects, therefore, results from their response to a combination of compounds which stimulate and those which discourage feeding and other behaviours (Kogan, 1977; Dethier, 1980).

In spite of these observations, the value of nutritive

components such as amino acids, proteins and carbohydrates in food rejection or acceptance has not received as much attention as secondary compounds in the feeding activities of *S. littoralis*.

Brodbeck and Strong (1987) have suggested that the low priority given to studies on the role of the nutritive contents of food plants in the population dynamics of insects may be due to the general acceptance that the nutritional requirements of insects are essentially similar. It has also been suggested that plants are similar in their nutritional value to insects (Harborne, 1988). Several studies have shown, though, that the nutritional factors of the food plants could affect the performance of insects. The rate of population growth in aphids is related to the nutritional status of the host plants and the composition of amino acids (van Emden, 1972; Jansson and Smilowitz, 1986). Thus nutrients such as free amino acids, in amounts below that required by aphids, could have a detrimental effect on the population.

In *Aedes egyptii*, oogenesis and fecundity were affected by both the quality and quantity of protein ingested from the blood meal (Broadway and Duffy, 1988; Briegel, 1990). Also, larval dispersal of *Choristoneura rosaceana* was correlated with the nitrogen content of its food plants (Carriere, 1992).

When snap bean cultivars were grown under similar conditions, cultivars with high carbohydrate content in their leaves had a higher percentage of leaf damage by the beetle *Adoretus sisicus* Burmeister (Furutani et al., 1993). These observations may suggest that the relative importance of plant nutritional status (plant primary chemistry) vs. plant secondary chemistry may vary from insect to insect.

The interaction between allelochemicals and nutrients may affect the suitability of plants as food plants. In some plants e.g. oak trees, tannins could block the availability of proteins by forming complexes with them and gossypol could decrease food assimilation in *Heliothis zea* (Hagen et al., 1984). Nevertheless, it is still necessary to relate an insect's growth and development to the levels of nutrients in a plant.

Studies on the feeding of *Spodoptera littoralis* have shown that there are some food plants that it does not feed on (Hosroy and Kotby, 1960; Moussa et al., 1960; Prasad and Bhattacharya, 1975). Methanol and ethanol leaf extracts from some of these plants such as *Dieffenbachia picta* and *Adhatoda vascia*, have been reported to inhibit larval feeding and development (Hegazy et al., 1992).

Similar deterrent effects of leaf extracts have also been observed in the fall armyworm, *Spodoptera frugiperda*

on petroleum ether and methanol extracts of Bermuda grass (Quisenberry et al., 1988); in *Pieris brassicae* on methanol, petroleum ether, chloroform and ethanol extracts of the ginkgo tree, *Ginkgo biloba* (Fu-Shun et al., 1990); in the weevil *Hypera brunneipennis* (Bernays and Cornelius, 1992); and in *S. exempta* on hexane extracts of a resistant maize variety (Okello-Ekochu and Wilkins, 1994).

3.1.1 EXPERIMENTAL OBJECTIVES

The experiments in this chapter attempted to determine the chemical factors, both nutritional and allelochemical, present in the leaves of the plants that are responsible for the high larval mortalities and the poor performance of *S. littoralis* on maize, millet and sorghum reported in the preceding chapter. Comparison was made with a broad-leaf species where possible.

3.2 MATERIALS AND METHODS

The food plants used in these experiments were maize, millet and sorghum (which were used at the 4 leaf stage) as well as cabbage or broad bean, which were used at the 6 leaf stage.

3.2.1 Effect of foliage of cereal food plants on some aspects of the biology of *S. littoralis*

Freeze dried samples were used to determine the extent to which the physical characteristics of the food plant contributed to the low larval food intake on the three

cereal food plants. Physical inhibition of feeding could be manifested by increased toughness of tissue (scleritisation), solidity of tissue, trichomes, accumulation of surface wax and other anatomical features (van Emden, 1987; Okello-Ekochu, 1990).

Foliage of maize, millet and sorghum were incorporated into a semi-artificial diet. The diet was prepared as follows: 4 g of agar was dissolved in 72 ml of water and the mixture was brought to the boil. On cooling, to about 60°C the agar was thoroughly mixed with 0.64 g ascorbic acid, 0.44 g methyl p-hydroxybenzoate and 23 g of milled foliage of each food plant. Small quantities of the diet were dispensed into plastic cups. One neonate larva was placed in each cup. The diet was changed after the first 4 days and, subsequently, after every 48 h until pupation. The immature and adult stages were maintained under the same conditions as outlined in chapter 2. Observations were made on the durations and weights of the larvae, pupae and adults.

3.2.2 Determination of total soluble carbohydrates

Soluble carbohydrates were determined following the method of the Ministry of Agriculture, Fisheries and Food (1973).

The soluble carbohydrates were extracted from freeze dried foliage with water. 0.2 g of well ground foliage was

transferred into a bottle and 200 ml of distilled water added. The mixture was shaken on a shaking machine for 1 hour after which it was filtered through a Whatman No.1 filter paper. The filtrate was retained for the determination of soluble carbohydrates. Four determinations were made for each food plant.

The soluble carbohydrate content was determined spectrophotometrically as the blue-green complex which is formed when carbohydrate is heated with anthrone in sulphuric acid.

The anthrone reagent was prepared by adding 760 ml sulphuric acid to 330 ml water. On cooling, 1 g thiourea and 1 g anthrone were added. The yellowish-green reagent was stored in a dark bottle at 4° C until needed.

A standard curve was prepared as follows: 0.65 g of anhydrous D(+)-glucose was dissolved in water. Glucose standard solutions of 0-0.2 mg/ml were then prepared. 2 ml of each standard solution were heated with 10 ml of anthrone for 20 min. The standards were cooled and the absorbance was measured at 620 nm. Spectrophotometric readings were taken with a Shimadzu UV-1201 spectrophotometer. The extracts of the foliage were treated in the same way as the glucose standards. The percentage of soluble carbohydrates in the sample (as glucose) was obtained by multiplying the difference in the absorbance

between the blank and the samples by 50.

3.2.3. Determination of free amino acids

20 mg of freeze dried leaf tissue was added to 10 ml of a methanol:chloroform:water (12:5:3) mixture (in a 20 ml glass tube) and shaken on a shaker for 8 h. The extract was filtered with a Whatman^R nylon filter and the filtrate was poured into a test tube. 2 ml of chloroform and 1.5 ml of water were added to the filtrate and the test tube shaken. The mixture in the test tube was then allowed to settle in order to separate into two layers. The clear upper layer (aqueous phase containing the amino acids) was siphoned off and filtered. This layer was used for the amino acid analyses. The lower phase (the chloroform layer containing the pigments) was discarded.

After extraction, the amino acids were analysed using reversed-phase high-performance liquid chromatography (HPLC) after automatic pre-column derivatization with o-phthalaldehyde (OPA) (Sigma Chemical Co.) and fluorometric detection. Proline is not detected by this method and cysteine is under-estimated because of its low fluorescence. AA-S-18 (Sigma Co.) amino acid mixture was used as the reference standard.

Chromatographic conditions were: two Shimadzu model LC-10AS pumps, a Shimadzu model SCL-10A system controller for automatic gradient generation, a Shimadzu model SIL-10A

auto injector/sampler, a Shimadzu RF-33 fluorescence monitor, and a Shimadzu C-R5A for the recording and the integration of chromatographic peaks. The column used was Beckman Ultrasphere ODS 5 μ m 4.6 x 25 cm. A guard column packed with Pellicular C18 material was fitted on to the column. Elution solvents were: A = tetrahydrofuran: methanol: 0.05 M sodium acetate (pH 7.2) 5:95:900 and B = methanol:0.05 M sodium acetate (pH 7.2) 800:200. The flow rate was 1.5 ml/min. the elution gradient was 0% B after 0.01 min, 50% B after 24 min, 100% B after 40 min, 0% B after 42 min. The fluorimeter detector was set at: Excitation wavelength = 330 nm and emission wavelength = 450 nm.

3.2.4. Elemental analyses of foliage

Samples of dried, milled leaves of maize, millet, sorghum, broad bean and cabbage were analysed for total C,H,N on a Carbo Erba 1106 Elemental Analyser in accordance with the manufacturers' instructions and weighed using a Mettler MT 5 microbalance.

3.2.5. Effect of the removal of leaf surface wax on larval feeding

3.2.5.1 Extraction of surface wax and bioassay procedure

The effect of the plant surface on insect feeding was quantified by observing the effect of altered leaf (leaf surface with surface wax removed) surface on the amounts of food ingested in a 24 h period. All solvents

used were of analytical grade and were purchased from Merck.

Four undamaged leaves (cut with the stalk or sheath) of maize, millet, sorghum, broad bean, cowpea and soybean and 2 undamaged leaves of cabbage were weighed and immersed in 100 ml of chloroform, at room temperature, for 15 s. The leaves were then air-dried in a fume cupboard for 15 min. Control leaves were not immersed in chloroform but were air dried for 15 min. The chloroform containing the wax extract was filtered with Alltech Nylon Membrane (47 mm diameter, 0.2 μ m pore size) and evaporated to dryness in a rotary evaporator. The dried residue was weighed as the total surface wax.

Weighed amounts of the leaves with the surface wax removed were offered to 4th instar larvae for 24 h. Uneaten leaves were oven dried at 60° C for 48h. The dry weight of leaves ingested was determined by the method of Waldbauer (1968) as outlined in Chapter 2.

3.2.5.2. Effect of wax extract on larval feeding

The wax extracts of the leaves were applied in the same proportion (w/w) as was obtained from the leaf surface (Table 3.5).

The appropriate amount of wax was dissolved in 500 μ l of chloroform and applied to filter paper (Whatman No 1,

1.5 cm diameter). The filter paper was then air dried. 1.5 mg of sucrose was then dissolved in 1 ml of water and applied to the filter paper mounted on a pin. It was then offered to the larvae in 10 cm diameter glass petri dishes. The petri dishes were placed in an incubator at 25°C and in total darkness for 24 h. The areas of the leftover filter papers were measured with a leaf area meter.

3.2.6 Sequential leaf extraction of cereal food plants

Freeze-dried leaves of maize, millet and sorghum at the 4 leaf stage were ground to a fine powder. 20 g of the samples were sequentially extracted with 1000 ml (250 mlx4) of petroleum ether, dichloromethane, acetone and methanol. The leaf sample was first extracted with petroleum ether and on filtering, the residue was then re-extracted with dichloromethane. The other solvents were used in sequence in the same fashion.

The extraction was carried out by placing 20 g of the leaf tissue in a 1 L flask. The appropriate amount of solvent was then added. The mixture was then placed on a magnetic stirrer and stirred continuously for 24 h, at room temperature. After filtering with an Alltech nylon membrane (74 mm diameter, 0.2 µm), the extracts were concentrated using a rotary evaporator at 30°C and dried in a stream of oxygen free nitrogen. The residue of the plant (after all the solvents had been used for the extraction) was air dried in a fume cupboard.

3.2.6.1. Bioassay procedure

Each extract was incorporated into an artificial diet, at 20 mg/g of diet (i.e. 20 mg of extract for each gram of artificial diet) and fed to 4th instar larvae. This was done using the appropriate amount of extract dissolved in 0.5 ml of methanol and added to 0.5 g alpha cellulose. The solvent was evaporated under a stream of oxygen-free nitrogen. The diet was then prepared as follows. 0.7 g agar and 1.8 g of glucose were dissolved in 40 ml of water and the mixture was brought to the boil. On cooling to about 60°C, 0.01 g methyl p-benzoic acid, 0.001 g of sorbic acid and the extract-containing alpha cellulose were added and thoroughly mixed. The diet, as 1 g discs, was offered to freshly moulted 4th instar larvae. Control diets contained alpha cellulose with only the solvent added. Detrimental effects of feeding were determined by comparing the weights of discs (agar containing extract) eaten with that of the controls (agar without extracts), after 24 h.

Petroleum ether (P), dichloromethane (Di), acetone (Ace), and methanol (Me) extracts as well as the residue (Res) and controls (Con) were used in the bioassay. The criteria used to determine extract activity were larval mortality, larval weight after 24 h and skin discoloration.

3.2.7. Extraction and bioassay of groups of compounds with potential feeding inhibition properties

The bioassay was carried out in order to detect the presence of feeding inhibitors or stimulants in each of the extracts. All filtration was carried out with an Alltech Nylon 66 membrane (47 mm diameter, 0.2 μ m pore size).

3.2.7.1. Bioassay of plant extracts

The plant extracts were applied such that each glass fibre disc contained the same proportion (w/w) of the extract as in the 5 g leaf samples from which the extracts had been obtained (Table 3.7). The plant extracts were offered to the 4th instar larvae on Whatman glass microfibre filters (GF/D 4.25 cm diameter and weighing 182.1 ± 2.6 mg when untreated).

Preliminary tests had showed that *S. littoralis* would not accept dry glass fibre discs with or without sucrose. Therefore, each extract was initially dissolved in 1 ml of methanol and applied to the disc. The disc was then air dried in a fume cupboard. When the discs were dry, 1.2 mg of sucrose was dissolved in 1 ml of water and applied to each disc. The control discs were treated with solvent and sucrose only. There were 1 larva per replicate and 10 replicates for each extract.

The treated discs were mounted on pins and placed in 10 cm diameter glass petri dishes. One larva was then introduced to each petri dish. The petri dishes were placed in an incubator at 25°C, 60-70% RH and in total darkness,

for 24 h, after which remaining discs were dried at 30°C for 12 h and weighed.

3.2.7.2. Lipid soluble constituents (terpenoids, lipids, waxes etc.)

Dried leaf tissue (5 g) was extracted continuously with petroleum ether (b.p 40-60°C) for 12 h in a Soxhlet extractor. After filtering, the extract was concentrated to dryness, at 30°C, with a rotary evaporator. The extract was redissolved in petroleum ether for the bioassay.

3.2.7.3. Aqueous methanol extract (soluble flavanoids, esters, amino acids, sugars, etc.)

The residue from the petroleum ether extract was re-extracted with methanol for 8 h in a Soxhlet extractor. The extract was filtered and then concentrated to dryness at 30°C using a rotary evaporator. The extract was redissolved in 80% methanol for the bioassay.

3.2.7.4. Alkaloids and related N-containing compounds

Dried leaf tissue (5 g) was extracted with 10% acetic acid in ethanol at room temperature for 4 h with continuous stirring. After filtration and concentration under vacuum in a rotary evaporator to about 25% of its original volume, any alkaloids were precipitated from the filtrate by the dropwise addition of concentrated NH_4OH . The precipitate was then centrifuged (at 4500 rpm for 5 min), washed with 1% NH_4OH and redissolved in pure ethanol for the bioassay.

3.2.7.5. Hydroxyaromatic acids bound as esters, glycosides or in the cell wall

Dried leaf tissue (5 g) was hydrolysed with 2 M HCl at 100°C (in a boiling water bath) for 45 min. After filtration and cooling, the filtrate was extracted twice with ether (150 ml x 2). The ether extract was then washed and dried with anhydrous sodium sulphate, refiltered and concentrated to dryness in a rotary evaporator at 30°C. The residue was redissolved in 80% methanol for the bioassay.

3.2.7.6. Aglycones of flavones and flavanols

Dried leaf tissue (5 g) was hydrolysed with 2 M HCl at 100°C (in a boiling water bath) for 45 min. After filtration and cooling the filtrate was extracted with ethyl acetate (150 ml x 2) and the combined extracts were evaporated to dryness in a rotary evaporator at 30°C. The residue was dissolved in 80% methanol for the bioassay.

3.2.7.7 Anthocyanidins from proanthocyanidins and glycoflavones

Dried leaf tissue (5 g) was hydrolysed with 2 M HCl at 100°C (in a boiling water bath) for 45 min. After filtration and cooling, the filtrate was extracted with amyl alcohol (150 ml x 2). The extract was concentrated to dryness by freeze drying. The residue was redissolved in 80% methanol for the bioassay.

3.2.7.8. Hydroxyaromatic acids bound as soluble esters

Dried leaf tissue (5 g) was hydrolysed with 2 M NaOH at room temperature, for 4 h. The extract was filtered and the filtrate reduced to pH 2 (with 2 M H₂SO₄) and then extracted with ether. The ether fraction was then washed, dried with anhydrous sodium sulphate and concentrated to dryness with a rotary evaporator. The residue was redissolved in 80% methanol for the bioassay.

3.2.7.9. Phenolic acids

Dried leaf tissue (5 g) was hydrolysed with 2 M HCl at 100°C (in a boiling water bath) for 45 min. After filtration and cooling, the filtrate was then extracted with ether. The ether phase was then washed with 2% aqueous sodium bicarbonate (pH 9). This removes the acids but not the free phenols. The acids were removed by acidifying the bicarbonate fraction (pH 2) and then shaking it with diethyl ether.

3.2.7.10. Free phenols

The free phenols were extracted from the ether fraction (obtained from 3.2.7.9. above) by shaking with 5% aqueous sodium hydroxide solution (pH 13). The phenols were removed by extraction into diethyl ether after acidification of the aqueous solution to pH 2 with 2 M H₂SO₄.

3.3 RESULTS AND DISCUSSION

3.3.1. Effect of foliage on some biological parameters of *S. littoralis*

When freeze-dried and ground foliage of the cereal food plants were incorporated into artificial diet and fed to the larvae, the larvae on the millet failed to survive to pupation.

On the artificial diet with maize and sorghum incorporated (Table 3.1), the percentage of larvae pupating and the number of adults emerging from the pupae were higher on the leaf containing diet than when the larvae were reared entirely on the food plants (Chapter 2).

However, there was no significant difference (at $P = 0.05$) in the proportion of larvae surviving to the adult stage between the larvae feeding on the fresh plant leaf and those feeding on the artificial diet with dried leaf for each plant. The findings indicate that the physical characteristics of the leaves of the plants (leaf hairs etc.) may have had only limited effect on the development of the immature stages reared on the food plants.

Mortality of the immature stage is the most important aspect of the population dynamics of any insect. Since the mortality was high on both fresh plant and artificial diet with leaf powder incorporated it could be concluded that the high mortality on the cereal food plants was due to

Table 3.1 Effect of artificial diet containing freeze-dried leaf on some aspects of the biology of *S. littoralis*

Parameter	Mean \pm SE		
	Maize	Sorghum	Control
Larval period (days)	20.2 \pm 2.1	23.4 \pm 2.5	20.2 \pm 3.4
Pupal period (days)	15.4 \pm 2.8	13.6 \pm 2.9	10.8 \pm 1.6
Pupal weight (mg)	192.7 \pm 11.6	183.2 \pm 13.8	248.2 \pm 6.6
Adult weight (mg)	120.7 \pm 10.3	99.5 \pm 8.7	143.4 \pm 12.6
Pupal formation(%)	31.3 \pm 2.4	22.5 \pm 2.7	63.4 \pm 4.5
Adult formation(%)	62.0 \pm 7.1	55.1 \pm 5.2	78.3 \pm 9.3
Ultimate survival(%)	7.3 \pm 4.2	9.8 \pm 1.3	49.5 \pm 5.4

chemical factors within them which either inhibited food intake and digestion or were toxic when ingested.

Artificial diets containing dried foliage have been used to indicate the presence of antifeedants in plant leaves. Davies (1976) observed no difference in the growth of the larvae of the fall armyworm *S. frugiperda* when it was fed artificial diet containing dried leaf foliage of both resistant and susceptible genotypes of corn. However, Williams et al. (1990) reported a significant reduction in the larval weight of *S. frugiperda* when it was fed artificial diet containing leaf foliage of a resistant corn genotype. They concluded that "addition of plant material

to a complete artificial diet would most likely affect larval growth adversely only in those cases where resistance resulted from the presence of a toxin or a strong antifeedant. Furthermore, ingredients present in the complete diet could easily mask differences between resistant and susceptible food plants if nutritional factors or mild antifeedants were involved".

3.3.2. Concentrations of soluble carbohydrates

Fig. 3.1 shows the standard curve used in the determination of total soluble carbohydrates in the food plants. Measurement of the concentration of soluble carbohydrates showed that the two dicotyledonous food plants tested cabbage and broad bean, had lower amounts than the cereal (monocotyledonous) food plants (Table 3.2).

The concentration found in maize (Dobidi) is higher than reported by Okello-Ekochu (1990) for some other maize varieties (Passat, Michioacan and BS 13). It was surprising to observe that the concentration in millet was lower than in maize and sorghum but similar to that of cabbage.

Generally, total carbohydrate content of leaves has been reported to vary between 3.5% and 10% depending on plant species (Wermelinger *et al.*, 1991) and at the concentration normally found in plant leaves, sugars play an important role in insect feeding by stimulating feeding activities.

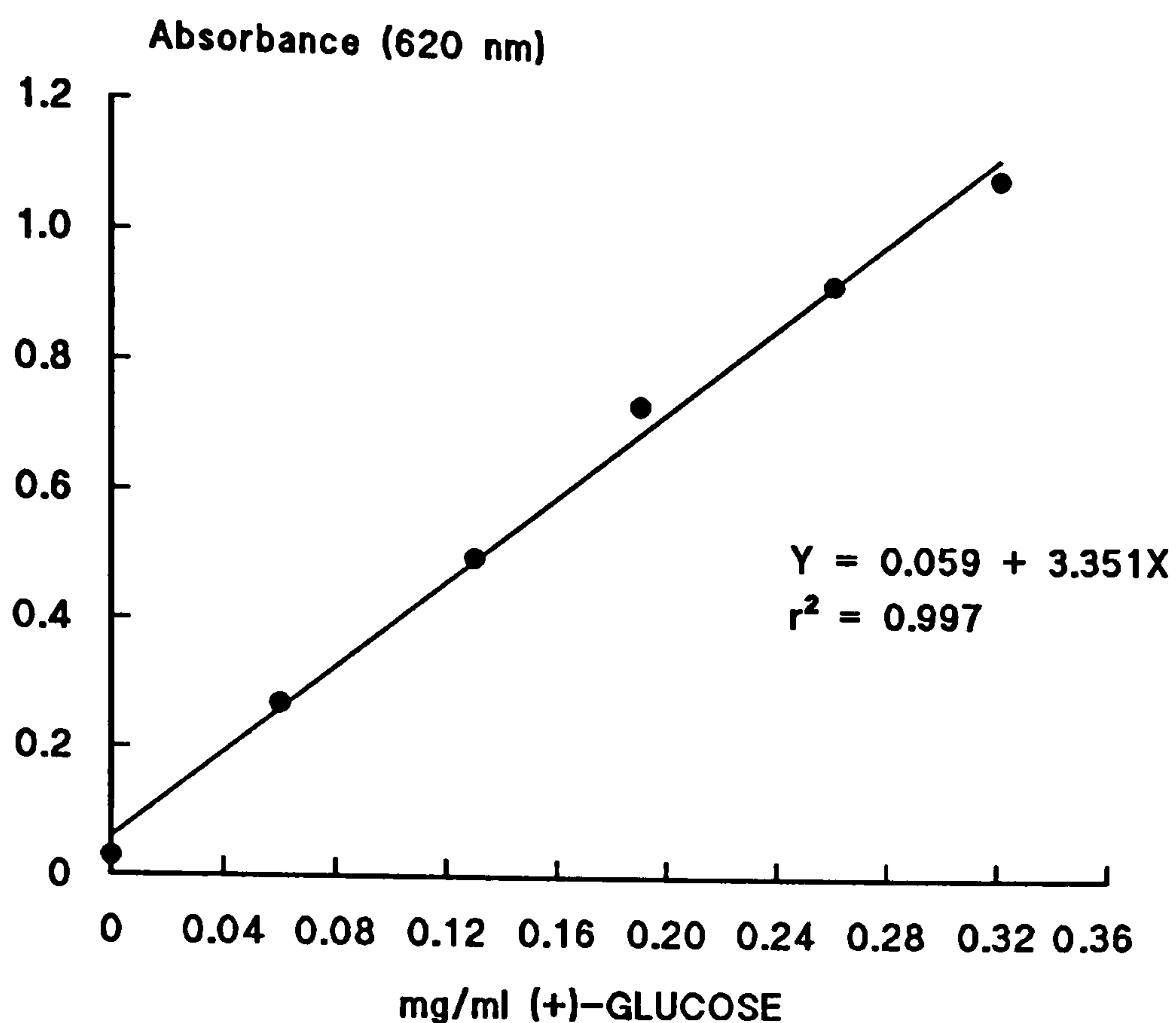


Figure 3.1. Standard curve for the determination of total soluble carbohydrates as mg glucose.

Table 3.2. Content (% of total dry weight) of soluble carbohydrates (calculated as glucose) of food plants.

Food plant	Soluble carbohydrate (%)
	Mean ± SE
Broad bean	2.1 ± 0.2 a
Cabbage	4.1 ± 0.3 b
Millet	4.4 ± 0.2 b
Maize	6.9 ± 0.1 c
Sorghum	7.3 ± 0.5 c

Means followed by the same letter are not statistically different at the 5% probability level (SNK).

Ishaaya (1986) suggested that the stimulation of the feeding activity is due to the stimulation of digestive enzymes in the gut of the insect. Other insect activities could be influenced by the carbohydrate content of the diet. Arita et al. (1993) reported that the Chinese rose beetle, *Adoretus sinicus*, prefers leaves with high carbohydrate content. Gunn and Gatehouse (1985) have suggested that in *Spodoptera exempta*, there is some evidence that sucrose in the adult diet could increase fecundity, especially in lighter moths reared on a suboptimal larval diet.

Variation in the concentrations of carbohydrates in the diets of insects could have effects on some insect activities. Chapman (1974) has reported that at high concentration, sugars could reduce feeding of some insects such as *Lepinotarsa decemlineata* and *Ostrinia nubilalis*. Gatehouse et al. (1987) have also observed that in the absence of other antimetabolic compounds, resistance in a variety (G12953) of the haricot bean (*Phaseolus vulgaris*) to *Acanthoscelides obtectus* was due to the high levels of a heteropolysaccharide.

It is unclear whether the levels that occurred in the cereal food plants, in this study, could be considered to be high in concentration. The trend in the soluble carbohydrate content of the foliage does not correlate with the amounts of food ingested by the larvae (observed in

Chapter 2). Larval food ingestion was higher on broad bean and cabbage while growth was poor on the cereal plants.

Two observations may explain why the apparently high carbohydrate content of the cereal plants had little effect on the growth and development of *S. littoralis* reared on them. Woodhead and Bernays (1978) have reported that sugars are not important in the inhibition of feeding of some insects such as *Locusta migratoria* (a polyphagous insect) when feeding on sorghum, and Schoonhoven (1990) has suggested that the balance between feeding stimulants and feeding inhibitors is more important in determining feeding activity than either factor acting alone. It is possible that even though the sugar content in plants may influence feeding, other factors could also play important roles.

It is possible that in the present study, there were greater amounts of feeding inhibitors in the maize, millet and sorghum than in the cabbage and broad beans.

3.3.3. Percentage of C,H and N in foliage

Table 3.3 shows the variation in the total C,H and N in the five food plants. Total nitrogen content was lower in the cereal food plants.

The effect of these elements on insects could vary. Janssen (1993) reported that nitrogen concentration in the food plant of *Spodoptera exempta* had no effect on its

Table 3.3. Concentrations (as % of sample) of C,H, and N of leaves of some food plants.

Food plants	Concentrations (% of sample)			
	Mean (SE)			
	N	C	H	C:N Ratio
Maize	2.6 (0.1)a	43.5 (0.5)c	5.8 (0.1)ab	16.7:1
Sorghum	3.7 (0.1)ab	40.9 (0.3)b	5.5 (0.0)a	11.1:1
Millet	4.0 (0.0)b	43.0 (0.6)c	5.9 (0.1)ab	10.8:1
Cabbage	5.7 (0.0)c	35.1 (0.3)a	5.1 (0.1)a	6.2:1
Broad bean	7.1 (0.1)d	43.9 (0.9)c	6.2 (0.1)b	6.2:1

Means followed by the same letter in a column are not significantly different at the 5% probability level (SNK).

growth and development. In general, however, it has been observed that insect growth, survival and fecundity depend on the nitrogen and water content of the food plants (Carriere, 1992; Slansky, 1992).

Insect activities affected by nitrogen and water contents of leaves include growth rate, such as in aphids on maize (Honek, 1994), and dispersal, e.g. *Choristoneura rosaceana* larvae on food plants (Carriere, 1992). Wu et al. (1988) estimated that about 45% of the nitrogen in the

female *Helicoverpa armigera* obtained from the food plant is used for egg production.

These observations would, in general, agree with the observations made in this study. High nitrogen and water content in the dicotyledonous plants correlated with the better performance of *S. littoralis* on these plants. Oviposition of the adult, food consumption, digestion and utilisation of the larvae were higher on all the broad leaf plants than on maize and sorghum.

It is difficult, though, to relate the performance of *S. littoralis* to the nitrogen levels in the three cereal plants because millet, which produced the highest mortality, also had the highest nitrogen content. In those three plants, nitrogen content relates to larval mortality. The water content of the three plants (Table 2.23) showed a reverse trend. It was lowest in millet and highest in maize. A definite conclusion on the water and nitrogen content cannot be made for the three cereal plants.

The C:N ratio indicates a high proportion of carbohydrates in the cereal food plants and it further indicates the comparatively poor nutritional status.

3.3.4. Free amino acid content of foliage

Table 3.4 shows the proportions of individual amino acids identified in the total amino acid pool in each

plant.

The results in Table 3.4 also show that of the 10 amino acids important to the nutrition of the insect, 9 showed a variation in concentration between the food plants. The cereal food plants had lower levels of six of the 9 essential amino acids (Met, Val, Phe, Ile, Leu and Lys). The low levels of the amino acids in these plants may have resulted in nutritional deficiencies in the larvae feeding on those food plants. Brodbeck and Strong (1987) have suggested that deficiencies in insects result from insufficient concentrations of essential amino acids ingested from the food plants rather than from lack of total nitrogen in the food plants.

There were variations in the proportions of the individual amino acids detected. There were higher levels of glutamic acid and alanine in the cereal plants. The dicotyledonous plants had higher levels of valine, phenylalanine, isoleucine, leucine, and lysine.

High levels of glutamic acid have also been reported in cereal plants (Weiner *et al.*, 1991; Douglass, 1993). Foyer *et al.* (1994) have observed levels of 21-24% glutamic acid in maize leaves. The higher levels of glutamic acid in the cereal plants may have contributed to the poor performance of *S. littoralis* on those plants.

Table 3.4 The concentration of individual amino acids expressed as a percentage of the total detected amino acid.

Amino acid	Percentage of amino acid in food plant				
	Maize	Millet	Sorghum	Broad bean	Cabbage
ASP	5.6	7.0	4.0	2.7	5.1
GLU	12.3	25.8	14.5	0.5	4.9
SER	17.7	8.4	10.4	9.8	9.2
HIS*	0.4	6.8	0.5	4.5	14.2
GLY	8.0	2.5	9.3	1.4	7.9
THR*	3.5	2.9	11.3	2.6	12.9
ARG*	0.6	0.8	2.2	3.1	0.3
ALA	42.7	40.2	38.1	24.0	19.5
TYR	1.5	1.2	1.2	6.8	0.1
MET*	0.6	0.2	0.4	2.9	1.8
VAL*	2.9	1.6	2.3	14.2	10.3
PHE*	0.9	0.9	1.3	6.4	4.5
ILE*	1.5	0.5	1.2	9.1	4.9
LEU*	1.4	0.8	2.4	10.1	3.7
LYS*	0.4	0.3	0.9	1.8	0.7

- ASP - Aspartic acid
GLU - Glutamic acid
SER - Serine
HIS - Histidine *
GLY - Glycine
THR - Threonine *
ARG - Arginine *
ALA - Alanine
- TYR - Tyrosine
MET - Methionine *
VAL - Valine *
PHE - Phenylalanine *
ILE - Isoleucine *
LEU - Leucine *
LYS - Lysine *

* Essential amino acids

Weibull (1988) and Douglass (1993) have reported that low suitability of cereals as food plants to the oat aphid, *Rhopalosiphon padi*, was related to high levels of glutamic acid in the plants. In the present study, millet, which had the highest level of glutamic acid, produced the highest larval mortality.

Variation in the amino acid profile has also been observed in other plants. Sadaka and Poinso-Balaguer (1987) and Douglas (1993) suggested that different plants allocate nitrogen differently among amino acids, proteins and other molecules. Thus total nitrogen may not be a useful indicator of plant quality. The amino acid composition would be much more reliable.

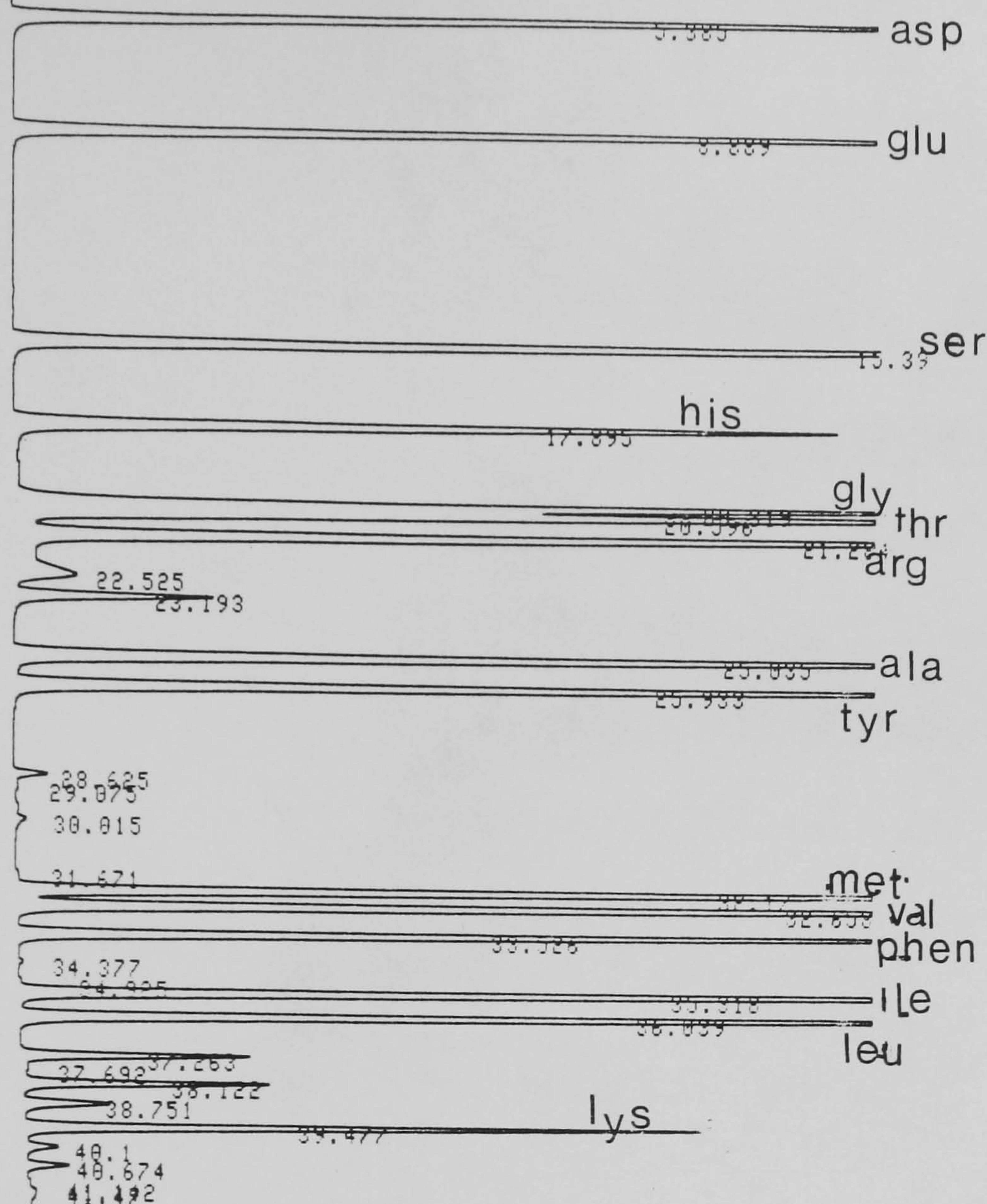
All insects require the same 10 amino acids that are important to humans. For other insects additional amino acids are important. Larvae of the flesh fly, *Phormia regina*, and the silkworm require proline and glutamic acid or aspartic acid as well (Hagen et al., 1984). However, there is hardly any data on the optimum levels of amino acids in plants required by insects for proper growth and development.

Some amino acids are phagostimulatory (Brodbeck and Strong, 1987). Lanza et al. (1993) have reported that the amino acid content of nectar could affect ant recruitment to plants. Some proteins as well as non-protein amino acids

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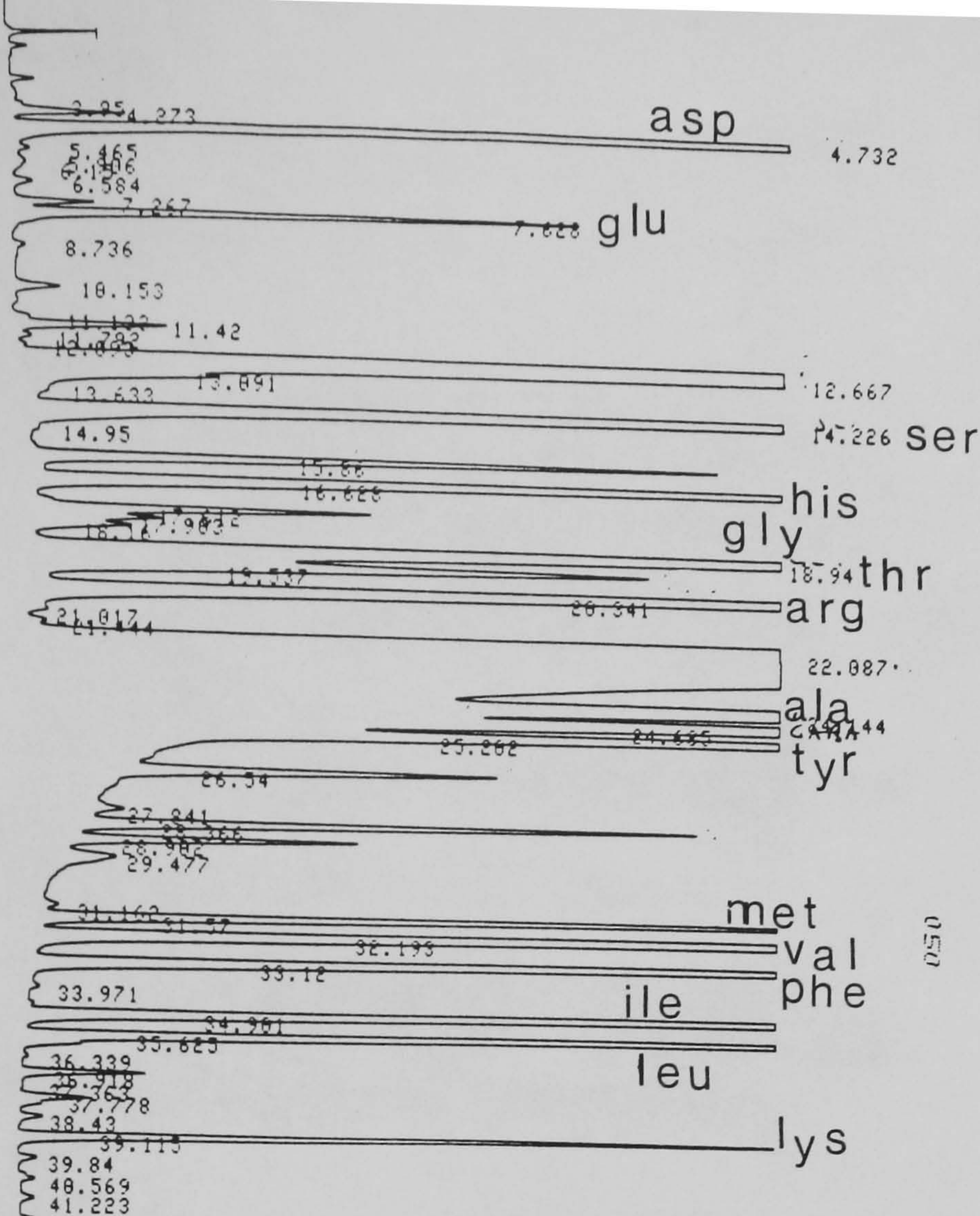
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REPORT NO 8

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METHOD 41

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1	5.385	63809			6.2965	
2	8.889	63198			6.2362	
3	15.39	79385			7.8335	
4	17.895	54080			5.3365	
5	20.319	60047			5.9253	
6	20.596	66257	V		6.5381	
7	21.284	77142	V		7.6122	
8	22.525	5920			0.5841	
9	23.193	11976			1.1818	
10	25.035	70067			6.9141	
11	25.933	65457	V		6.4591	
12	32.17	71228	V		7.0286	
13	32.658	73432	V		7.2461	
14	33.526	60216	V		5.942	
15	35.318	70261	V		6.9332	
16	36.039	61648	V		6.0833	
17	37.263	11098			1.0952	
18	38.122	12155	V		1.1994	
19	38.751	5135	V		0.5067	
20	39.477	30892			3.0483	

Figure 3.A. HPLC chromatogram of standard solution of amino acids.



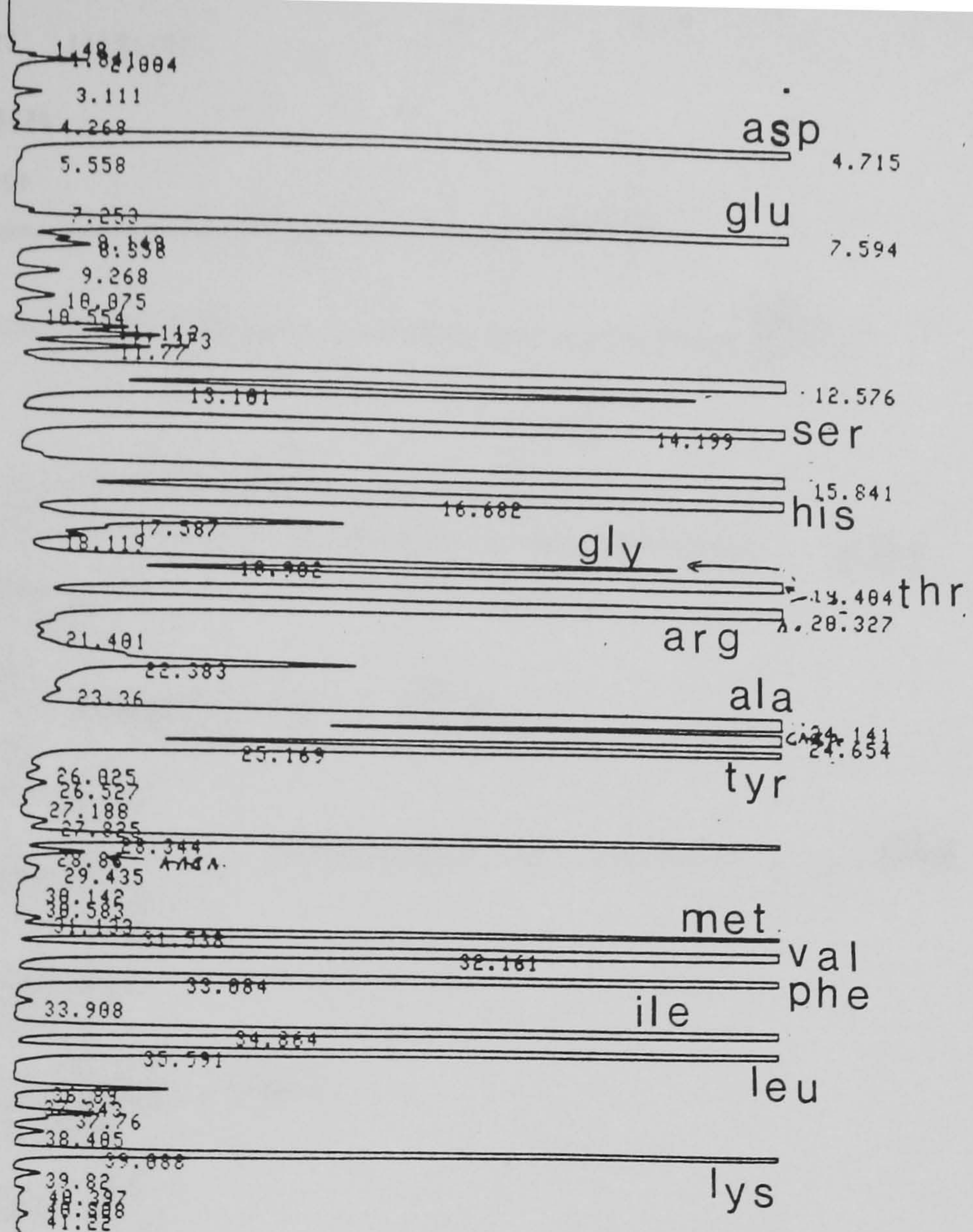
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FILE 0
METHOD 41

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1	4.273	5711	V		0.0977	
2	4.732	207080			3.5433	
3	7.628	31289	V		0.5354	
4	11.42	8960	V		0.1533	
5	12.667	994178	SV		17.0112	
6	14.226	270996	SV		4.637	
7	15.86	50440	V		0.8631	
8	16.628	125915	V		2.1545	
9	17.612	27536	V		0.4712	
10	17.903	8687	V		0.1486	
11	18.16	9246	V		0.1582	
12	18.94	158460	V		2.7114	
13	19.537	58269	V		0.997	
14	20.341	262351	SV		4.489	
15	22.087	1600605	V		27.3877	
16	24.144	556974	V		9.5303	
17	24.685	289183	V		4.9482	
18	25.202	198019	V		3.3883	
19	26.54	64218	V		1.0988	
20	27.841	15900	V		0.2721	
21	28.366	48573	V		0.8311	
22	28.902	25211	V		0.4314	
23	29.477	26074	V		0.4462	
24	31.57	63926	V		1.0938	
25	32.193	300118	V		5.1353	
26	33.12	153549	V		2.6273	
27	34.901	138918	V		2.377	
28	35.625	99274	SV		1.6987	
29	36.918	6342	T		0.1085	
30	39.84					
31	40.569					
32	41.223					

Figure 3.B. HPLC chromatogram of solution of amino acids (Broad bean).



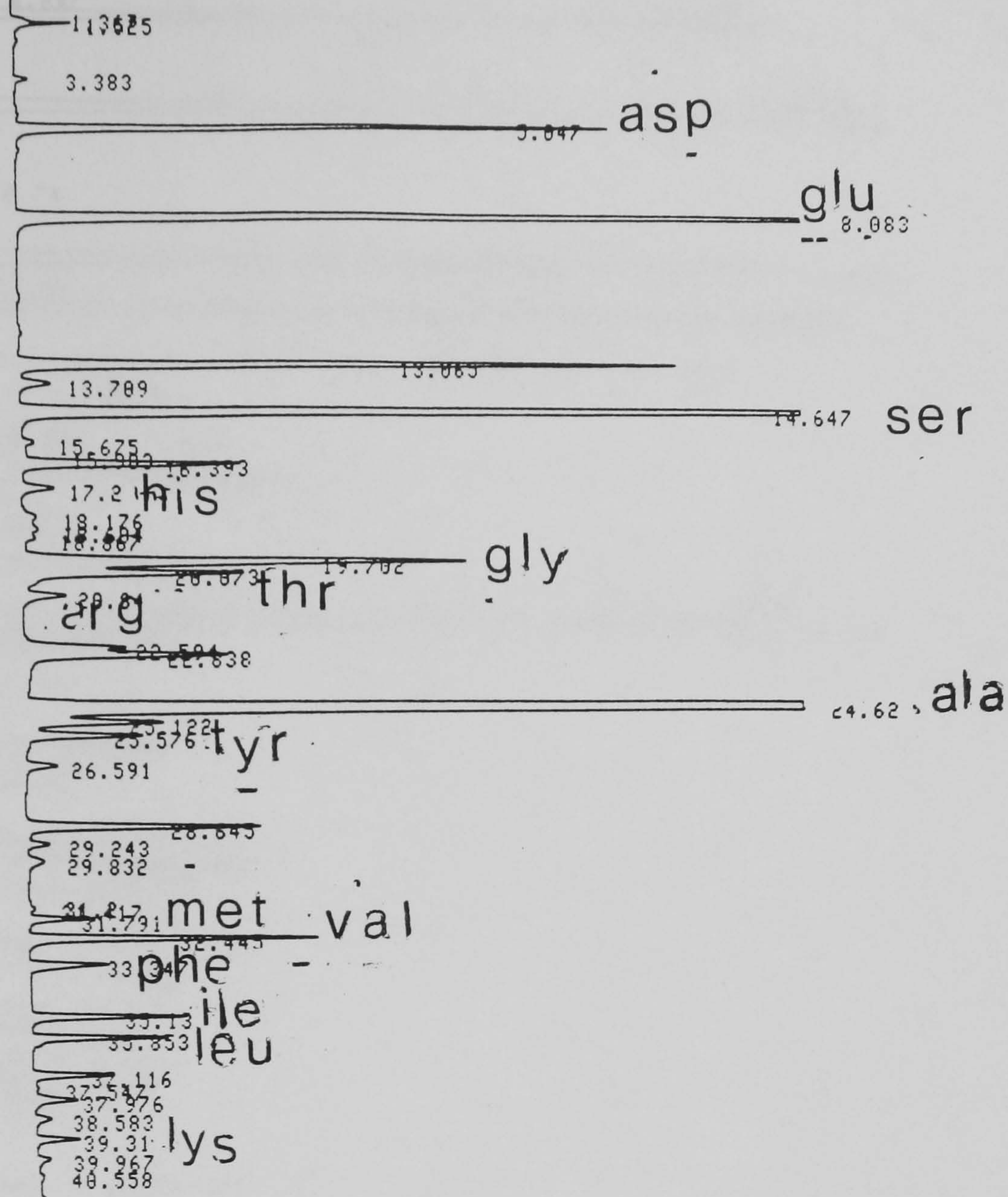
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METHOD 41

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1	4.715	184362			3.5958	
2	7.594	194605	SV		3.7956	
3	11.112	6790	T		0.1324	
4	11.373	8766	TV		0.171	
5	11.77	7627	TV		0.1488	
6	12.576	622774	V		12.1466	
7	13.101	50640	V		0.9877	
8	14.199	302329	V		5.8966	
9	15.841	492874	V		9.613	
10	16.682	163497	V		3.1888	
11	17.587	30584	V		0.5965	
12	18.119	5413	V		0.1056	
13	18.902	53504	V		1.0435	
14	19.404	245082	V		4.7801	
15	20.327	494456	SV		9.6438	
16	22.383	37225	T		0.726	
17	24.141	677747	V		13.2187	
18	24.654	492264	V		9.6011	
19	25.169	104678	SV		2.0416	
20	28.344	67526	V		1.317	
21	28.86	5071	V		0.0989	
22	31.538	51598	V		1.0064	
23	32.161	330992	V		6.6117	
24	33.084	138890	V		2.7089	
25	34.864	162881	V		3.1768	
26	35.591	103705	V		2.0227	
27	36.89	8720	V		0.1701	
28	37.76	5997	V		0.117	
29	39.088	68570	V		1.3374	
TOTAL		5127162			100	

Figure 3.C. HPLC chromatogram of solution of amino acids (Cabbage).



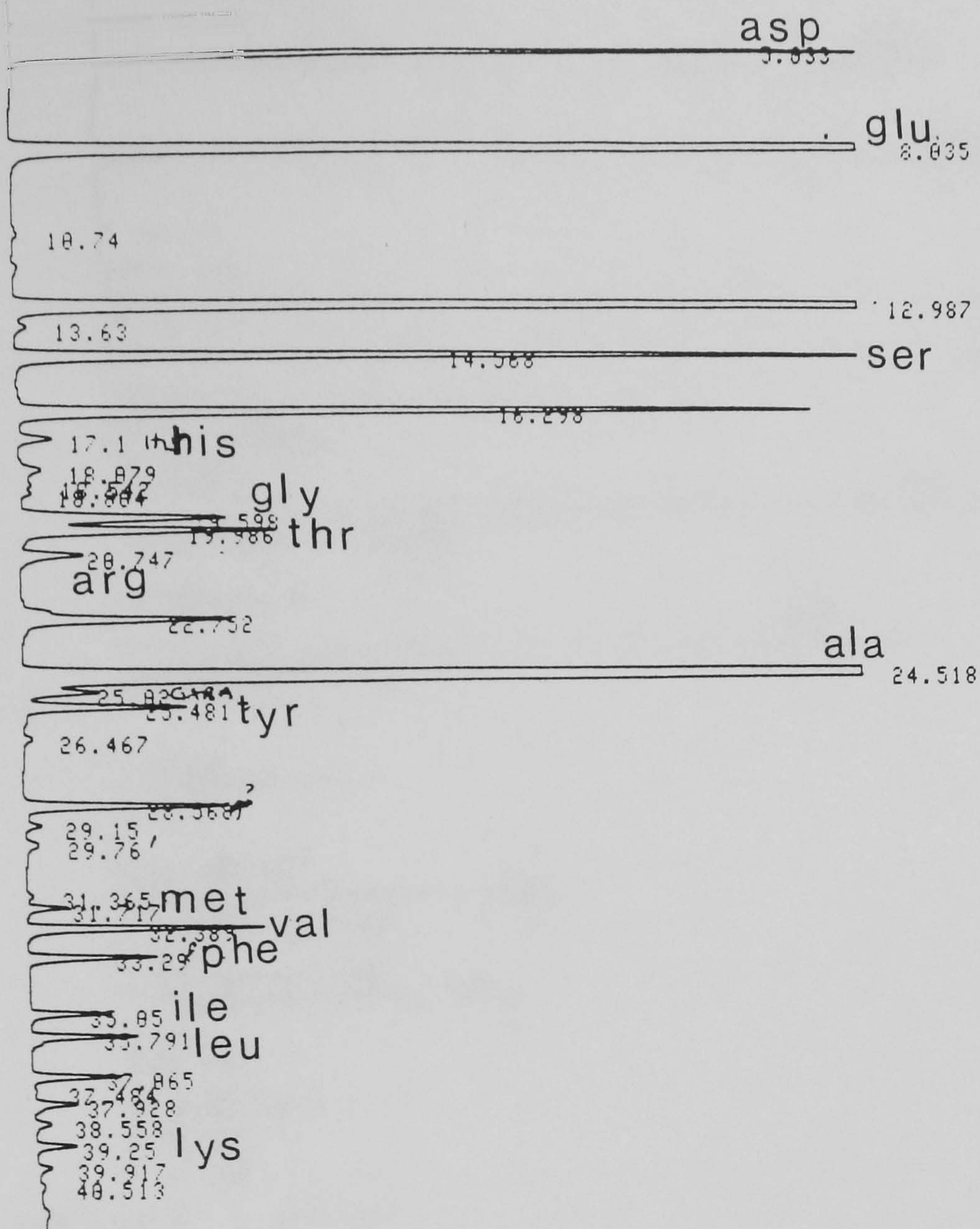
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FILE 0
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1	5.047	27396			4.8461	
2	8.083	61400			10.8611	
3	13.065	37355			6.6078	
4	14.647	80243	V		14.1943	
5	16.393	13309	V		2.3543	
6	19.702	33577			5.9395	
7	20.073	15003	V		2.668	
8	22.594	5649			0.9993	
9	22.838	13605	V		2.4066	
10	24.62	220037			38.9227	
11	25.122	8788	V		1.5545	
12	25.576	6650	V		1.1763	
13	28.645	13204			2.3356	
14	32.445	14564	V		2.5762	
15	35.13	7526			1.3312	
16	35.853	6933			1.2264	
TOTAL		565318			100	
95/07/25		12:41:31				

Figure 3.D. HPLC chromatogram of solution of amino acids (Maize).



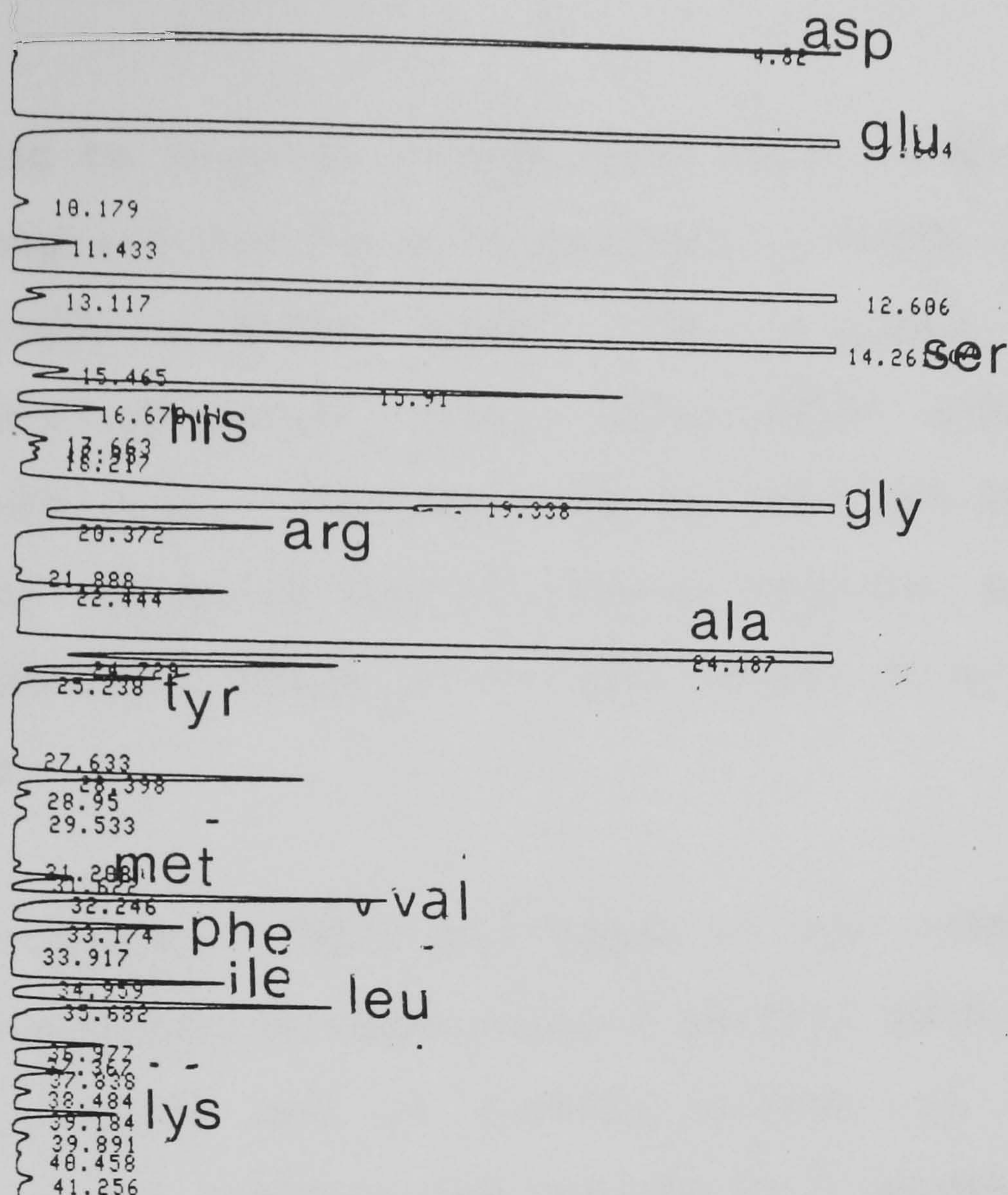
CHROMATOGRAM 1 MEMORIZED

C-R5A CHROMATOPAC
CHANNEL NO 1
SAMPLE NO 0
REPORT NO 12

FILE 0
METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	5.033	38501	Y		5.2272	
2	8.035	151542			20.5745	
3	12.987	111747			15.1717	
4	14.568	49045			6.6587	
5	16.298	44951			6.1029	
6	19.598	15141	Y		2.0557	
7	19.986	16205	Y		2.2001	
8	22.752	14246			1.9342	
9	24.518	253405			34.4041	
10	25.481	8695	Y		1.1305	
11	28.568	10726			1.4562	
12	32.389	11142			1.5127	
13	33.29	6097			0.8277	
14	35.791	5110			0.6938	
TOTAL		736553			100	
95/07/25		13:31:55				

Figure 3.E. HPLC chromatogram of solution of amino acids (Millet).



CHROMATOGRAM 1 MEMORIZED

C-R5A CHROMATOPAC

CHANNEL NO 1

SAMPLE NO 0

REPORT NO 13

FILE 0
METHOD 41

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1	4.82	39477			3.8469	
2	7.664	123254			12.0187	
3	12.606	130821			12.7482	
4	14.261	87206			8.498	
5	15.91	35825	V		3.4911	
6	16.678	5776	V		0.5629	
7	19.338	175850	V		17.136	
8	20.372	18375	V		1.7906	
9	22.444	13082			1.2743	
10	24.187	299553			29.1906	
11	24.729	18837	V		1.8356	
12	25.238	8145	V		0.7937	
13	28.398	16655	V		1.623	
14	32.246	18248			1.7782	
15	33.174	8910	V		0.8682	
16	34.959	9634			0.9388	
17	35.682	16550	V		1.6127	

Figure 3.F. HPLC chromatogram of solution of amino acids (Sorghum).

are, however, toxic to insects. Though they occur in many plant species, they are particularly present in seeds of legumes. L-DOPA found in *Mucuna* seeds (6-9%) is toxic to *Spodoptera eridania* (Harborne, 1988). Other amino acids also play important roles. Phenylalanine is required for sclerotisation and melanisation of insect cuticle and cysteine is an important source of sulphur (Hagen *et al.*, 1984) for insects.

Scriber and Slansky (1981) and Hagen *et al.* (1984) have suggested that since nitrogen plays a central role in all metabolic processes and in genetic coding, it is possible that it is the quantity and quality of N (protein and/or amino acids) available that generally limits growth and fecundity of insects. In both respects maize, millet and sorghum are inferior food plants. Their nitrogen and amino acid levels were generally low and may have contributed to the poor growth and development of *S. littoralis*.

3.3.5. Feeding activity on leaf with surface wax removed

The plant surface is an important defence against insect attack due to (among other things) a thin layer of surface lipids (epicuticular waxes) that cover the entire surface of the plant (Städler, 1988; Sugayama and Salatino, 1995). The chemical composition of the epicuticular wax is distinctive for each plant species and some of the compounds present in the wax could, potentially, be critical in food

plant selection (Baker, 1982). With *S. littoralis*, there is little information available on the interaction with surface lipids of its food plants.

There were variations in the quantities of wax extracts obtained from the plants (Table 3.5).

Table 3.5. Mean leaf surface wax content (mg/g of leaf).

Food plant	Wax content (mg/g)
	Mean \pm SE
Maize	0.14 \pm 0.01 a
Cowpea	0.23 \pm 0.02 a
Soybean	0.32 \pm 0.08 b
Millet	0.48 \pm 0.05 c
Broadbean	0.51 \pm 0.10 c
Cabbage	0.57 \pm 0.03 c
Sorghum	1.15 \pm 0.07 d

Means followed by a common letter are not significantly different at the 5% probability level (SNK).

When leaves with altered surfaces (surface wax removed) were offered to the larvae, there were variations in the amounts of food ingested by the larvae (Fig. 3.2).

The increases in the consumption of each food plant suggest that the epicuticular wax of the food plants reduced larval food intake when the larvae fed on the fresh plants. Since larval growth was inhibited only on the cereal plants, the long term effect of such feeding inhibition, due to the surface wax, was negligible on the broad leaf plants.

There was a noticeable correlation between wax content and the effect of wax removed on feeding on the leaves. Cabbage, however, had more surface wax than broad bean, yet the increase in weight eaten of the leaf of cabbage was less than broad bean.

The differences in the responses of the larvae to the altered leaf surfaces could be explained by the fact that the wax extracts obtained from different plant species are chemically different, and therefore would have different effects on the larvae.

The level of nutrients could, in combination with the surface wax, also have affected the level of feeding of the larvae. When the wax extract obtained from the leaves was applied to glass fibre discs, there were variations in the amounts of filter paper ingested (Fig. 3.3).

Even though the relationship between waxiness and susceptibility of food plants to damage has not been demonstrated unequivocally in all insect species

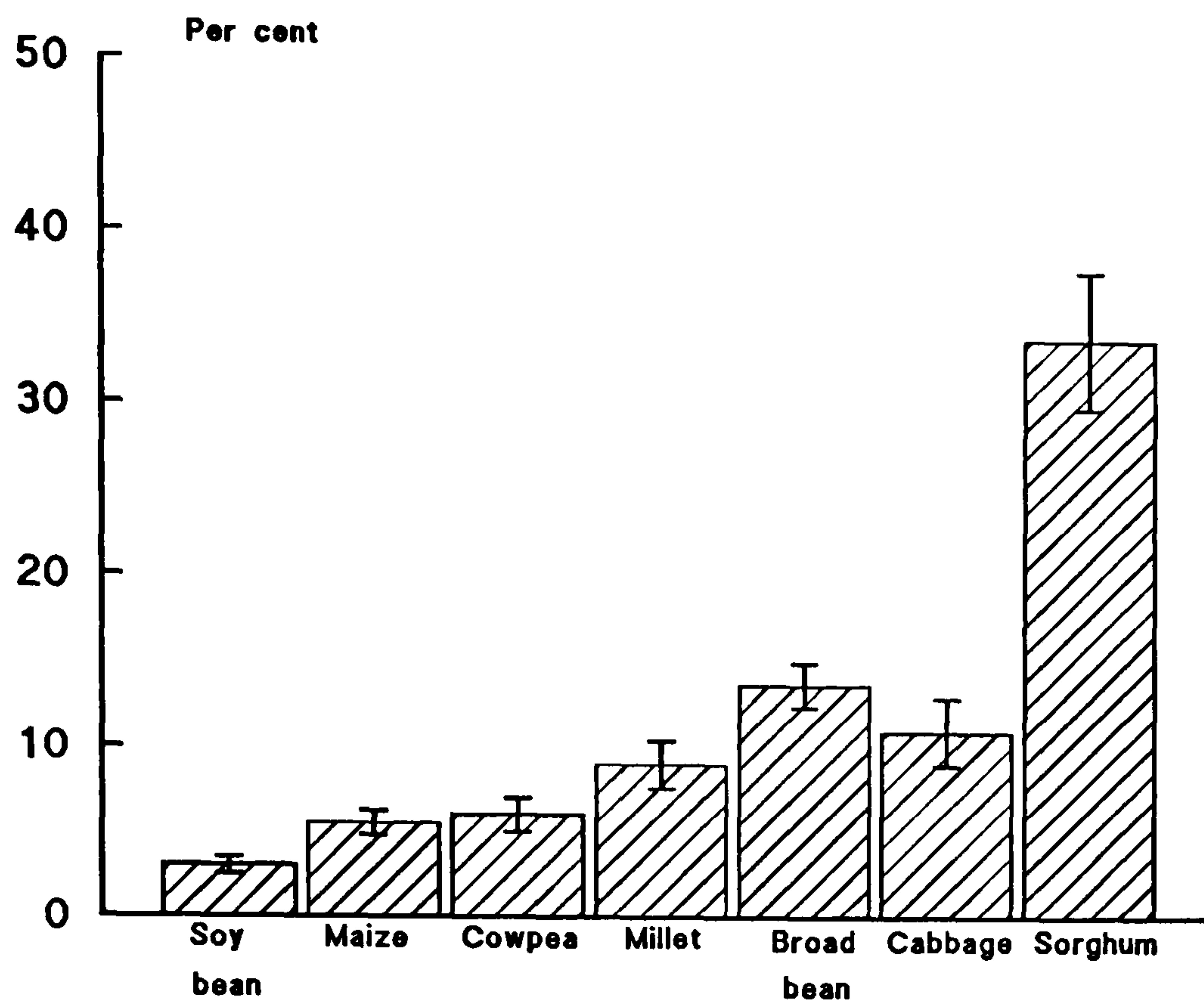


Figure 3.2. Increase in consumption of leaves following removal of surface wax (Difference in weight of food ingested)/(weight of food given) X 100 Bars represent Standard Errors.

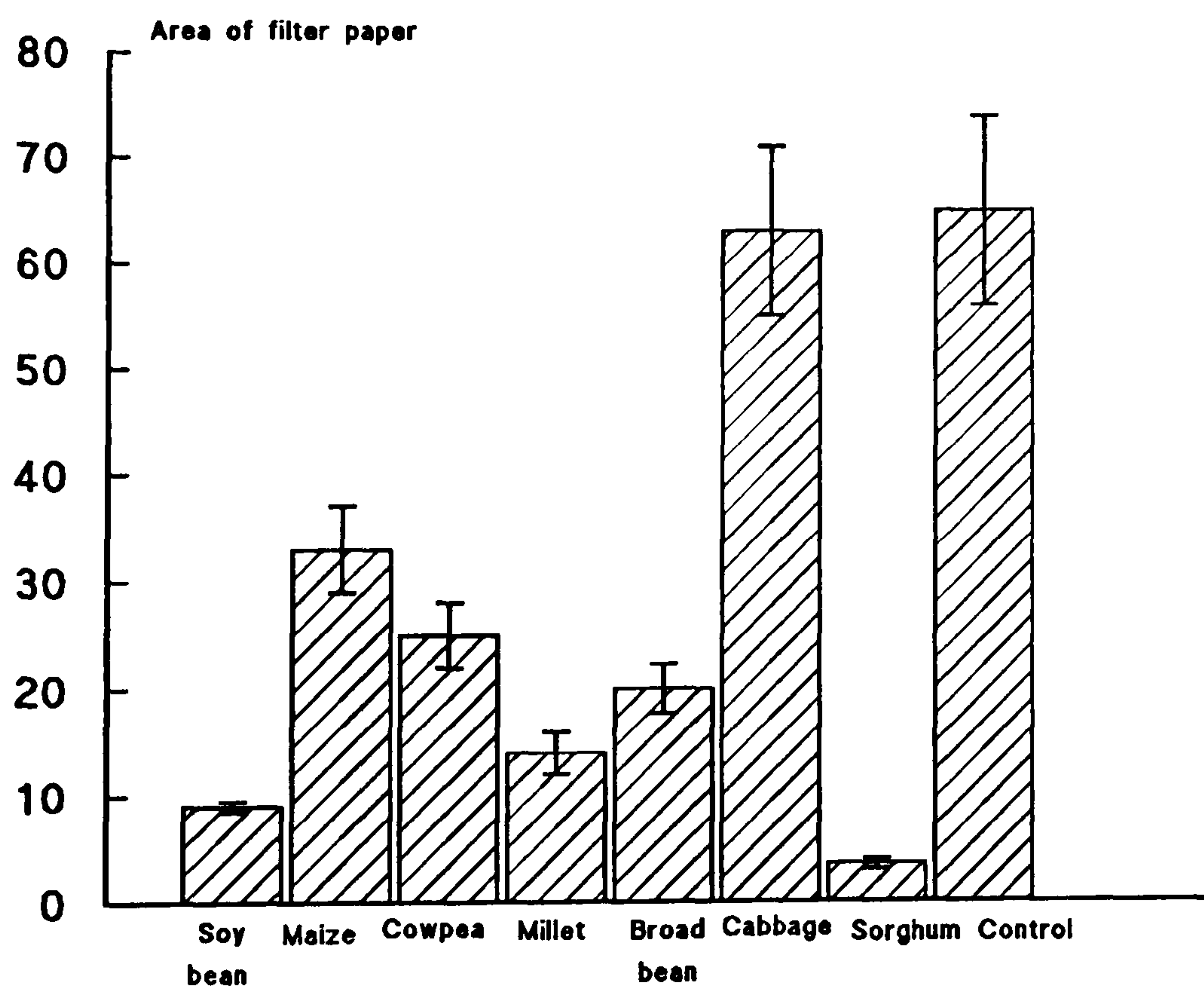


Figure 3.3. Area (mm²) of filter paper (Whatman 1: 4.25cm) impregnated with extracted surface wax ingested by larvae. Bars represent Standard Errors.

(Eigenbrode and Espelie, 1995), it has been demonstrated in some (Woodhead and Bernays, 1978) and surface leaf extracts may have an inhibitory (Woodhead and Padgham, 1988) or stimulatory (Bernays et al., 1976) effect on larval feeding. Leaf lipids extracted from both host and acceptable non-host plants stimulated feeding by larvae of *Manduca sexta* (de Boer and Hanson, 1988). However, application of surface chloroform extracts of non-host plants to wheat flour discs inhibited feeding in the grasshopper *Chorthippus parallelus* due to deterrent factors in the epicuticular lipids (Bernays and Chapman, 1975).

The chemical composition of the surface wax contents differs in the various plant species and therefore, different compounds may be involved in the inhibition of larval feeding. In sorghum, Woodhead (1983) obtained some n-alkanes, esters, free fatty acids, free fatty alcohols and p-hydroxybenzaldehyde from the wax extract that had inhibited feeding in *L. migratoria*. Sugayana and Salatino (1995) have reported that it was the chemical composition, rather than the physical traits, of the surface wax that could affect the feeding of the ant *Atta sexdens rubropilosa*.

The results obtained in this study do not conclusively show that surface waxes of the plants inhibited the feeding of the larvae. They did show, though, that the

surface wax may have an influence on the feeding of the larvae.

3.3.6. Biological activity of extracts of sequential extraction

In the experiment 20 mg of extract for each gram of artificial diet was used because it was the minimum concetration that had any effect on larval feeding. There is the possiblity that the lack of response to the extracts (below 20 mg/g) might have been due to the sucrose level being to ahigh and masking the effects of the extracts.

Table 3.6 shows the weight of each extract obtained by sequential extraction of 20 g of leaf tissue of the three cereal food plants.

Table 3.6 Mean yields of extracts (% of dry weight) of maize, sorghum and millet leaves.

Extracts	Yields (%)		
	Maize	Sorghum	Millet
Petroleum ether	3.07	3.94	2.15
Dichloromethane	2.04	2.37	6.94
Acetone	1.33	1.17	1.92
Methanol	14.31	13.92	10.42

No comparison was made with a dictotyledonous plant because low larval feeding was observed only on the monocotyledonous plants. There were greater amounts of material in petroleum and methanol extracts of maize and sorghum than in millet while in millet, there was a greater amount of material dichloromethane and acetone extracts than in the other plants.

Different crude extract fractions, when incorporated in an artificial diet, had varying feeding inhibitory effect on the larvae. The crude petroleum extract of the maize had the greatest inhibitory effect on larval feeding (Fig. 3.4) and also on the larval weight after ingesting the extract (Fig 3.5). For millet, the dichloromethane extract caused the greatest inhibitory effect on feeding (Fig. 3.6) and also on the larval weight (Fig 3.7). On sorghum, the dichloromethane and methanol extracts and the residue-incorporated-diet caused a reduction in larval feeding (Fig.3.8). The effect on larval weight is shown in Fig. 3.9.

The results obtained in this study suggest that different non-polar allelochemicals may be responsible for the inhibition of feeding of the larvae in maize and millet. In sorghum, in addition, there may be some inhibitory substances that are water-soluble and still present in the residue. Similar effects of leaf extracts have been reported in other insect species.

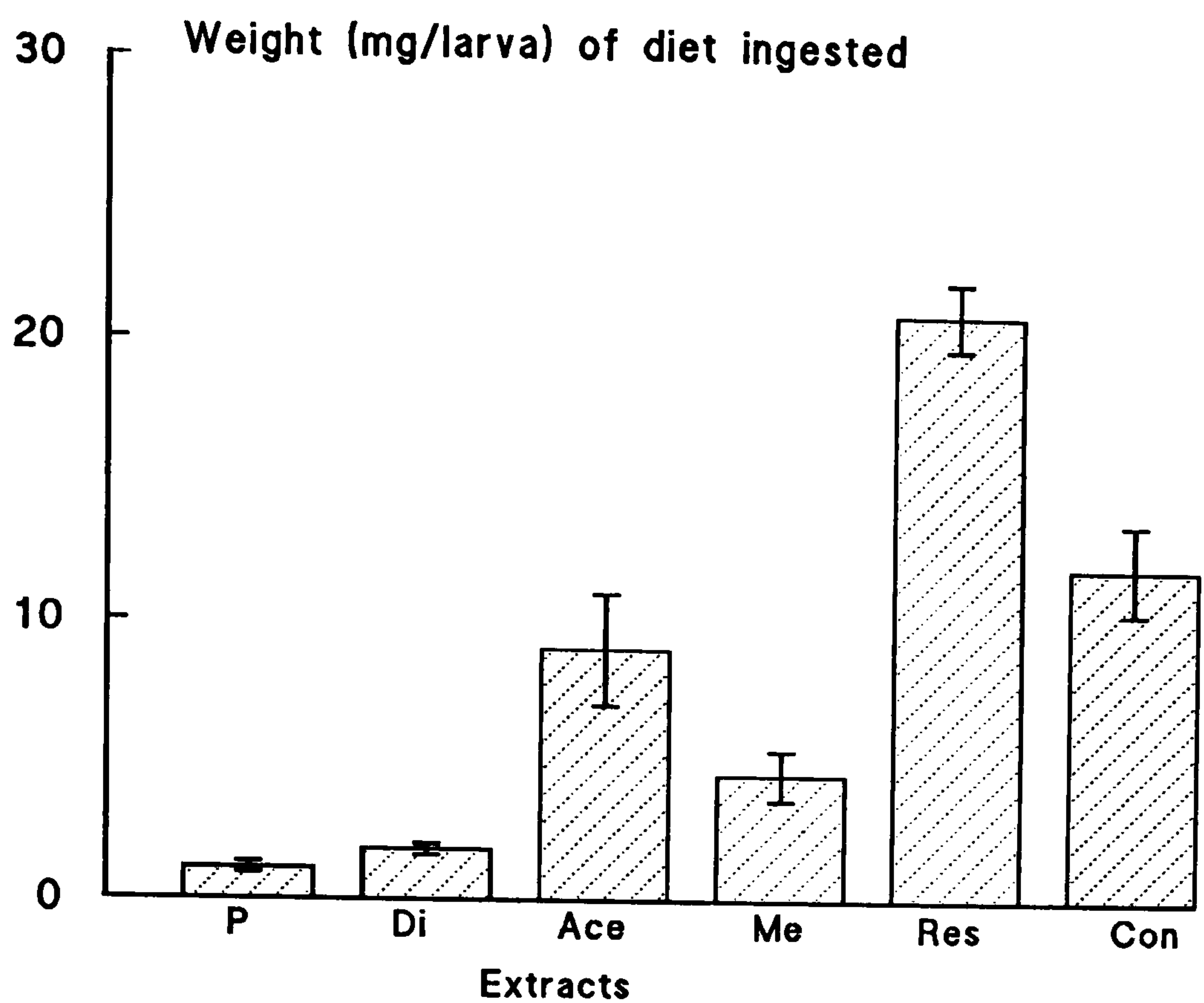


Figure 3.4. Feeding response of larvae to maize extracts at 20mg/g of artificial diet. Bars represent Standard Errors.

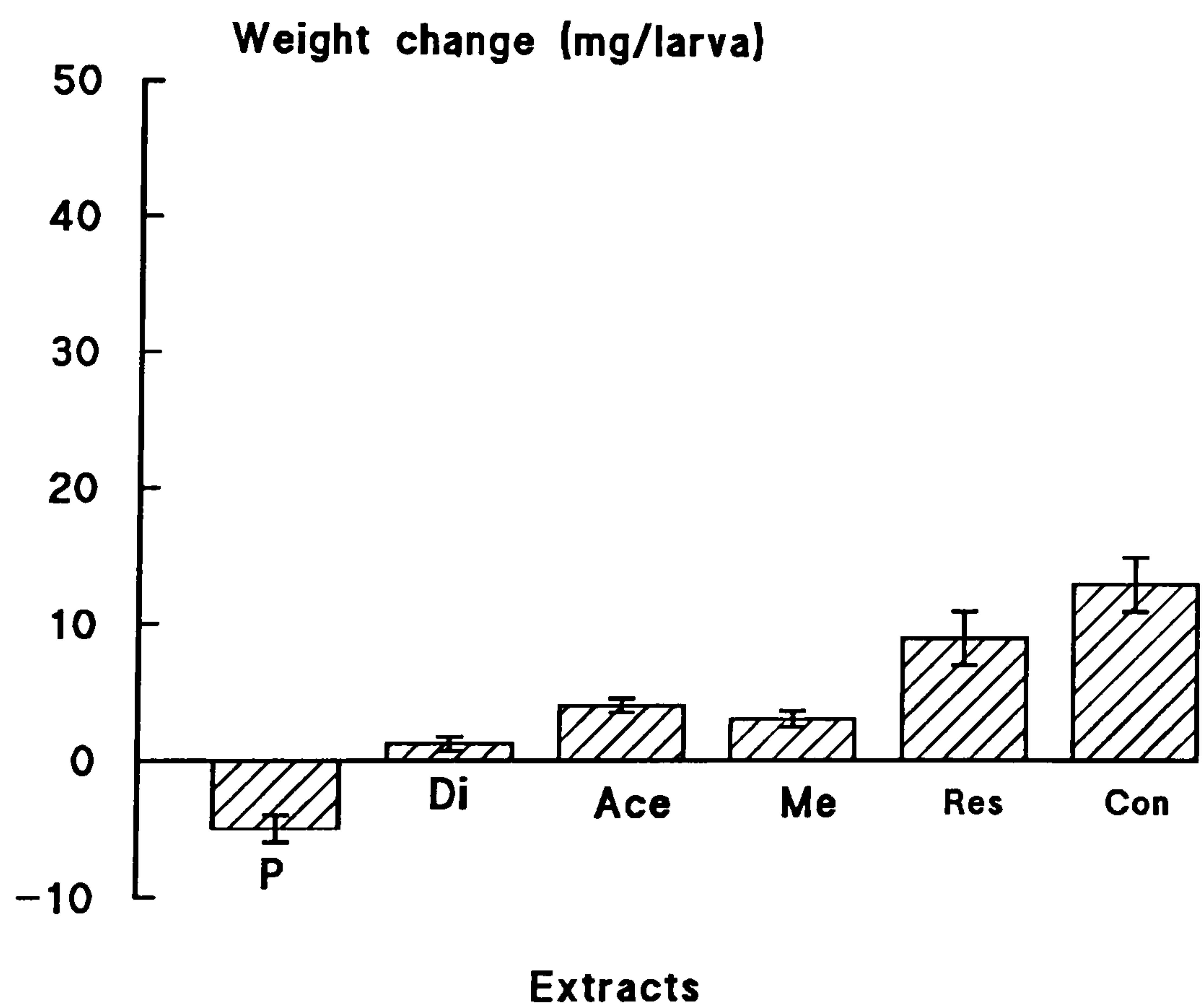


Figure 3.5. Weight (mg) gain/loss in 24 h of larvae fed maize extracts at 20mg/g of artificial diet. Bars represent Standard Errors.

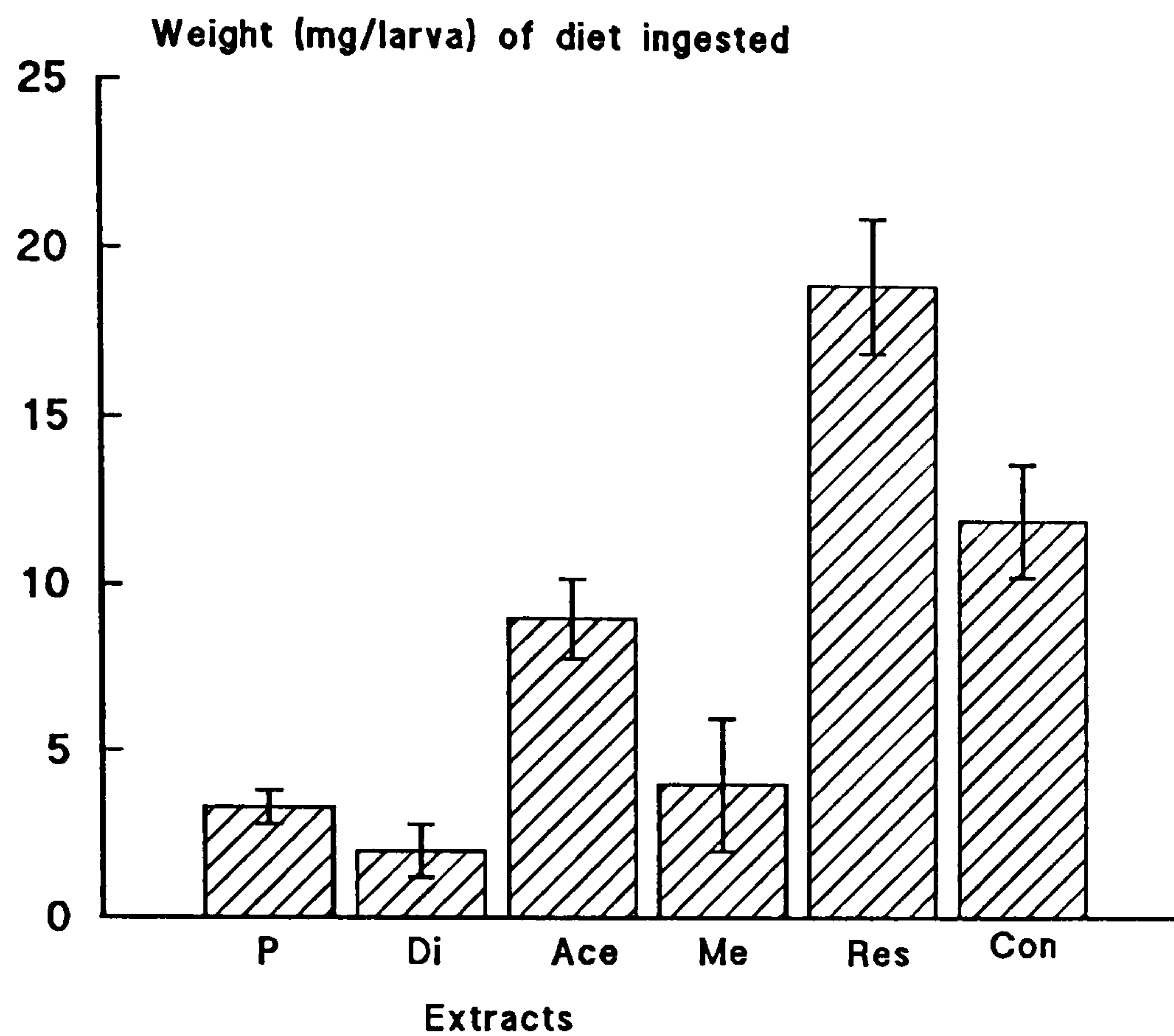


Figure 3.6. Feeding responses of larvae on millet extracts at 20mg/g of artificial diet. Bars represent Standard Errors.

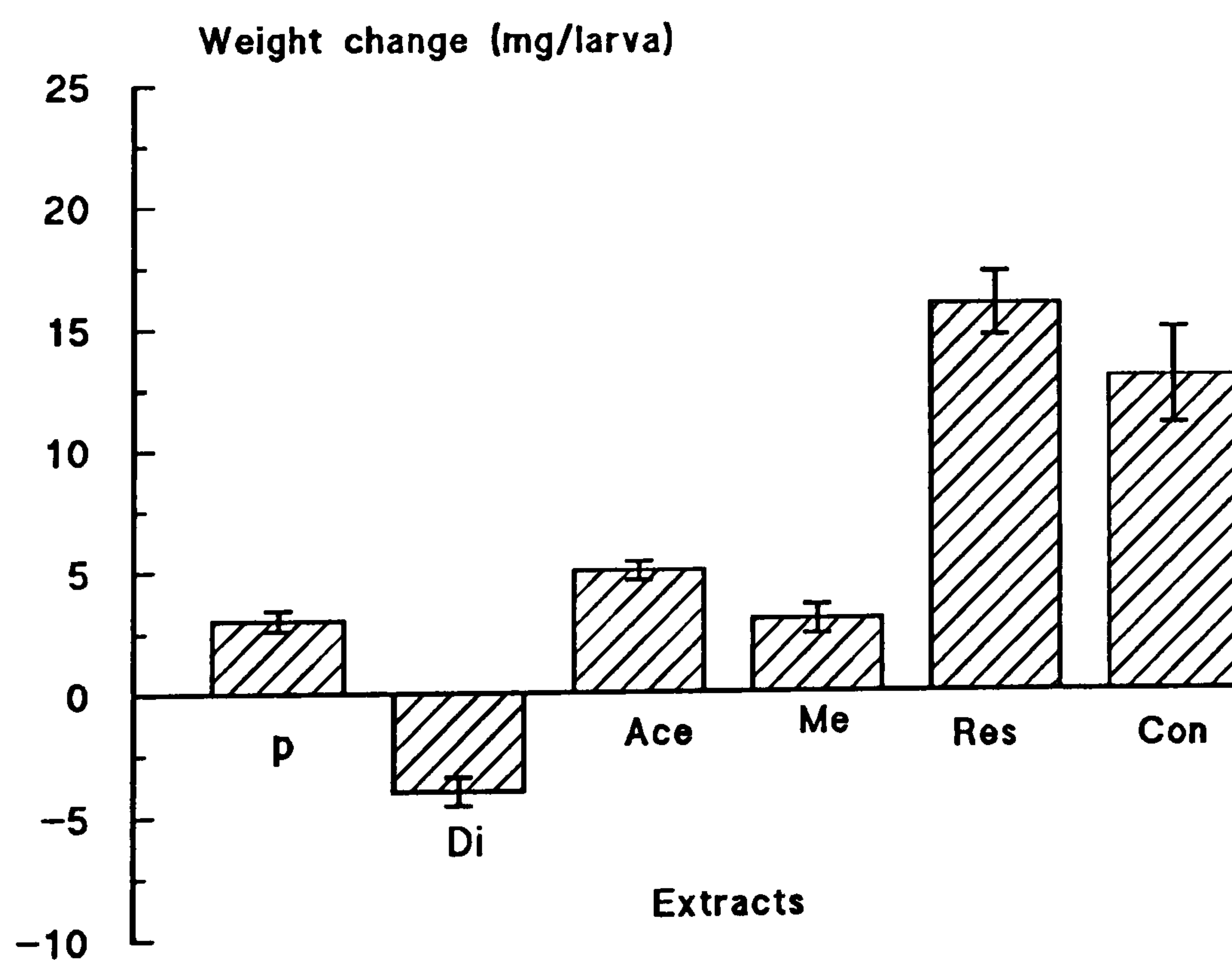


Figure 3.7. Weight (mg) gain/loss in 24 h of larvae fed millet extracts at 20 mg/g of artificial diet. Bars represent Standard Errors.

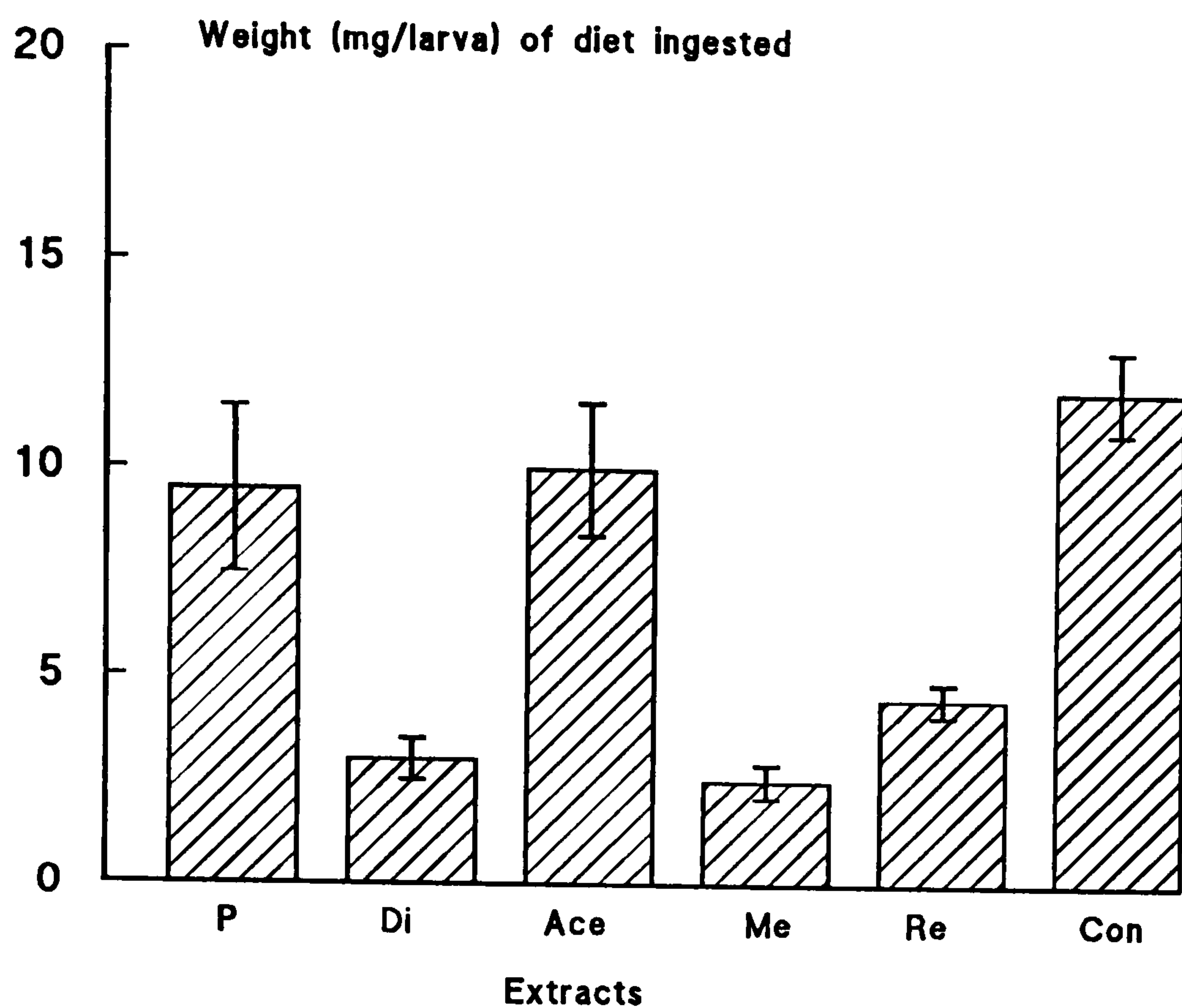


Figure 3.8. Feeding responses of larvae on sorghum extracts at 20mg/g of artificial diet. Bars represent standard Errors.

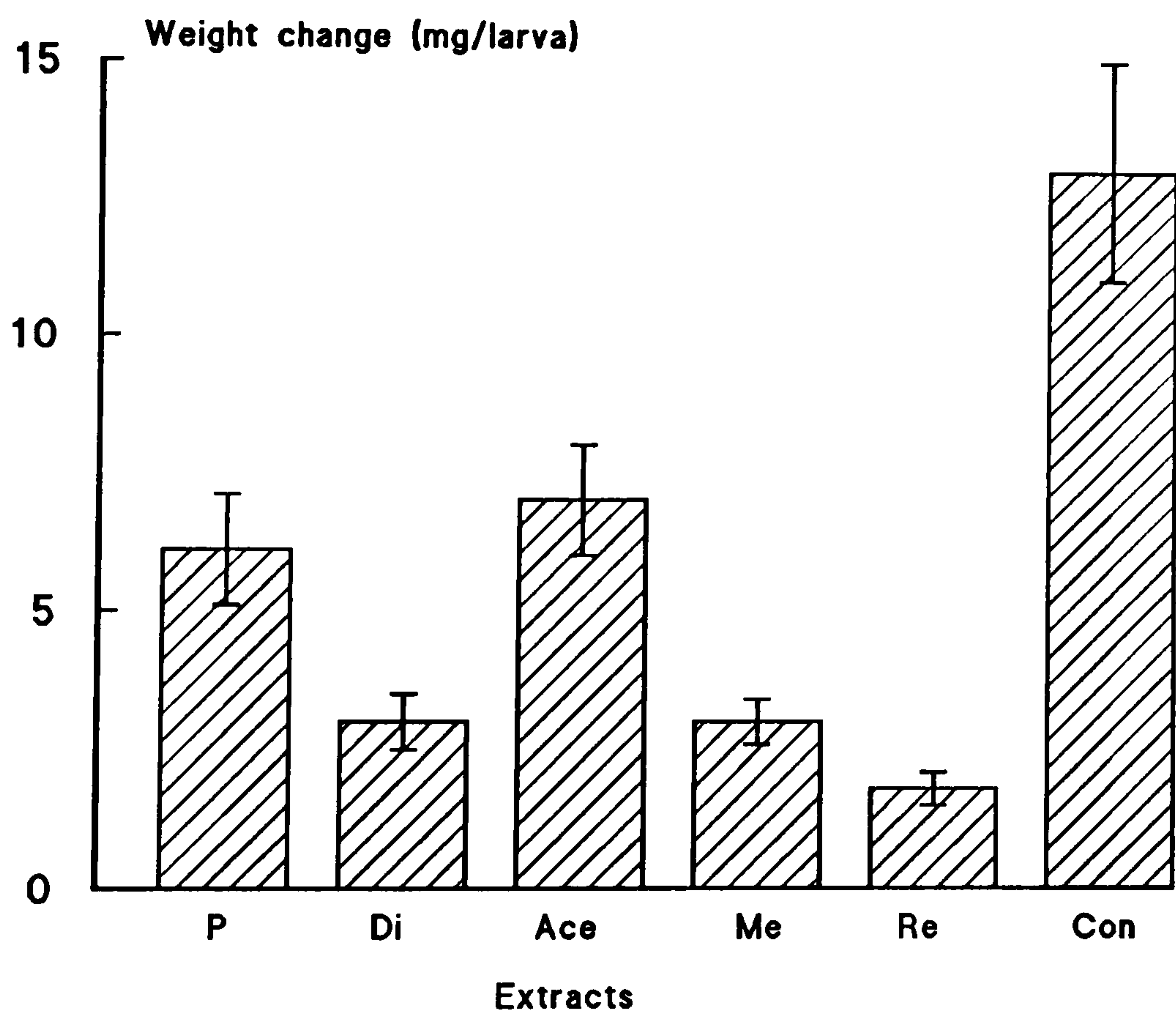


Figure 3.9. Weight (mg) gain in 24 h of larvae fed sorghum extracts at 20mg/g of artificial diet. Bars represent Standard Errors.

Meisner et al. (1981) observed inhibition of larval feeding in *S. littoralis* in a methanol extract of *Catharanthus roseus*. Quisenberry et al. (1988) reported high mortality in *S. frugiperda* when the larvae were fed petroleum ether and dichloromethane extracts of resistant bermuda grass varieties, and high mortality from the methanol fraction of a susceptible variety. There were no differences in the mortalities obtained with the water extracts. Saxena et al. (1992) observed inhibition of feeding, low fecundity and high larval mortalities in *Callosobruchus chinensis* fed petroleum and methanol extracts.

3.3.7. Biological activity of groups of compounds with potential feeding inhibitory properties.

Nine crude extracts were made from the leaves of the three cereal food plants (maize, millet, sorghum) and one dicot plant (for the purposes of comparison). The nine extracts were I: terpenoids, lipids, waxes, etc.; II: soluble flavonoids, esters, amino acids, sugars, etc.; III: alkaloids and other N-containing compounds; IV: hydroxyaromatic acids; V: aglycones of flavonols and flavones; VI: anthocyanidins from proanthocyanidins and glycoflavones; VII: aglycones of esters; VIII: free phenols, and IX: phenolic acids.

Table 3.7 shows the variation in the weights of the extracts obtained from 5 g of freeze dried sample of leaves

of the various food plants from which the extracts were made.

Table 3.7 Yields* of various groups of compounds that could contribute to inhibition of larval feeding

Extract	Weight (mg dry weight) of extracts			
	Millet	Sorghum	Maize	Cabbage
I	122.5	173.5	194.9	156.7
II	221.6	314.3	101.6	326.1
III	5.5	8.8	10.4	6.2
IV	12.2	22.9	16.4	26.0
V	322.5	133.6	231.2	209.4
VI	17.5	21.2	27.4	19.2
VII	295.8	423.5	549.2	236.2
VIII	4.2	6.8	7.1	4.3
IX	34.8	90.1	52.1	48.3

* Dry weight of extract obtained from 5 g of freeze-dried leaf

There were variations in the effects of the crude extracts of maize (Fig. 3.10), millet (Fig. 3.11), sorghum (Fig. 3.12) and cabbage (Fig. 3.13) on the feeding activity of the 4th instar larva.

Woodhead and Bernays (1978) tested extracts I-VII of

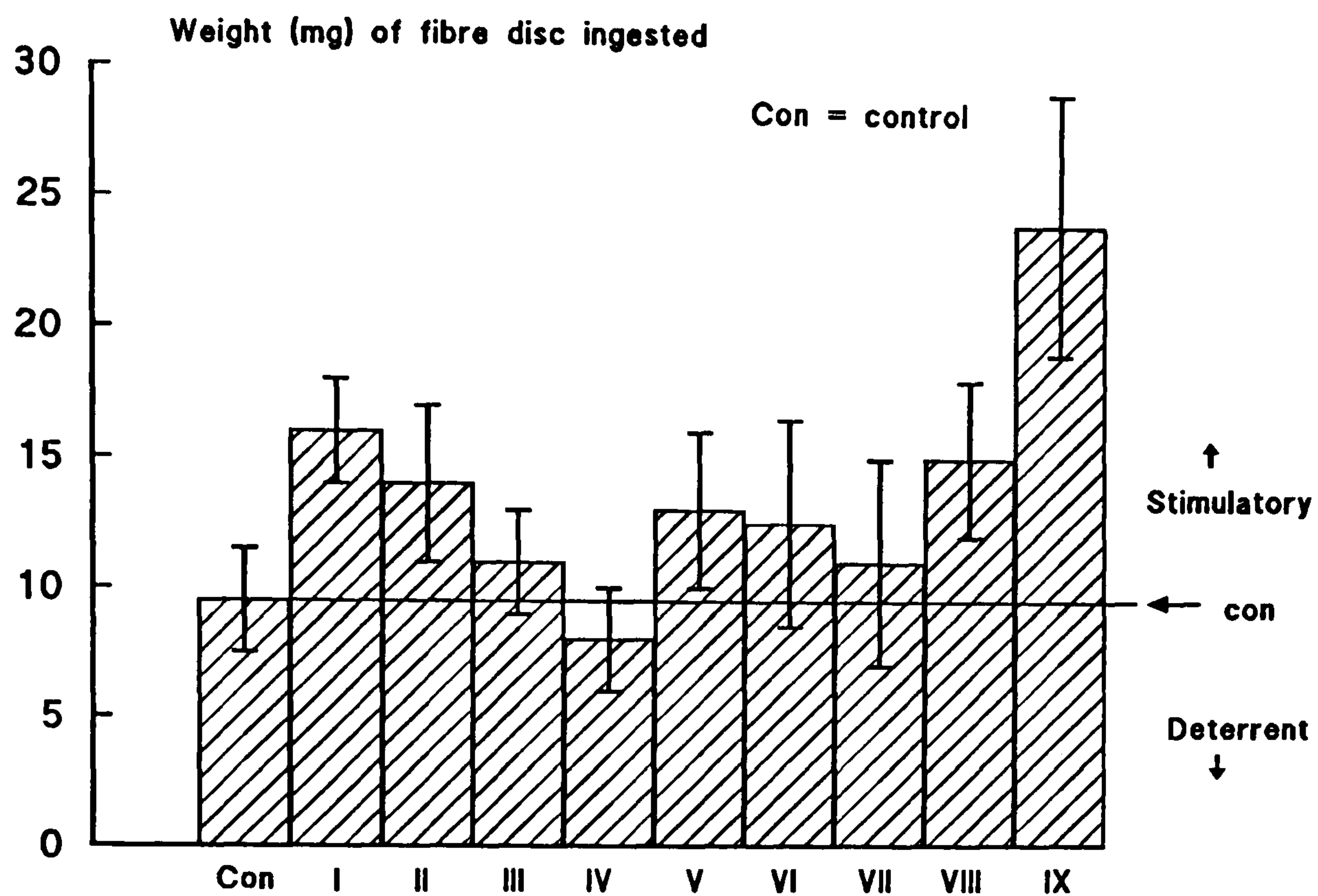


Figure 3.10. Effect of crude leaf extracts of maize on feeding of 4th instar larvae. Bars represent Standard Errors.

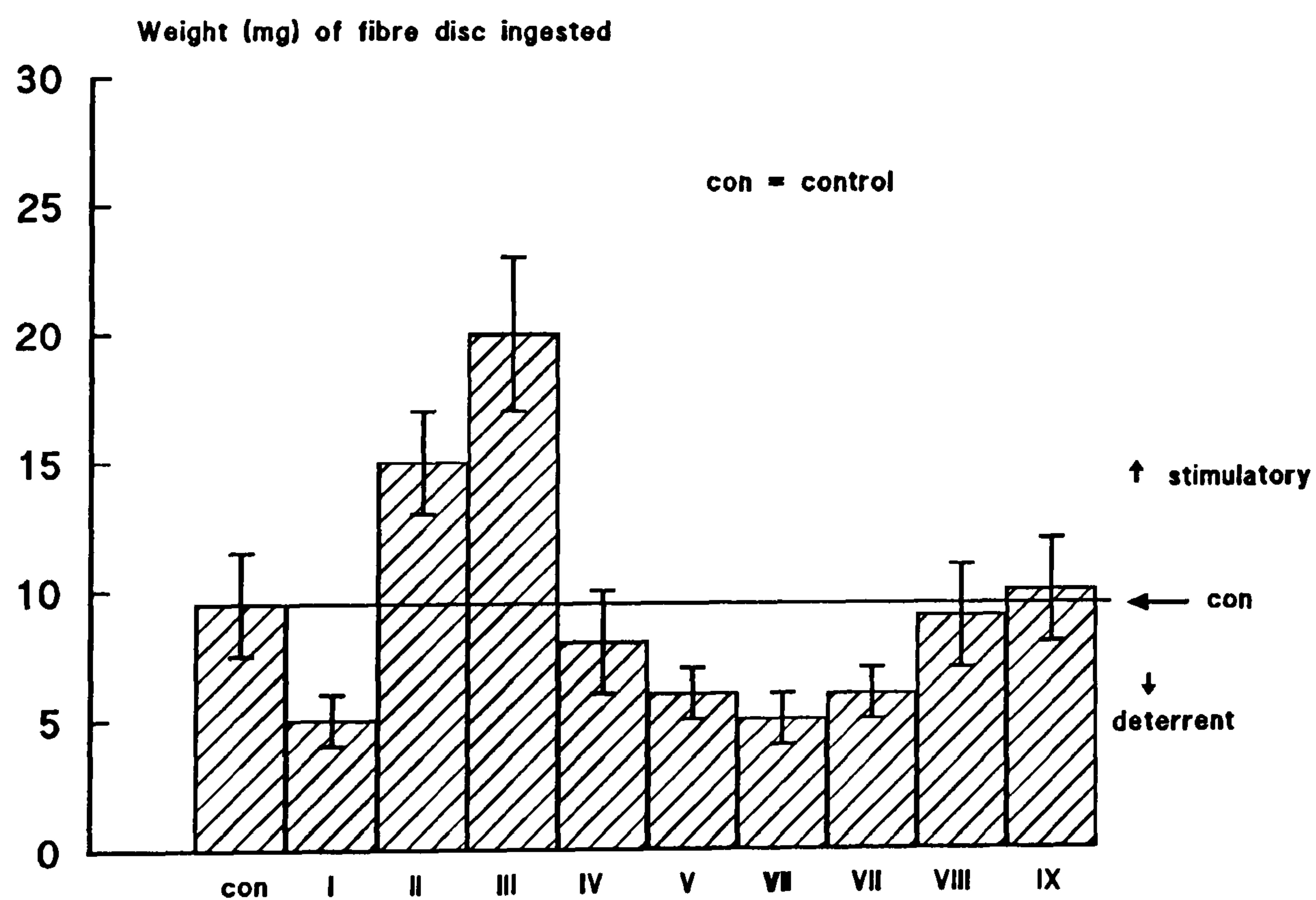


Figure 3.11. Effect of crude leaf extracts of millet on feeding of 4th instar larvae. Bars represent Standard Errors.

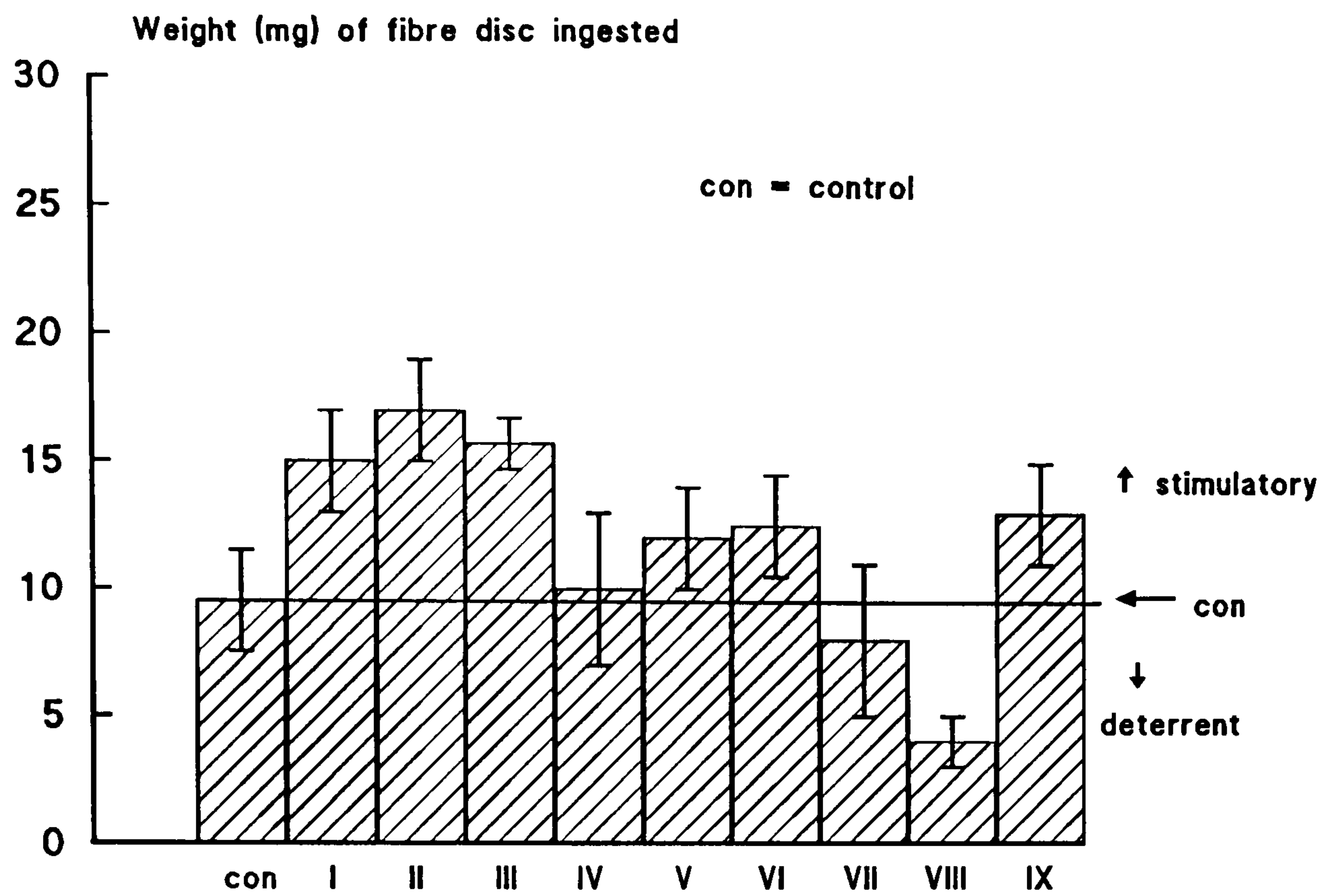


Figure 3.12. Effect of crude leaf extracts of sorghum on feeding of the 4th instar larvae. Bars represent Standard Errors.

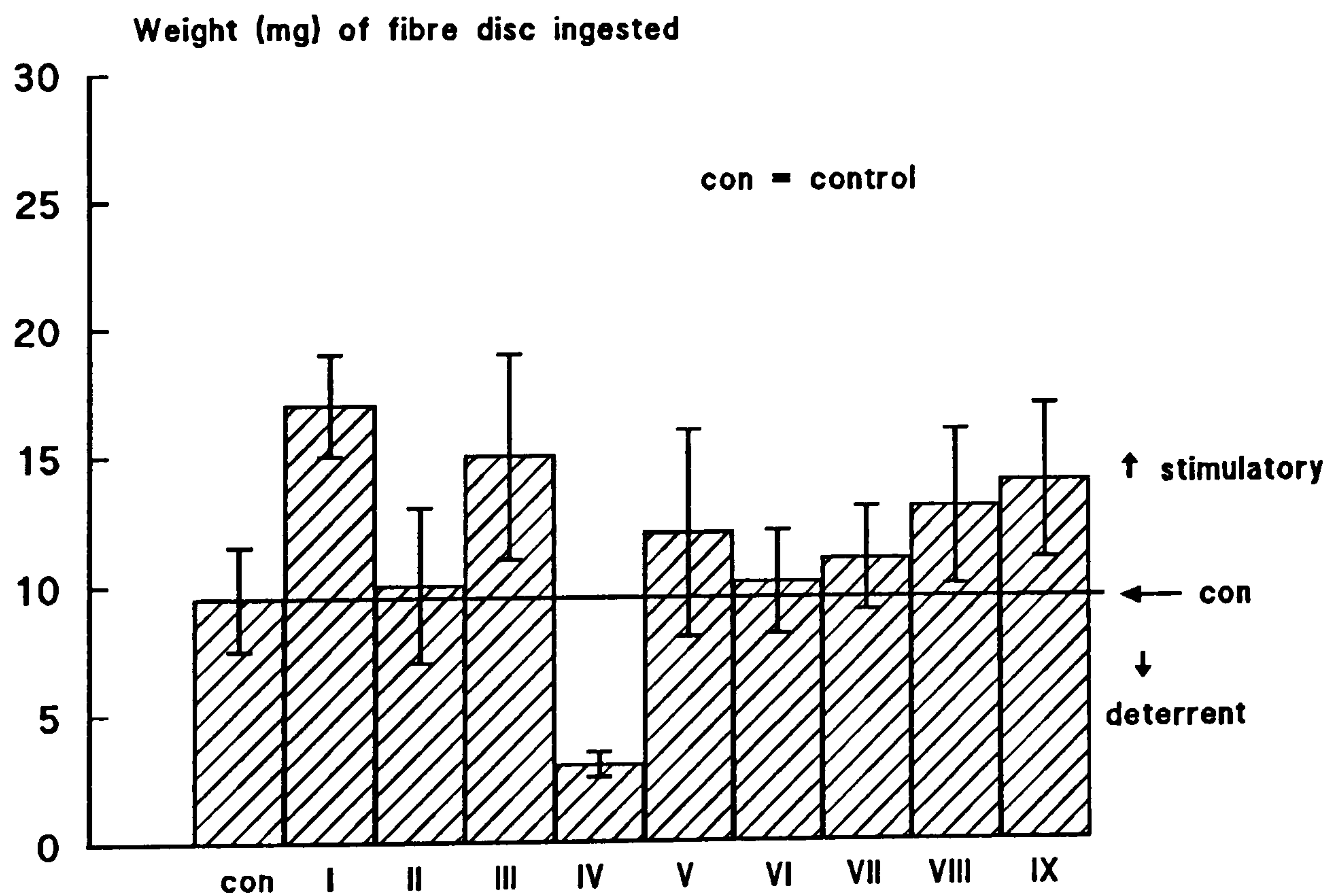


Figure 3.13. Effect of crude leaf extracts of cabbage on feeding of the 4th instar larvae. Bars represent Standard Errors.

sorghum against *Locusta migratoria*. Extract I was significantly deterrent in the youngest plants (corresponding to the growth stage of the cereal plants used in this study) while in older plants it was stimulatory. Extracts II, III and VI had little effect at any stage. Extract IV was inhibitory at any stage. Extracts V and VII were also inhibitory, though less inhibitory than extract IV. Further analyses of extract IV showed that it contained a mixture of hydroxybenzoic and cinnamic acids (phenolic acids).

The results of Woodhead and Bernays (1978) differ from those observed in this study. In the present study, extracts II and III had little effect on larval feeding in all the food plants. Extract IV was not deterrent in sorghum and extract VII was deterrent in millet and possibly sorghum. The free phenols (extract VIII) were deterrent only in sorghum. In general, millet had more inhibitory extract fractions than the other food plants.

On a resistant maize variety (Bastille), Okello-Ekochu (1990) did not observe any deterrent effect with extracts I-IX in the African armyworm, *S. exempta*. In this experiment, extract IV was deterrent in maize.

Various compounds have been isolated and tested on insects to determine their feeding inhibition properties. The results have varied depending on the insects. The

unpalatability of Gramineae seedlings was reportedly due to the presence of alkaloids (Bernays et al., 1974), phenolic acids and a lipid soluble material (Woodhead and Bernays, 1978). The alkaloid gramine has been observed to contribute to feeding inhibition in some varieties of seedling barley to the aphid *Schizaphis graminum* (Zuniga et al., 1985). Aasen et al. (1969) have suggested that the "staggers" condition observed in livestock is due to the alkaloids that were present in the ryegrass that they fed on. While α -tomatine (a steroidal alkaloid) has been found to be highly toxic to *Heliothis zea* indole alkaloids were found not to contribute to the resistance of winter wheat to *Sitobion avenae* (Isman and Dufey, 1983).

Phenolic constituents of tomato have been shown to inhibit early larval growth of the *H. zea* (Elliger et al., 1980). Singh and Rana (1989) reported a correlation between the levels of total phenols and resistance to the feeding of *Chilo partellus*. However, Torto et al. (1991) found phenolic compounds to be phagostimulatory to freshly moulted 3rd instar larvae of *C. partellus*. The authors hypothesised that the feeding behaviour of the insect may change with larval development, resulting in changes in larval behaviour.

Interestingly, extract IV was most inhibitory in cabbage. A possible explanation is that the higher levels of nutrients in the cabbage may have masked the effects of

the deterrent factors in the plant. Further studies involving fractionation and bioassay may reveal whether a factor or factors common to all the cereal plants used in the study may be present in the plants.

Various other compounds have been implicated in the resistance of plants to insect feeding. Okello-Ekochu and Wilkins (1994) obtained two deterrent compounds from the hexane extract of a resistant maize variety which were toxic to *S. exempta*. An aglucone, 2,4-dihydroxy-7-methoxybenzoxazin-3-one (DIMBOA) which is present in uninjured maize leaf, is released from a glucoside when the leaf is injured. DIMBOA has been reported to impart resistance to the European corn borer (*Ostrinia nubilalis*) (Klun and Brindley, 1966). The presence of DIMBOA was not determined in this study. However, Woodhead and Bernays (1978) failed to detect DIMBOA in sorghum but detected the release of HCN and some phenolic acids during the feeding of *L. migratoria*. These compounds inhibited larval feeding.

3.4 CONCLUSIONS

1. Artificial diet containing freeze dried foliage of maize and sorghum did not reduce mortalities of the immature stages significantly when compared to mortalities on fresh leaves. Therefore, the physical characteristics of the leaves may not have adversely affected the feeding of the larvae.

2. There were variations in the nutrient contents of the plants. Carbohydrate content was higher in the cereal plants. Nitrogen and the proportions of most of the important amino acids were lower in the leaves of the cereal plants. Differences in the nutritional composition of the food plants, in all probability, played a role in the larval performance on the plants.

3. Non nutritional factors also influenced larval feeding and overall performance on the food plants.

4. Larval food consumption increased on leaves with the surface wax removed. No definite relationship was established between the wax content of the plant, its nutritional quality and the consumption of foliage.

5. There were variations in the effects of the crude leaf extracts on larval feeding indicating the involvement of different compounds of the leaves in the inhibition of larval feeding.

6. The poor performance of the moth on the cereal plants was probably due to both nutritional and allelochemic factors.

CHAPTER FOUR

INFLUENCE OF FOOD PLANTS ON LARVAL SUSCEPTIBILITY TO
TOPICALLY APPLIED INSECTICIDES AND TOTAL CONTENT OF
CYTOCHROME P-450

4.1 Introduction

Phytophagous insects respond to variations in plant chemical compounds by behavioural, physiological and biochemical mechanisms which reduce the deleterious consequences of exposure to such compounds. Insects, usually, respond by using more than one mechanism. The most important mechanism, however, is biochemical (Hodgson *et al.*, 1993).

One of the biochemical responses involves reactions catalysed by enzyme systems such as mixed function oxidases, glutathione transferases and esterases. One of the most important enzymatic reactions involves oxidation by the mixed function oxidases (MFO) (Gibson and Skett, 1994).

The diversity of reactions catalysed by MFO make them important, not only for the metabolism of toxic substances and secondary plant chemicals found in plants, but also for the metabolism of endogenous substrates such as fatty acids (Agosin 1985), as well as foreign compounds such as insecticides (Cohen *et al.*, 1992).

The characteristics of MFOs that make them specially adapted for general purpose protection in herbivorous insects include a remarkable degree of non-specificity for lipophilic xenobiotics, the location of the enzymes

primarily in the major portals of entry into the body e.g. the gut and their induction by a wide variety of foreign compounds (Wilkinson, 1983).

4.1.1 Properties of mixed function oxidases

Mixed function oxidases are a group of enzymes which require O_2 and NADPH for the reactions that they catalyse. In the reaction, one atom of a molecule of oxygen is reduced to water while the other is used to oxidise the substrate. The reaction can be represented as follows:

$NADPH + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + ROH$, where RH is the oxidisable substrate (Gibson and Skett, 1994).

Since half of the oxygen is incorporated into the product and the other half into water, the reaction is referred to as a mixed function oxidase (Agosin, 1985). The MFOs therefore, represent an electron transfer system and cytochrome P-450 is the terminal end of this electron transfer system. In the reaction, the foreign substrate forms a complex with the oxidised form of cytochrome P-450. The complex is then reduced by an electron from the NADPH. The reduced P-450/substrate complex then reacts with an activated molecular oxygen. The resulting oxygenated complex then breaks down to yield a product and water.

The reactions catalysed by the MFOs include aromatic, aliphatic and alicyclic hydroxylation, epoxidation of double bonds, dealkylation of ethers and substituted

amines, desulphuration and dehalogenation (Hodgson *et al.*, 1993).

The substrates metabolised by the insect MFO include insecticides (Yu, 1986a; Wheelock and Scott, 1990); endogenous and synthetic steroids (Feyereisen 1993); endogenous and synthetic juvenile hormones and insect pheromones (Wilkinson, 1983).

The most important of the metabolic activities, as far as pest control is concerned, is the metabolic conversion of insecticides. MFO are capable of metabolising insecticides with different chemical specificities. Because of this, they represent, possibly, the major detoxication mechanism available to insects exposed to an insecticide. Such insecticide conversion could lead to the activation of the insecticide. Generally, though, the reaction leads to the conversion of the parent compound to a less toxic form. It is this conversion which often leads to the development of insect resistance (Agosin, 1985; Valles *et al.*, 1994).

4.1.2 Occurrence

By the early 1950s, it was known that insects were capable of metabolising insecticides and other xenobiotics (Agosin, 1985). The early studies of MFO were carried out in mammals, where they occur mainly in the liver. Later studies revealed that MFOs with properties similar to the mammalian MFO occur in arthropods, plants, bacteria, fish,

and birds (Wilkinson, 1983; Hodgson et al., 1993).

MFOs are associated with the microsomal fractions of tissue homogenates. The two most important components of MFO are the cytochrome P-450 and NADPH-cytochrome P-450 reductase (Agosin 1985). The key component of the MFO is cytochrome P-450 and the reactions catalysed by P-450 result in the transformation of the usually apolar parent compound into less lipophilic products which are more easily excreted.

Studies have shown that cytochrome P-450 corresponds to a single polypeptide, of which multiple forms (isozymes) with molecular weights of 30 000 - 70 000 Da exist (Stanton et al., 1978). The broad spectrum activity observed in P-450 is due to the presence of these multiple forms (Hodgson et al., 1993). There are at present over 200 known forms of P-450 (Gibson and Skett, 1994) and they confer somewhat different but overlapping substrate specificities to the MFO. Each isozyme has a preferred narrow range of substrates (Terreier, 1984). *H. virescens* larvae resistant to nicotine exhibited isozyme specific increases in metabolism because, in two of the six substrates measured for enzyme activity, metabolism was significantly greater than could be accounted for strictly on the basis of increased P-450 content (Hodgson et al., 1993).

4.1.3 Functions

MFOs perform several functions. These functions include metabolism of xenobiotics and fatty acids and the biosynthesis of pheromones and juvenile hormones (Yu, 1986b).

MFOs play a key role in the resistance of insects to insecticides due to insecticide metabolism. In a population of *H. virescens* resistant to pyrethroids an application of piperonyl butoxide, an inhibitor of the MFO, resulted in a 520-fold increase in insecticide toxicity, suggesting the involvement of the mono-oxygenase system (Hodgson et al., 1993).

MFOs probably play a role in the adaptation of insects to multiple food plants (Krieger et al., 1971; Hung et al., 1990). Although direct evidence of the role of cytochrome P-450 in insect food plant adaptation does not exist, indirect evidence that MFO play a role in the feeding of phytophagous insects is based on the following observations (Yu, 1983).

1. MFO activity in polyphagous larvae is higher than in oligophagous and monophagous larvae. Krieger et al. (1971) demonstrated a correlation between the number of plant families fed on and the MFO activity in the larval midgut, in 35 species of Lepidoptera. Yu (1986b) has also demonstrated that MFO activity toward a variety of

allelochemicals tested was higher in *S. frugiperda* (a polyphagous insect) than in the velvetbean caterpillar *Anticarsia gemmantalis* (an oligophagous insect). These observations provide strong evidence that MFOs play an important role in the detoxication of plant toxins and hence food plant selection in herbivorous insects.

2. MFO activity is higher in actively feeding stages than in non feeding stages. In three species of *Drosophila*, total P-450 levels were significantly higher in adults (more actively feeding stage) than in larvae by up to 20-fold (Danielson et al., 1994).

4.1.4 Factors affecting MFO activity

Several factors affect the activity of MFOs. These include:

1. Species of insects. Hodgson (1974) listed 16 different insect species in which cytochrome P-450 had been demonstrated. The levels varied from 0.04 nmol/mg protein in *Heliothis zea* to 0.37 nmol/mg protein in *Heliothis virescens*.

2. Developmental stage of the insect. In *S. frugiperda*, MFOs in younger larvae were less inducible by food plants than in older larvae (Yu, 1983). In the German cockroach, MFO differed between the nymphs and the adults (Valles et al., 1994).

3. Food plants fed on by the insect. Hung *et al.* (1990) reported higher levels of MFO in two lepidopterous species as compared to five homopterous insects. The lower levels in the homopterous insects were probably due to the fact that they fed only on water soluble materials in the plant sap.

4.1.5 Induction of cytochrome P-450

An important general characteristic of cytochrome P-450 is its ability to be induced by chemicals. Induction is a process in which the metabolising activity of a detoxication system is increased by a chemical stimulus (Terriere, 1984). The chemicals that can induce P-450 include insecticides, insect hormones, growth regulators, and allelochemicals present in food plants (Brattsten 1988; Gibson and Skett, 1994). Terriere (1984) has suggested that organisms can produce several forms of P-450 in their life time and that not all the forms are present all the time.

Several studies have shown that plant allelochemicals, and thus food plants, can induce or inhibit enzymes involved in the metabolism of insecticides and thus influence insecticide toxicity (Yu, 1983; Rose *et al.*, 1988).

The ability of the oxidase system to be induced by a wide variety of xenobiotics would allow the insect to

respond rapidly to periods of unusually severe chemical stress and would be sufficient to ensure immediate survival of the insect (Wilkinson, 1983). The phenomenon of induction has been reported in other insects (Yu, 1986a) though not yet reported for *S. littoralis*.

4.1.6 Susceptibility of larvae to insecticides

Insecticide toxicity is influenced by a variety of factors. These factors include the physiological state of the insects and the food plant fed on (Brattsten, 1988; Rose *et al.*, 1988; Moldenke *et al.*, 1992). The effects of the food plants would depend to a great extent on effects on the detoxifying enzymes.

The use of resistant crop plants is now an important ingredient in integrated pest management systems. Such crop resistance is usually due to enhanced levels of plant chemicals. Therefore, the influence of crops on the efficacy of insecticides is important. It is possible that the chemicals imparting resistance to the plants could also induce the detoxifying enzymes. For instance, P-450 involved in the metabolism of xanthotoxin is inducible by xanthotoxin (Cohen *et al.*, 1992). Also the use of crop plants capable of reducing an insect's tolerance to insecticides could reduce the level of insecticide use on farms.

Studies have demonstrated significant variation in the

susceptibilities of insects to insecticides due to the effects of food plants (Moldenke et al., 1992). It is important to conduct more studies on the influence of food plants on the susceptibility of *S. littoralis* to insecticides in order to understand better its development of resistance to insecticides.

4.1.7 Experimental Objectives

The experiments in this chapter were conducted for three reasons:

1. To determine the response of the larvae to topically applied insecticides.
2. To determine the effects of food plants used in the previous study on the total cytochrome P-450 content of larval midguts.
3. To determine the effects of sublethal doses of insecticides on larval feeding.

4.2 MATERIALS AND METHODS

4.2.1 Insecticides and solvents

All the solvents used were of analytical grade and the insecticides were of technical grade. The cypermethrin (91% pure) and permethrin (94.1% pure) were supplied by Shell Research Limited. Barbital, malathion (95% pure) and β -naphthoflavone were purchased from Sigma Chemical

Company. Carbofuran (98.1% pure) was supplied by Zeneca.

4.2.2 Treatment of insects

Two groups of larvae were used. One group consisted of larvae reared from the first instar to the beginning of the sixth instar on maize, sorghum, cabbage, broad bean, soybean and cowpea. Four groups of 15 larvae were randomly selected from larvae reared on each of the plants. The larvae were starved for 6 h and then microsomes were prepared from their midguts.

The second group consisted of larvae reared on the semi-artificial diet up to the beginning of the sixth instar. This group of sixth instar larvae were then randomly divided into groups of 15. Each group represented a replicate. Four groups of larvae were fed leaves of the food plants for 48 h. Larvae which fed on the artificial diet were used as controls. At the end of the 48 h feeding period, the larvae were starved for 6 h and then used for the extraction of the enzyme.

4.2.3 Extraction of enzyme

The larvae were weighed after the 6 h starvation period, then the head and the tip of the abdomen were cut off. The alimentary canal was then drawn out and the fore and hind gut removed. The contents of the midguts of larvae were then removed.

The midgut, (400 mg) were then washed in ice cold 1.15% KCl and homogenised, for 30 s, in 25 ml of ice cold 0.1 M sodium phosphate buffer, pH 7.5, in a hand operated glass tissue homogeniser with a teflon pestle. The homogenising was done on packed ice. The homogenate was then filtered through cheesecloth and centrifuged at 10,000 g_{max} for 15 min at 4°C. The pellets were discarded and the supernatant was filtered through glasswool and recentrifuged at 100,000 g_{max} , at 4°C, for 1 h in a Centrikon T-1055 ultracentrifuge. The supernatant was discarded. The microsomal pellets were resuspended in 0.1M sodium phosphate buffer containing 30% (v/v) glycerol (to give a microsomal suspension) (Yu, 1983).

The total cytochrome P-450 content was measured by the methods of Omura and Sato (1964) and Gibson and Skett (1994). The microsomal suspension (3 ml) was pipetted into each of two cuvettes. The suspension in one cuvette was to act as the sample and the other as the reference. A few grains of solid sodium dithionite were added to each cuvette, with gentle stirring. Both cuvettes were placed in a Philips PU 8820 split beam spectrophotometer. After obtaining the base line, carbon monoxide gas was passed through the suspension in the sample cuvette, at a rate of 1 bubble/second for 1 min. Spectrophotometric readings were taken at room temperature at 450 nm.

4.2.4 Determination of protein content

Protein contents were determined by the method of Bradford (1976) using bovine serum albumin as the standard. The protein reagent was prepared as follows: 50 mg of Coomassie brilliant Blue G-250 was dissolved in 25 ml of 95% ethanol. 50 ml of 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 500 ml.

Standard bovine serum albumin solutions (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mg/ml) were prepared. The standard protein solutions (0.1 ml) were pipetted into test tube and 5 ml of the protein reagent was then added. The contents were thoroughly mixed by vortexing. The absorbance was then measured, after 2 min, at 595 nm, with a Shimadzu UV-1201 spectrophotometer. The reagent blank was prepared with only the buffer and 5 ml of protein reagent. Microsomal samples were treated in the same way as the standard protein solutions.

4.2.5 Calculation of total cytochrome P-450 content

The cytochrome P-450 concentration of larval midgut was determined according to Gibson and Skett (1994) as follows:

$$P = \frac{A \times 1000}{B}$$

Where :

P = cytochrome P-450 content (nmol/mg protein)

A = Difference in absorbance at 450 nm

B = Extinction coefficient ($91 \text{ cm}^{-1} \text{ mM}^{-1}$)

Specific content of cytochrome P-450 is given by:

$$S = \frac{P}{D}$$

Where :

S = P-450 concentration of microsome preparation

D = Protein concentration of microsome preparation

4.2.6 Effect of barbital, β -naphthoflavone and piperonyl butoxide on larval feeding

Larvae reared on the semi artificial diet were used for the feeding tests. 6th instar larvae were randomly selected and divided into groups of 10. Each group represented a replicate. 4 groups were used for each food plant.

The larvae were topically treated with either piperonyl butoxide (Pbo) (21.2 ug/g larva), a known inhibitor of cytochrome P-450, β -naphthoflavone (19.5 ug/g of larva) and barbital (19.5 $\mu\text{g/g}$ of larva) known inducers of cytochrome P-450. The compounds were applied in acetone in 1 μl aliquots, with a Burkhard Pax 100 micro applicator fitted to a 1 ml glass syringe. The solutions were applied to the dorsal thoracic region. Prior to the application of

the solutions, the larvae were anaesthetised with CO₂ for 1 min. Control larvae were treated with only acetone.

Two hours after the application of the compounds, the larvae were weighed and each larva was placed in a 10 cm (diameter) petri dish with moist filter paper placed at the bottom of the petri dish. Each larva was provided with weighed leaves of one of the food plants. Feeding was allowed to continue for 48 hours. After 48 h, the larvae were weighed. The leftover leaves were oven dried and weighed. The amounts of food ingested were calculated on a dry weight basis by the method of Waldbauer (1964) as outlined in 2.2.7.3.

4.2.7 Insecticide treatment of insects

The insecticides used were malathion, permethrin and cypermethrin. Two groups of larvae were used for the insecticide bioassay. Fourth instar larvae were used because the high larval mortality on the maize, millet and sorghum of the older larvae. One group consisted of larvae reared entirely on the semi-artificial diet. The second group of larvae had been reared from the first instar to the fourth instar on each of the food plants.

Fourth instar larvae reared on the semi-artificial diet were randomly placed in groups of 15. Each group of larvae was given leaves of the food plants and allowed to feed on them for 24 h. Control insects were allowed to feed

on the artificial diet.

After 24 h the larvae were removed for insecticide bioassay. The appropriate amounts of the insecticide in acetone were applied in 1 μ l aliquots with a Burkard Scientific Pax 100 microapplicator. The insecticides were applied to the prothoracic dorsum of previously anaesthetised larvae. Controls were treated with 1 μ l acetone only. There were 15 larvae per replicate and 4 replications for each of the 5 concentrations of insecticides used for each plant.

After the treatments, the larvae were provided with fresh foliage in petri dishes. Mortality was determined by the inability of the larvae to respond to a pin prick applied to the prothorax after feeding on the food plants for 48 h. Mortality data were analysed by probit analyses using a PC.

To evaluate the effect of β -naphthoflavone and piperonyl butoxide on larval susceptibility to insecticides, 4th instar larvae reared on the semi-artificial diet were used. The inducer and the inhibitor were applied first. The insecticides were applied 2h later. The larvae were then offered leaves and mortality was determined after 48 h.

4.2.8 Effect of sublethal doses of insecticides on larval

feeding

On determination of the LD₅₀ values for all the insecticides (4.2.7.) a sub lethal dose, i.e. LD₅, was applied to the larvae reared only on artificial diet. The effect of the sub lethal dose on larval feeding was determined. The experimental conditions were the same as outlined in 4.2.6.

4.2.9 SDS-Polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was run in order to find out whether the variations in the P-450 contents of larvae observed in the previous experiments were associated with changes in the peptide profiles of microsomes prepared from the larval midguts.

Three groups of sixth instar larvae were used. These were larvae reared on the artificial diet to the 6th instar and then fed on food plants for 48 h, larvae reared on the artificial diet up to the 6th instar and then treated with piperonyl butoxide, barbital or β -naphthoflavone, and larvae reared from the egg stage to the 6th instar on each of the food plants.

Microsomes were prepared from the gut of the larvae as outlined in 4.2.3. The microsomal suspensions were used in SDS polyacrylamide gradient gel electrophoresis, with a 5% stacking gel and 10%-20% linear gradient acrylamide resolving gel.

4.2.10 Preparation of stock solutions

With the exception of water, all the materials were purchased from SIGMA Chemical Company. The pH was adjusted by adding concentrated HCl. The stock solutions were prepared as follows (D. Mantle, pers. communication; Becker et al., 1990):

A. 22.2 g acrylamide
0.6 g bis-acrylamide
made in 200 ml of water

B. 22.2 g acrylamide
0.6 g bis-acrylamide
made in 100 ml of water

C. 22.2 g acrylamide
0.6 g bis-acrylamide
made in 50 ml of water

D. Stacking gel buffer, 0.5 M Tris-Hcl, pH 6.8
Tris 6.0 g
Water to make 100 ml

E. Resolving gel buffer, 1.5 M Tris-HCl, pH 8.8
Tris 18.15
Water to make 100 ml

- F.** Sodium dodecyl sulfate, 10%
- | | |
|-------|----------------|
| SDS | 10 g |
| Water | to make 100 ml |
- G.** TEMED (N,N,N'N'-tetramethylethylenediamine)
Added last and just before loading sandwich.
- H.** Tank buffer, pH 8.3
- | | |
|---------|-----------------|
| Tris | 3.0 g |
| Glycine | 14.4 g |
| SDS | 1.0 g |
| Water | to make 1000 ml |
- I.** Sample buffer
- | | | | |
|---------------------------|----------|--------|------------|
| Tris | 0.0625 M | pH 6.8 | 50 μ l |
| 10% SDS | | | 10 μ l |
| 2-Mercaptoethanol | | | 3 μ l |
| 20% Glycerol | | | 76 μ l |
| Bromophenol blue (0.001%) | | | 10 μ l |
- J.** Staining solution
- | | |
|--------------------------------|--------|
| Coomassie brilliant blue R 250 | 1.25 g |
| Glacial acetic acid | 400 ml |
| Methanol | 400 ml |
| Water | 200 ml |
- K.** Destaining solution
- | | |
|----------|--------|
| Methanol | 400 ml |
|----------|--------|

Glacial acetic acid	400 ml
Water	200 ml

4.2.11 Preparation of resolving gel

To prepare the separating gel, the following solutions were pipetted into two separate 100 ml beakers (**Y and Z**)

	Y	Z
Stock solution E (1.5 M Tris/HCl, pH 8.8)	7.5 ml	7.5 ml
Water	7.5 ml	7.5 ml
Acrylamide solution	C - 13.5 ml	B - 13.5 ml
Ammonium persulphate (7.5 mg/ml)	1.5 ml	1.5 ml
20% SDS	0.6 ml	0.6 ml
TEMED	30 μ l	15 μ l

4.2.12 Preparation of stacking gel

To prepare the stacking gel, the following were used:

Stacking gel buffer	D	7.5 ml
Stock solution	A	13.5 ml
(0.5 M Tris/HCl, pH 6.8)		
Water		7.5 ml
Ammonium persulphate (15m g/ml)		1.5 ml
TEMED		50 μ l
20% SDS		0.3 ml

4.2.13 Casting of gels

Wearing gloves to prevent contaminating the glass plates with grease, the surfaces of the glass plates were cleaned with ethanol. The plates were assembled using 1.5 mm spacers. In forming the resolving gel, 15 ml of the mixture in beaker **Y** was poured into space A and 15 ml of the mixture in beaker **Z** was poured into space B (Fig. 4.A).

The peristaltic pump was then started. The peristaltic pump flow rate was 100 ml/min. On filling the glass sandwich to the required level, the top of the gel was layered with water. The layer of water was poured out after the gel had polymerised and before the stacking gel was poured into the sandwich.

Electrophoresis was performed according to Laemmli (1970) using a 5% acrylamide stacking gel and a 10%-20% linear acrylamide gradient separating gel. There were 10 wells per gel in a 16 cm X 18 cm gel slab. Gels were run at 50 mA per gel in a tap water cooled Hoefer SE600 vertical slab gel electrophoresis unit.

4.2.14 Preparation of sample

Microsomal samples were suspended in 1 ml 0.0625 M Tris-HCl, pH 6.8, and the protein content was determined. The microsomal samples were diluted in the sample buffer and boiled for 3 min in a water bath. The amount of buffer used depended on the protein content of the microsomes.

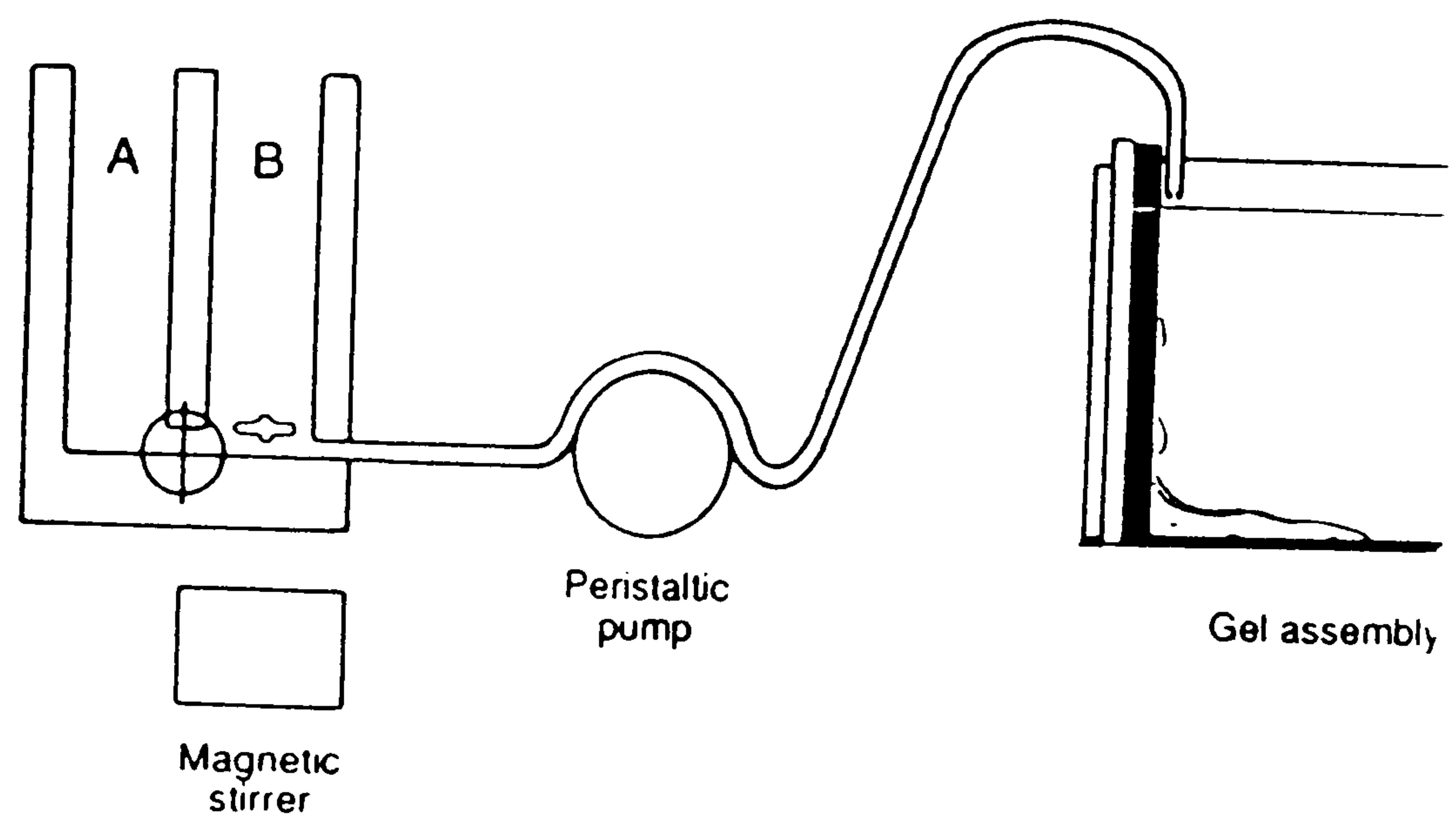


Figure 4.A. Apparatus for the preparation of a gradient gel
(Bollag and Edelstein, 1991)

50 μ l, of 50 μ g protein content, of the sample was loaded in each well. Proteins used as standards were carbonic anhydrase (29 kDa), egg albumin (45 kDa), bovine plasma albumin (66 kDa), phosphorylase B (97.4 kDa), β -galactosidase (116 kDa) and myosin (205 kDa).

Following electrophoresis, the gels were fixed by immersion in 20% (w/v) trichloroacetic acid for 30 min, stained in the staining solution for 30 min and then destained by frequently washing the gel with the destaining solution.

4.2.15 Gel scanning

The gels were scanned with a Joyce-Loebl Chromoscan 3 flat-bed densitometer with a 0.1 mm slit width and with associated driving/control software. A 3.0 A (grey level 255) measurement range factor was used. The profiles were edited using a PC Midas software with an autozero background, with Gaussian smoothing and a Gaussian half-width.

4.1 RESULTS AND DISCUSSION

4.3.1. Amount of foliage ingested by the 6th instar larvae feeding on the food plants for 48 h

The cumulative amounts of foliage ingested by the 6th instar larvae during the 48 h feeding period show that lower amounts of the monocot plants were ingested as compared to the amounts of dicots ingested (Table 4.1).

The mean dry weights of faecal pellets produced by the larvae are shown in Table 4.2. The significance of the variations of food ingested by the larvae has been discussed in 2.3.20.

Table 4.1. Mean dry weight of leaves ingested by 6th instar larvae feeding on food plants for 48 h.

Food plants	Weight (mg)	
	Mean \pm SE	
Sorghum	19.3 \pm	1.1 a
Maize	22.5 \pm	2.5 a
Cowpea	81.3 \pm	5.1 b
Soybean	115.2 \pm	13.9 c
Broad bean	122.1 \pm	10.4 c
Cabbage	124.5 \pm	9.7 c

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Table 4.2 Mean dry weight of faecal pellets produced by 6th instar larvae feeding on food plants for 48 h.

Food plants	Food plants	
	Mean \pm SE	
Maize	4.7 \pm	0.2
Sorghum	8.2 \pm	0.4
Broad bean	51.8 \pm	2.7
Cabbage	47.7 \pm	0.9
Cowpea	41.8 \pm	2.0
Soybean	35.3 \pm	2.6

4.3.2 Total cytochrome P-450 content

Figure 4.1 shows the calibration curve for the molecular weight markers used during electrophoresis. Figure 4.2 shows the standard curve for the determination of protein content (Bradford, 1976) (4.2.4).

Even though the enzyme activity was not determined in the present study, several reports have indicated that the activity of P-450 is related to the total P-450 content of microsomes prepared from larvae (Moldenke *et al.*, 1992; Lindroth *et al.*, 1993). The results of this study show that the total P-450 content in the midgut was dependent on the species of plant that the larvae fed on.

Table 4.3 shows the body weight of the larvae at the time when the microsomes were prepared. This was lower on the monocots than on the dicots. Table 4.3 also shows that the total content of cytochrome P-450 was not greatly increased on the monocots when sixth instar larvae were fed on the food plants for only 48 h. Compared to the artificial diet, there was a 2-4 fold increase in P-450 content in the dicots.

Table 4.4 shows the variation in body weight of the larvae and the total content of P-450 in larval midguts when the larvae were reared on the food plants from the first to the sixth instar. The total P-450 content was lower in larvae feeding on the monocot plants.

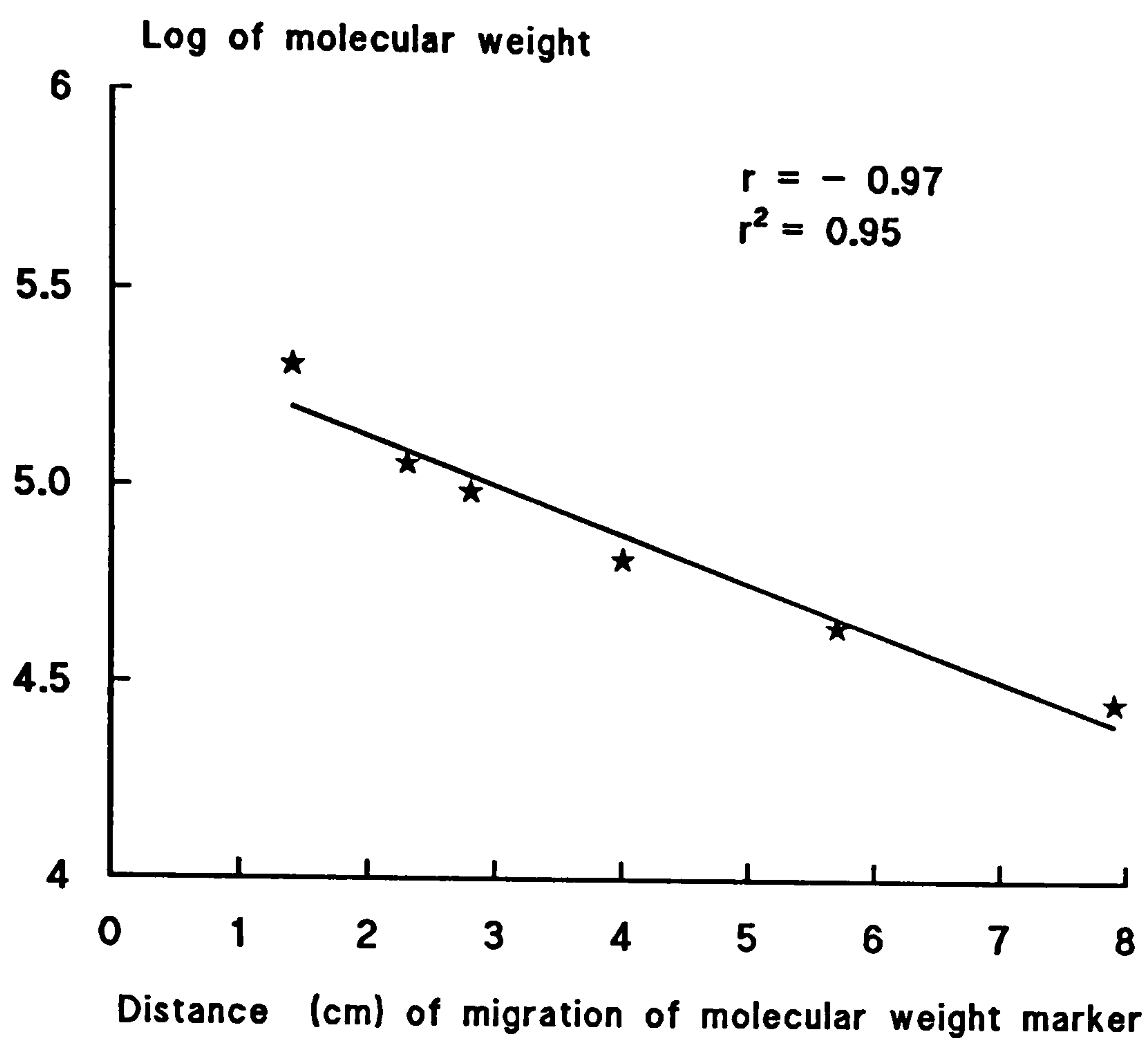


Figure 4.1. Calibration curve for molecular weight standards

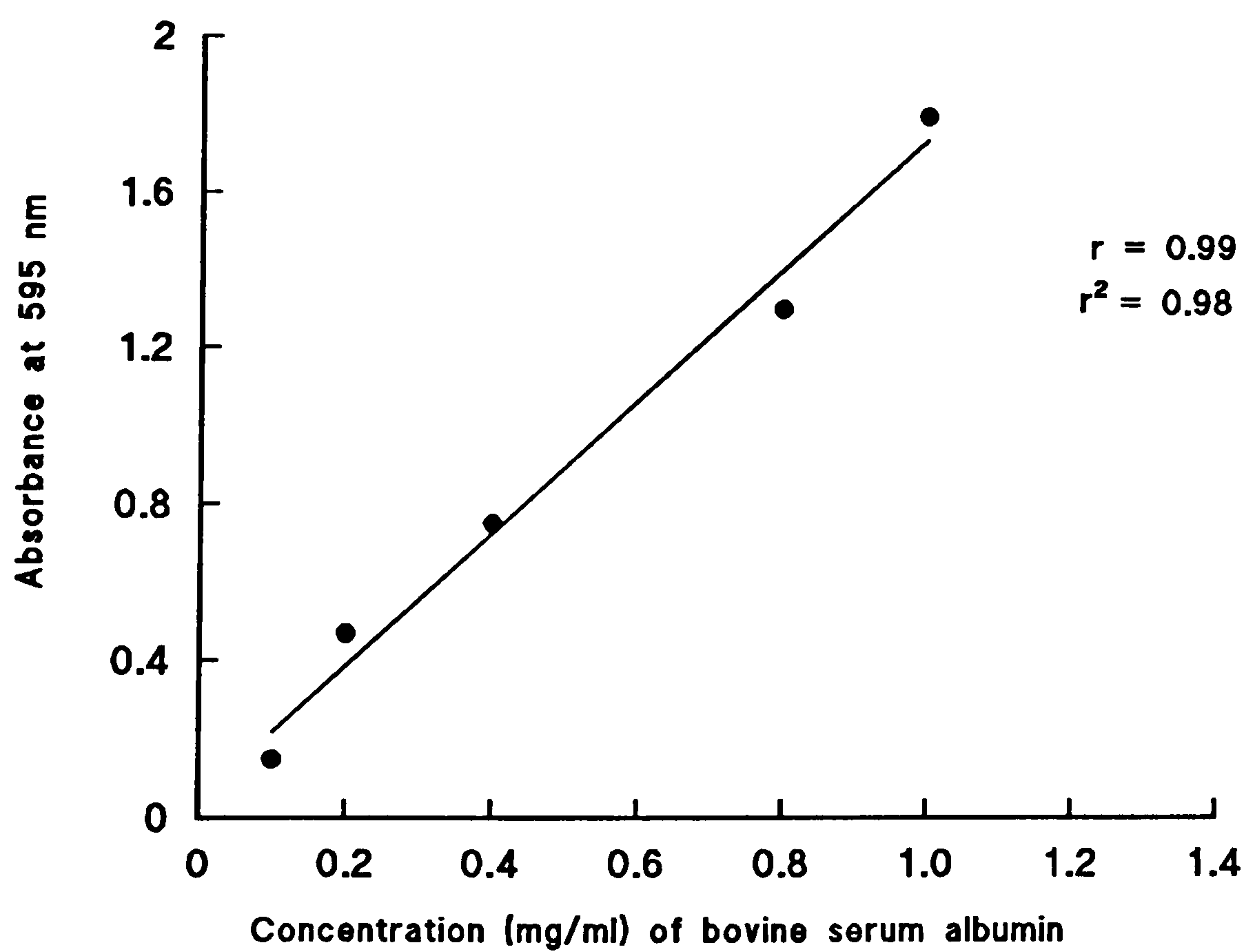


Figure 4.2. Standard curve for the determination of protein content.

Table 4.3. The total content of cytochrome P-450 of 6th instar larval midgut microsomes after larval feeding for 48 h on food plants

Food plants	P-450 content	
	nmol/mg protein	Body weight (mg)*
	Mean \pm SE	Mean \pm SE
Artificial diet	0.053 \pm 0.003 a	455.3 \pm 15.1
Maize	0.060 \pm 0.004 a	363.8 \pm 10.2
Sorghum	0.065 \pm 0.003 a	358.1 \pm 12.7
Cowpea	0.090 \pm 0.004 b	529.0 \pm 20.5
Broad bean	0.115 \pm 0.003 c	496.4 \pm 28.5
Cabbage	0.122 \pm 0.003 c	522.6 \pm 21.0
Soybean	0.145 \pm 0.005 d	507.2 \pm 25.3

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

*** weight when microsomes were prepared**

The variation in P-450 content in Table 4.3 shows that food plants influence the total content of cytochrome P-450 in larve. The amounts of P-450 obtained in this study are very low compared to the amounts reported in literature (Agosin, 1985; Yu, 1983). There are two possible reasons. The P-450 level in the last larval instar is lower than in the earlier instars because of the onset of pupation (A. McCaffery, per. communication).

Table 4.4 The total content of cytochrome P-450 of larval midgut microsomes when larvae were reared from the first to the sixth instar on the food plants.

Food plants	P-450 content	
	nmol/mg protein	
	Mean ± SE	Body weight *
		Mean ± SE
Artificial diet	0.083 ± 0.003 b	343.43 ± 36.42
Sorghum	0.043 ± 0.003 a	180.82 ± 25.45
Maize	0.060 ± 0.016 a	271.65 ± 43.90
Broad bean	0.063 ± 0.003 a	303.39 ± 59.18
Cowpea	0.087 ± 0.003 b	321.02 ± 38.83
Soybean	0.097 ± 0.009 b	408.21 ± 59.65
Cabbage	0.143 ± 0.006 c	414.68 ± 72.06

Means in a column followed by the same letter are not significantly different at the 5% probability level (SNK).

* weight when microsomes were prepared.

Use of the penultimate larval instar might have yielded better results. The second reason could be that proteolytic enzymes may have acted on the midgut tissue prior to homogenisation and affected the content of P-450. It is possible also that the experimental techniques used in the extraction of the enzyme may have affected the content of P-450. In spite of these shortcomings, it is still apparent that the food plants influenced the P-450 content of the larvae.

The specific P-450 contents in larval midgut

microsomes were lower in all cases when larvae were reared from the 1st instar to the beginning of the 6th instar on the food plants than when the larvae were initially reared on the artificial diet up to the 6th instar and then fed on the food plants for 48 h. There could be three possible reasons.

Firstly, the differences in the P-450 content may have been due to variation in the body weights of the larvae on the plants. In mammals, for instance, increase in MFO activity is associated with an increase in liver weight (Goldberg cited by Wilkinson and Brattsten, 1973). If it could be assumed that the proportions of the various parts of the insect's body are related to the overall body weight, then the differences in body weight of the larvae at the time of extracting the enzyme could influence the content of P-450.

Secondly, one of the factors that could affect the body weight of the insect is the level of nutrients present in its food plants. The monocots used in the study had lower levels of total nitrogen and some of the essential free amino acids than the dicots (3.3.3 and 3.3.4). P-450 is a protein and therefore, its induction would represent an increase in protein synthesis (Wilkinson and Brattsten, 1973). If the protein levels in the larvae were low at all times because, the monocot plants on which they had fed on had lower levels of nitrogen and amino acids (both of which

could give an indication of the total protein concentration of the plants being ingested) then, the level of induction in the larvae feeding on the monocots would be lower than in the ones feeding on the dicots. This suggestion could be supported by the observation that DDT treatment of resistant adult houseflies resulted in an increase in the incorporation of ^{14}C -labelled amino acid into total protein and microsomal protein (Ishaaya and Chefurka cited by Wilkinson and Brattsten 1973). Gibson and Skett (1994) reported that feeding rats on a 5% protein (casein) diet resulted in a decrease in the activity of MFO due to a decrease in the microsomal protein (when compared to normal diet with a protein content of 20%).

Thirdly, one of the characteristics of P-450 is that the presence of high levels of the enzyme in the tissues is associated with periods of maximum foreign compound exposure (Hung *et al.* 1990). Therefore, the differences in the P-450 content between the two groups of insects could be because when reared on the food plants, the larvae become adapted to the chemical compounds present and therefore, allelochemicals present in the plants which would require detoxication and excretion could be detected in time for the metabolic responses to be activated (Terriere, 1984). When larvae were exposed to the toxins for a limited period of time (as was the case when the larvae were fed on the food plants for 48 h) a different response was necessary and that may have accounted for the

higher levels of P-450 in the larvae fed for 48 h on the food plants.

The larval development on maize, millet and sorghum, observed in Chapter 2 suggest that these plants are not natural food plants of *S. littoralis*. Therefore, when exposed to these plants, the larvae may not have the biochemical mechanism to detoxify some of the compounds present in the plants. If the hypothesis that MFO originally evolved to detoxify naturally occurring toxins in plants (Hodgson et al. 1993) is assumed, then it would have been expected that a polyphagous insect, such as *S. littoralis*, would have been able to develop normally on these plants. This is because polyphagous insects have high levels of MFOs (Yu, 1983).

The variation in the content of P-450 could also have been due to the amounts of food ingested. If the inducing factors were present in the food plants and the amounts of the inducers were related to the amounts of food ingested, then when the amounts of food ingested were low, as in maize and sorghum, the levels of the inducing factors in the larvae would be low. It has been suggested that the level of the inducing agent in lepidopterous larvae needs to be very high before any effect can be observed (Agosin, 1985). Yu (1983) reported that the amounts of food ingested by the various instars of *S. frugiperda* correlated with the level of inducibility of MFO in the larvae.

Variation of the total concentration of P-450 in larval midguts due to feeding on food plants has also been reported by other workers. Yu (1983) obtained total cytochrome P-450 contents of 0.25 (soybean), 0.42 (sweet potato), 0.52 (potato), 0.58 (corn) and 0.26 (artificial diet) nmol/mg protein in *S. frugiperda* when the larvae were fed on the plants for 48 h. Moldenke et al. (1992) reported lower levels of total cytochrome P-450 in larvae of the gypsy moth *Lymantria dispar* when they were fed on artificial diet than when they were fed on foliage of alder (*Alnus rhombifolia*) and douglas fir (*Pseudotsuga menziesii*). Lee and Boo (1993), obtained lower activity of P-450 on artificial diet than on food plants, in all cases where 4 insect species had been reared on both artificial diet and plants. P-450 has also been implicated in plant utilisation by at least three species of *Drosophila* that are endemic to the Sonoran Desert in Mexico (Danielson et al. 1994).

4.3.3 Gel electrophoretic profiles of microsomes

SDS-polyacrylamide electrophoresis is a useful technique for the characterisation of P-450 in mammalian and insect microsomes (Fuchs et al., 1993). There are, however, some limitations in the use of the method. Stanton et al. (1978) list one of the limitations as the non-resolution of proteins with similar molecular weights. In this study, electrophoresis was used to establish whether variation in the total P-450 concentration was associated

with changes in the protein profiles of microsomal preparations from larval midguts of larvae fed on the food plants.

Even though the manufacturer's (Sigma Co.) label had indicated that there were six proteins in the molecular weight standard, gel electrophoresis yielded more than 6 peaks, indicating the presence of more than 6 proteins. The peaks (Fig 4.3) that correspond to the standard proteins are 3 (myosin, 205 kDa), 4 (β -galactosidase, 116 kDa), 5 (phosphorylase B, 97.4 kDa), 6 (albumin bovine plasma, 66 kDa), 8 (egg albumin, 45 kDa) and 10 (carbonic anhydrase, 29 kDa).

There were some difficulties in the interpretation of the profiles. The software for the Chromoscan densitometer that was used for scanning the gels does not give the areas of the individual bands that were identified as peaks. It is unable to label all the peaks in a scan unless maths processed. As a result of the maths processing, some of the peaks were combined. In spite of these shortcomings, the scans did show differences in the peptide profiles of the microsomes. Representative gels are shown in Appendix 3.

There was some variation in the exact number and positions of peptide bands (as detected by the software) in each microsomal profile, when microsomes from similarly treated larvae, but from different batches, were used in

repeat experiments. The larvae that were used were randomly selected. They may not have been of exactly the same age, and The proportions of male to female in each batch of larvae were not determined. Since the P-450 content of microsomes would be affected by age and sex of larvae (Agosin, 1985), these factors may have contributed to the variation in the peptide profiles of similarly treated larvae from different batches.

Despite the variation in repeat experiments the overall peptide profile of the microsomal preparations from larvae subjected to each treatment did show some consistency. It is unlikely that all the peptide bands are P-450 species (the number of P-450 proteins in any insect is unknown). However, the scans do show that the variations in the total content of P-450 that occur when the larvae are reared on the plants or treated with chemicals are associated with changes in the number and intensity of the peptides present in the microsomes. It is probable that at least part of the variation in the peptide profile is due to the effect of the food plants and chemical treatments on the P-450 content of the microsomes.

Treatment of the larvae with β -naphthoflavone, barbitol or PBO changed the intensities of some of the peptide bands as compared to microsomes from the control (larvae reared on the artificial diet and not treated with any chemical) (Fig. 4.3). Variations in the peptide

profiles also occurred in larvae reared on the food plants (Fig. 4.4) and on larvae initially reared on the artificial diet and then fed on the food plants for 48 h (Fig. 4.5). The larvae fed continuously on the dicot plants (Fig. 4.4) a great deal more change than the larvae reared on the monocots or fed on the plants for only 48 h.

The electrophoretic pattern of β -naphthoflavone treated larvae showed an increase in peptides with molecular weights of about 27-50 kDa compared to the artificial diet alone. Barbital treated larvae showed an increase in peptides of molecular weights of 29 kDa or less. Treatment with piperonyl butoxide resulted in a general decrease of intensities of all the peptides. Compared to the profile of the control, the reduction in intensities of individual bands in PBO treated larvae appear to be associated with an increase in number of peptide bands. It is possible that the total concentration of P-450 may not have been affected by PBO.

Application of barbital and β -naphthoflavone generally resulted in an increase of peptides of lower molecular weights. The number and intensity of peptide bands were greater in the β -naphthoflavone treated larvae than in the barbital treated larvae. The differences in the intensity of the peptide bands may have been because not all the peptide bands react in the same way to the various inducers.

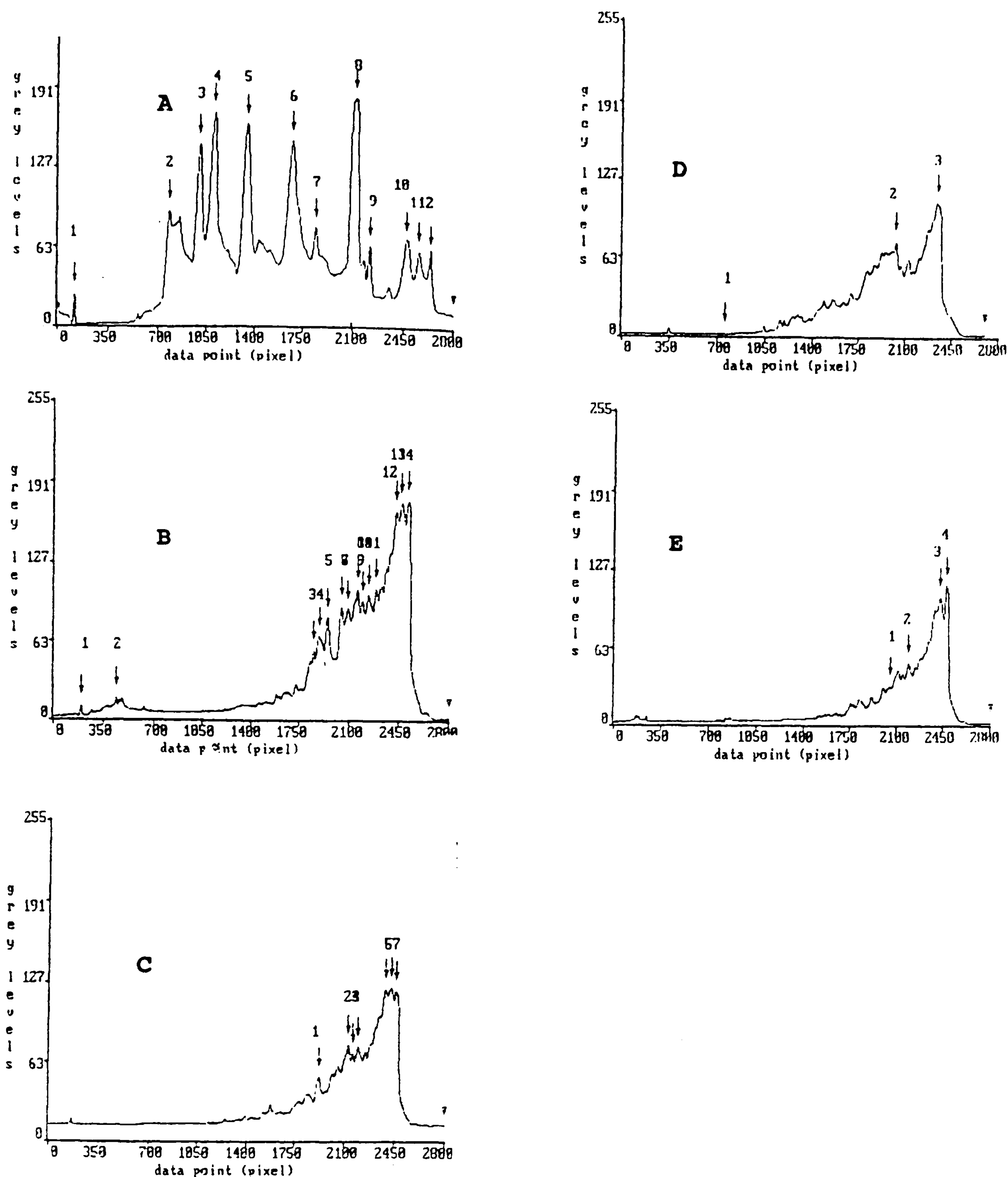


Figure 4.3. Electrophoretic profiles of standard molecular weight markers (A) microsomes from larvae treated with β -naphthoflavone (B) barbital (C) untreated (D) PBO (E). Data point (pixel) refers to time of scan along a gel track.

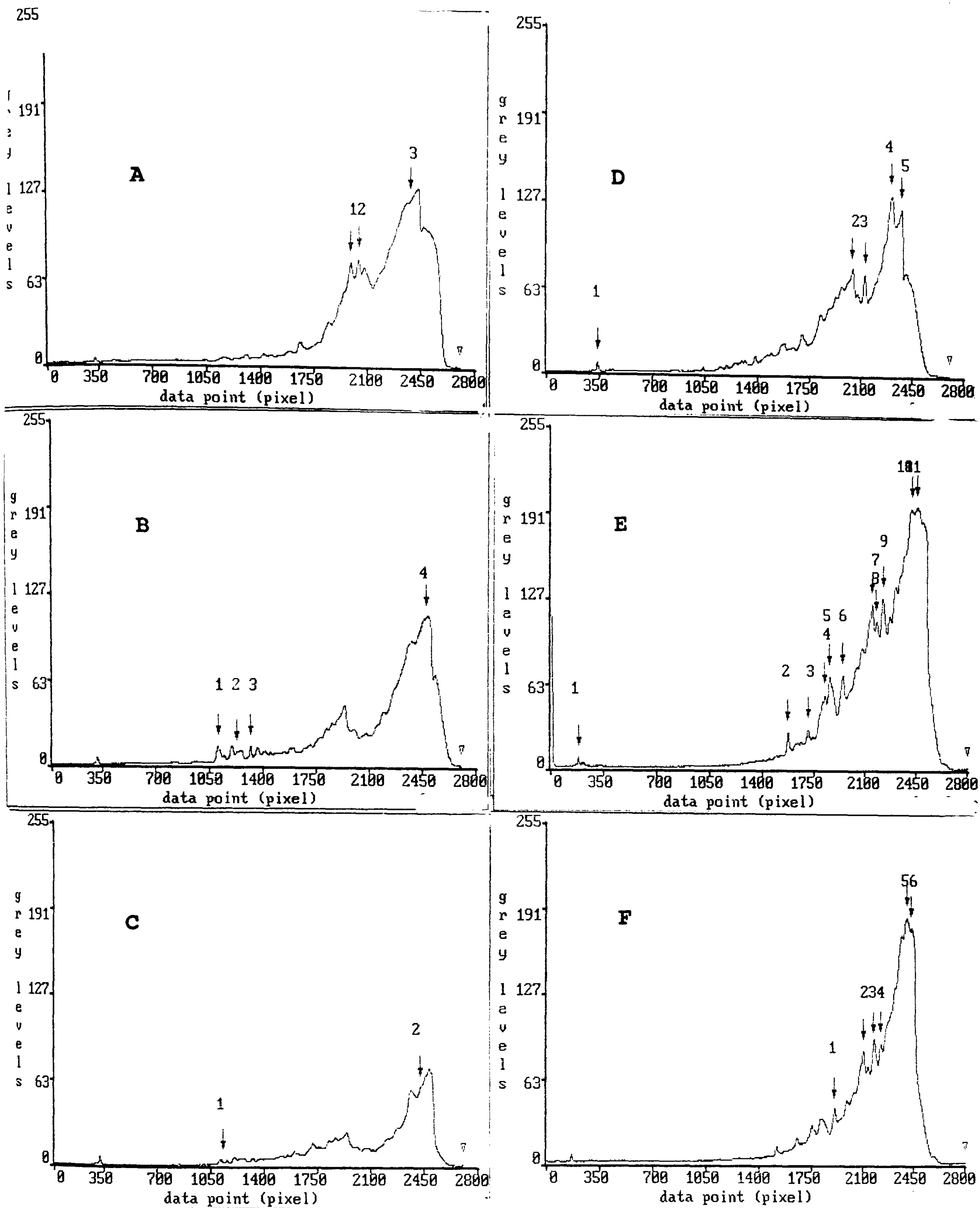


Figure 4.4. Electrophoretic profile of microsomes from larvae reared on cowpea (A) maize (B) sorghum (C) cabbage (D) soy bean (E) and broad bean (F). Data point (Pixel) refers to the time along a gel track.

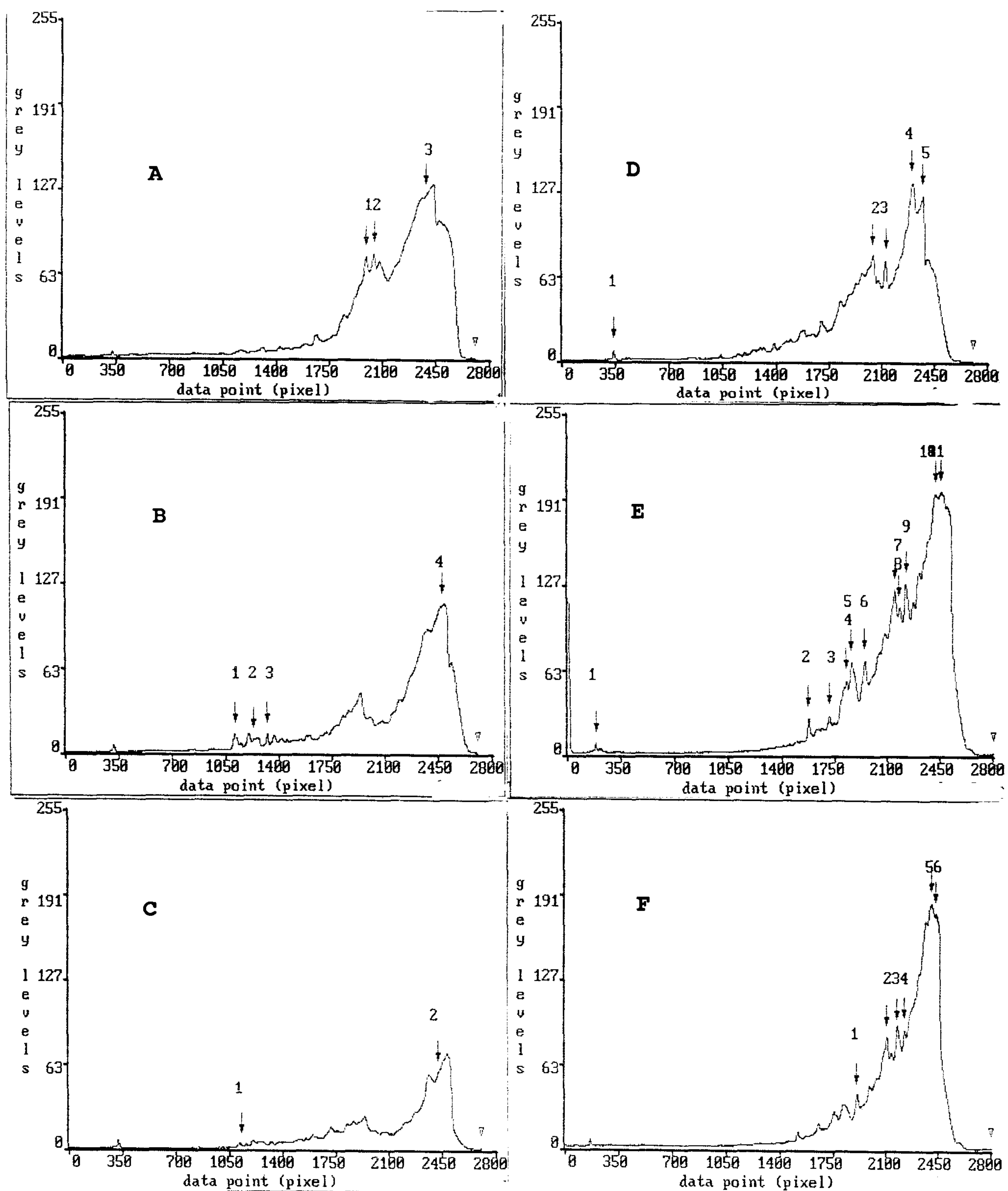


Figure 4.4. Electrophoretic profile of microsomes from larvae reared on cowpea (A) maize (B) sorghum (C) cabbage (D) soy bean (E) and broad bean (F). Data point (Pixel) refers to the time along a gel track.

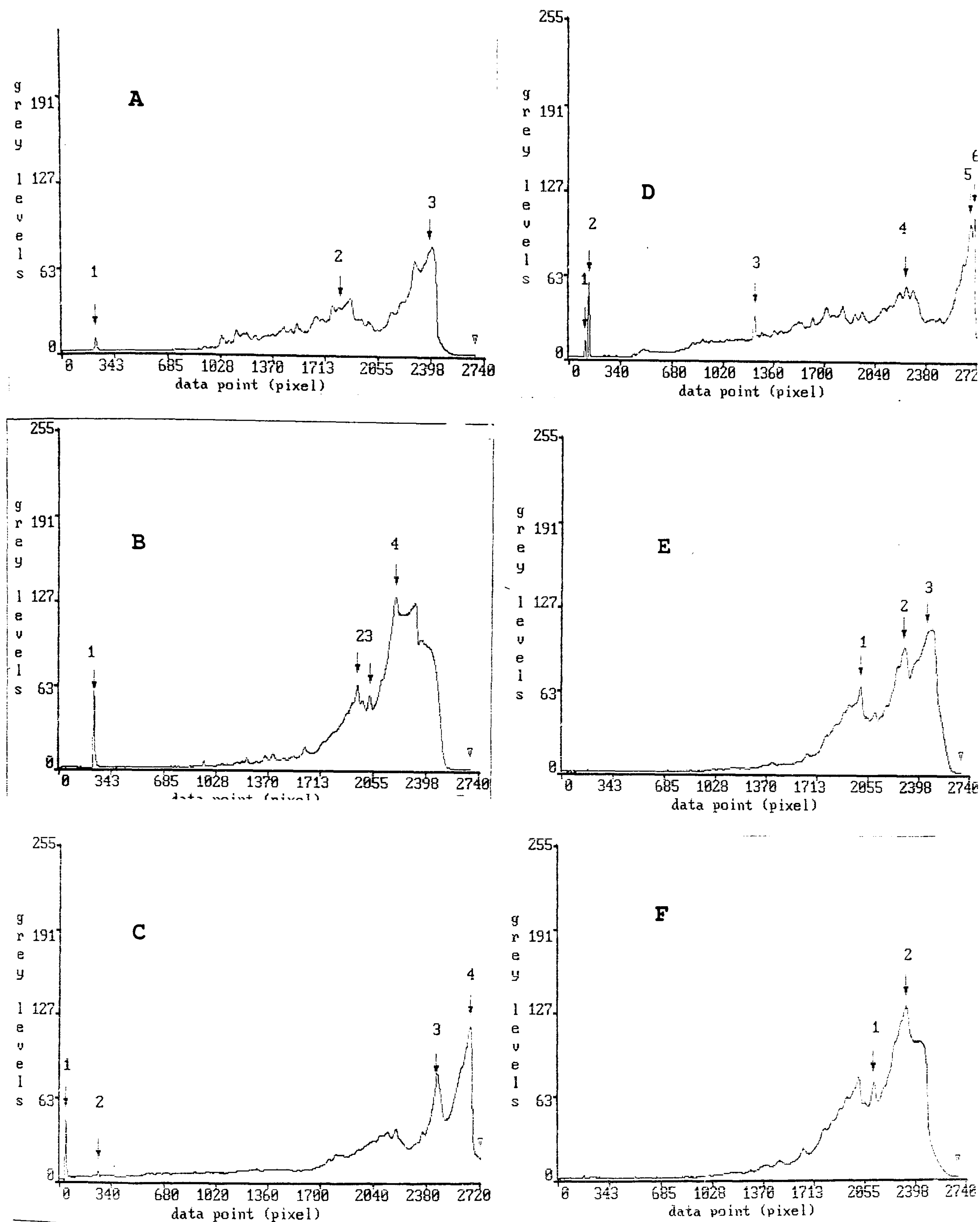


Figure 4.5. Electrophoretic profiles of microsomes from larvae reared on artificial diet and fed for 48 h on cabbage (A) soy bean (B) cowpea (C) broad bean (D) sorghum (E) and maize (F). Data point (pixel) refers to the time along a gel track.

4.3.4 Effect of β -naphthoflavone, barbital and piperonyl butoxide on larval feeding.

When the larvae were pretreated with barbital, piperonyl butoxide or β -naphthoflavone, there were variations in the concentrations of P-450 in the larval mid- guts (Table 4.5). Stanton et al. (1978) also observed variations in P-450 in the housefly when it was treated with various inducers. The large variation in the results would mean that the observed changes in the concentrations of P-450 may not be due entirely to the chemicals that were applied.

Table 4.5. Cytochrome P-450 content in microsomes prepared from larvae pretreated with piperonyl butoxide, barbital β -naphthoflavone and fed on artificial diet.

Treatment	P-450 content (nmol/mg protein)
	Mean \pm SE
Control	0.073 \pm 0.002 b
PBO	0.051 \pm 0.001 a
Barbital	0.082 \pm 0.004 bc
β -naphthoflavone	0.095 \pm 0.07 c

Means followed by the same letter are not significantly different at the 5% probability level (SNK).

Fig. 4.6 shows the relative increases (difference in weight between treated and control larvae on each food plant) in the weights of foliage ingested. Fig. 4.7 shows the variations in the fresh weights (difference between the weights of the control larvae and the treated larvae on each food plant) of the larvae treated with β -naphthoflavone and feeding on the food plants for 48 h.

Fig. 4.8 shows the variations in weight of foliage ingested when the 6th instar larvae were treated with barbital and Fig. 4.9 shows the variations in the larval fresh weight.

Fig 4.10 shows the variations in weight of foliage ingested when the 6th instar larvae were treated with piperonyl butoxide and Fig. 4.11 shows the variations in larval fresh weight.

Since β -naphthoflavone and barbital induce and PBO inhibits MFO in insects (Agosin, 1985; Feyereisen, 1993), it could be inferred that the variation in larval food consumption on treatment with the compounds was associated with the variations in the P-450 levels.

The higher levels of P-450 in the barbital and β -naphthoflavone treated larvae are likely to enable the larvae to detoxify toxins present in the plants. If this is the case, then the relative increase in feeding should

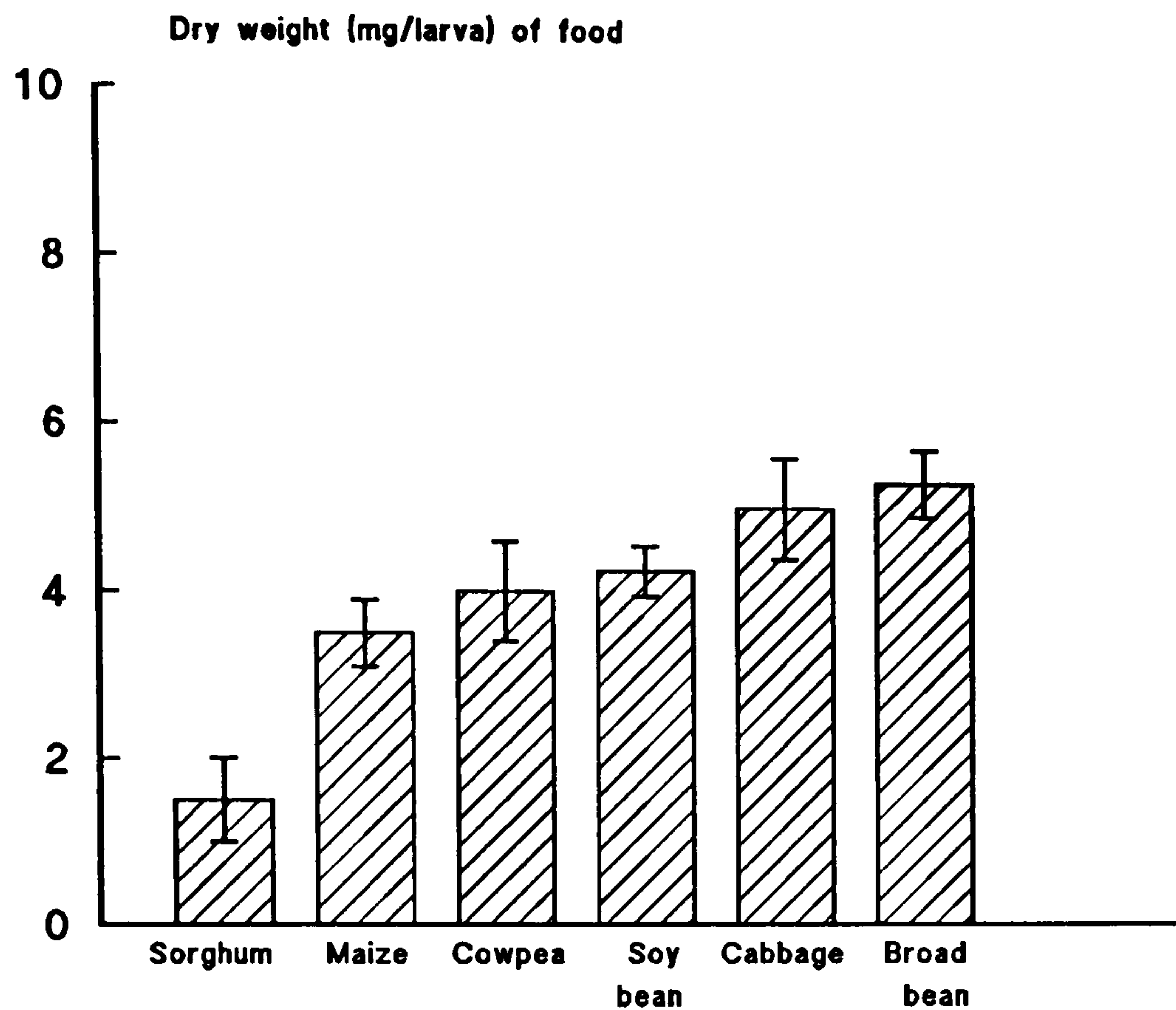


Figure 4.6. Increase in weight (mg) of foliage ingested by 6th instar larvae treated with β - Naphthoflavone. Bars represent Standard Errors.

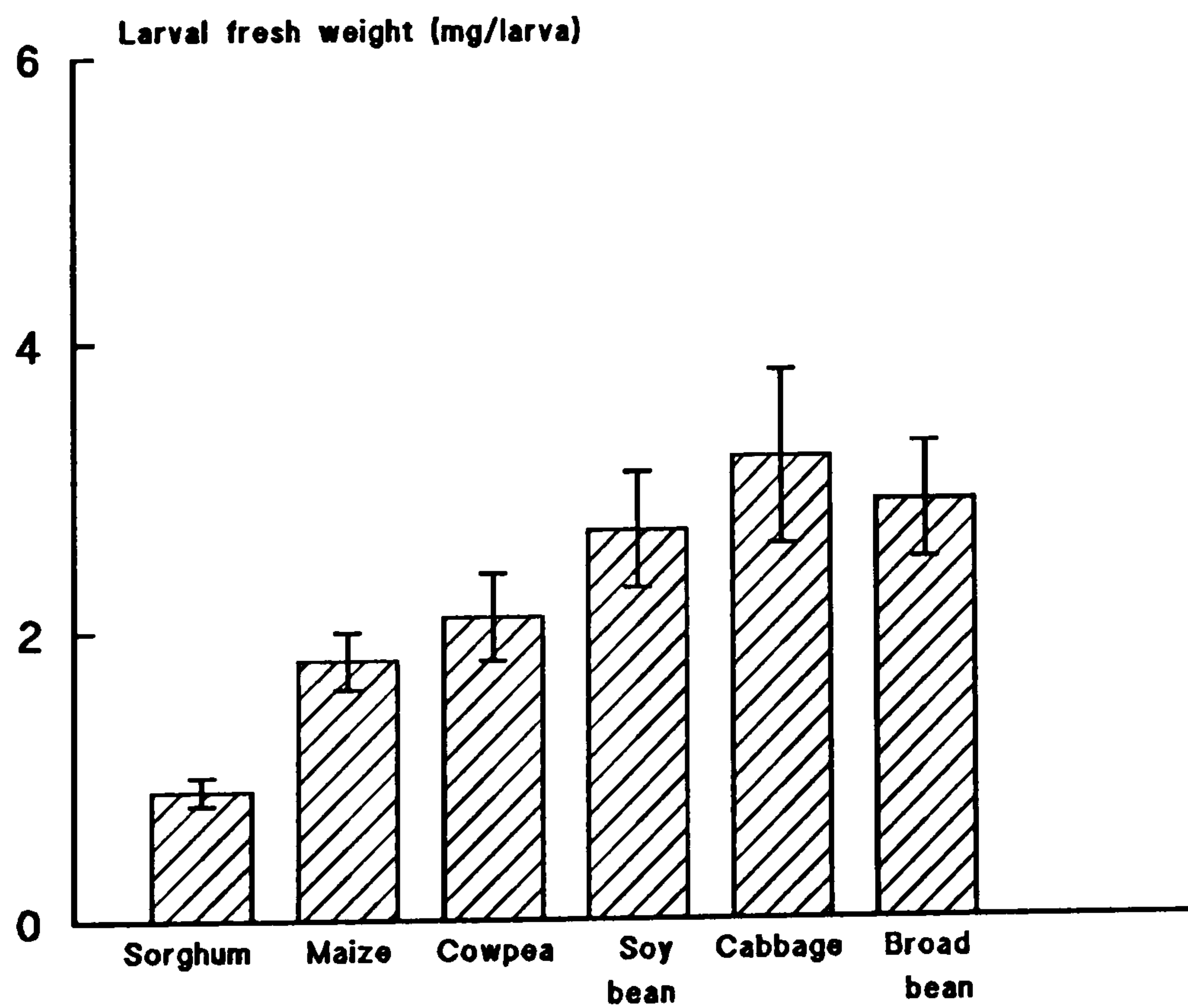


Figure 4.7. Increase in fresh weight (mg) of 6th instar larvae treated with β - Naphthoflavone. Bars represent Standard Errors.

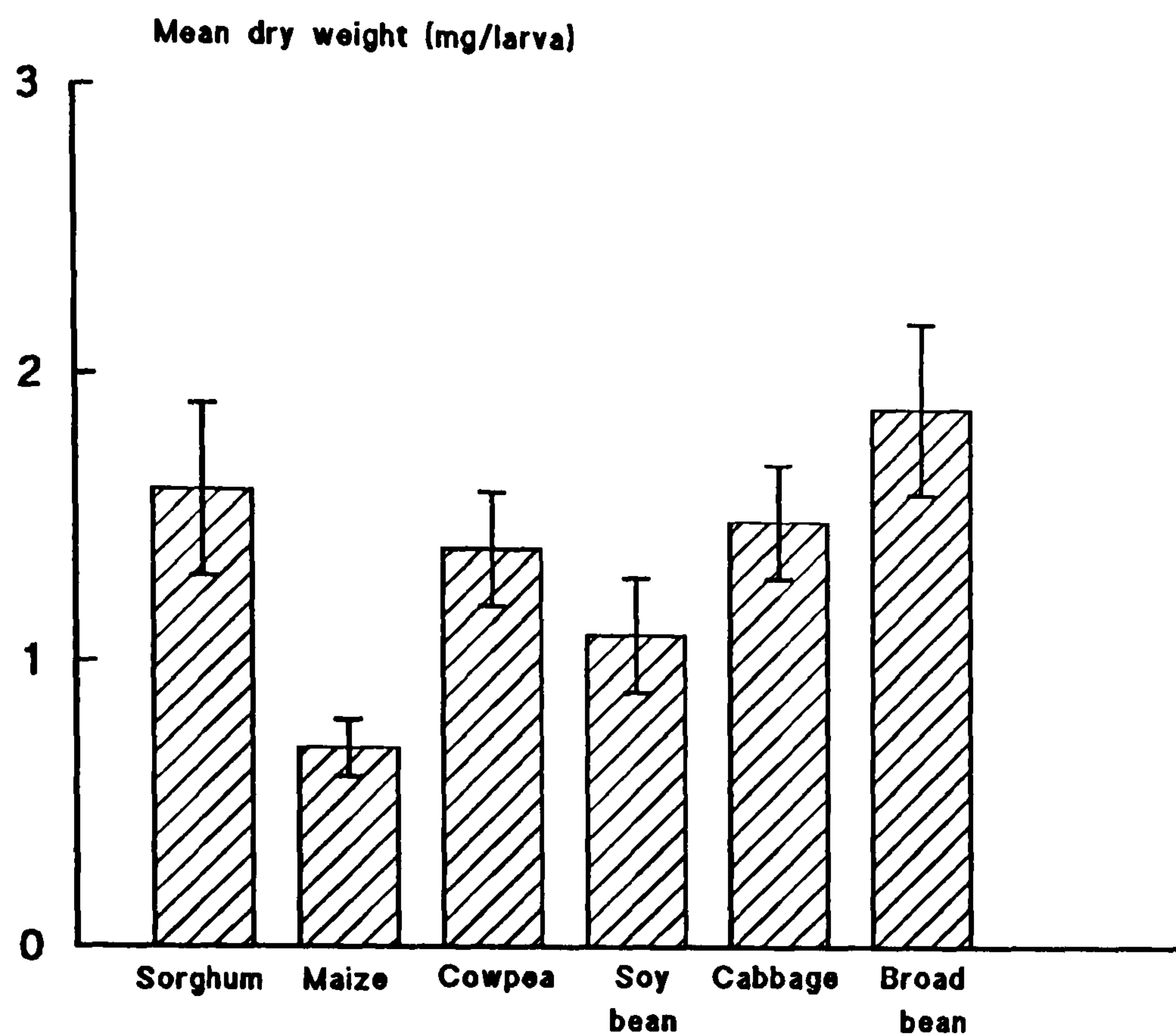


Figure 4.8. Increase in weight (mg) of foliage ingested by 6th instar larvae treated with barbitol. Bars represent Standard Errors.

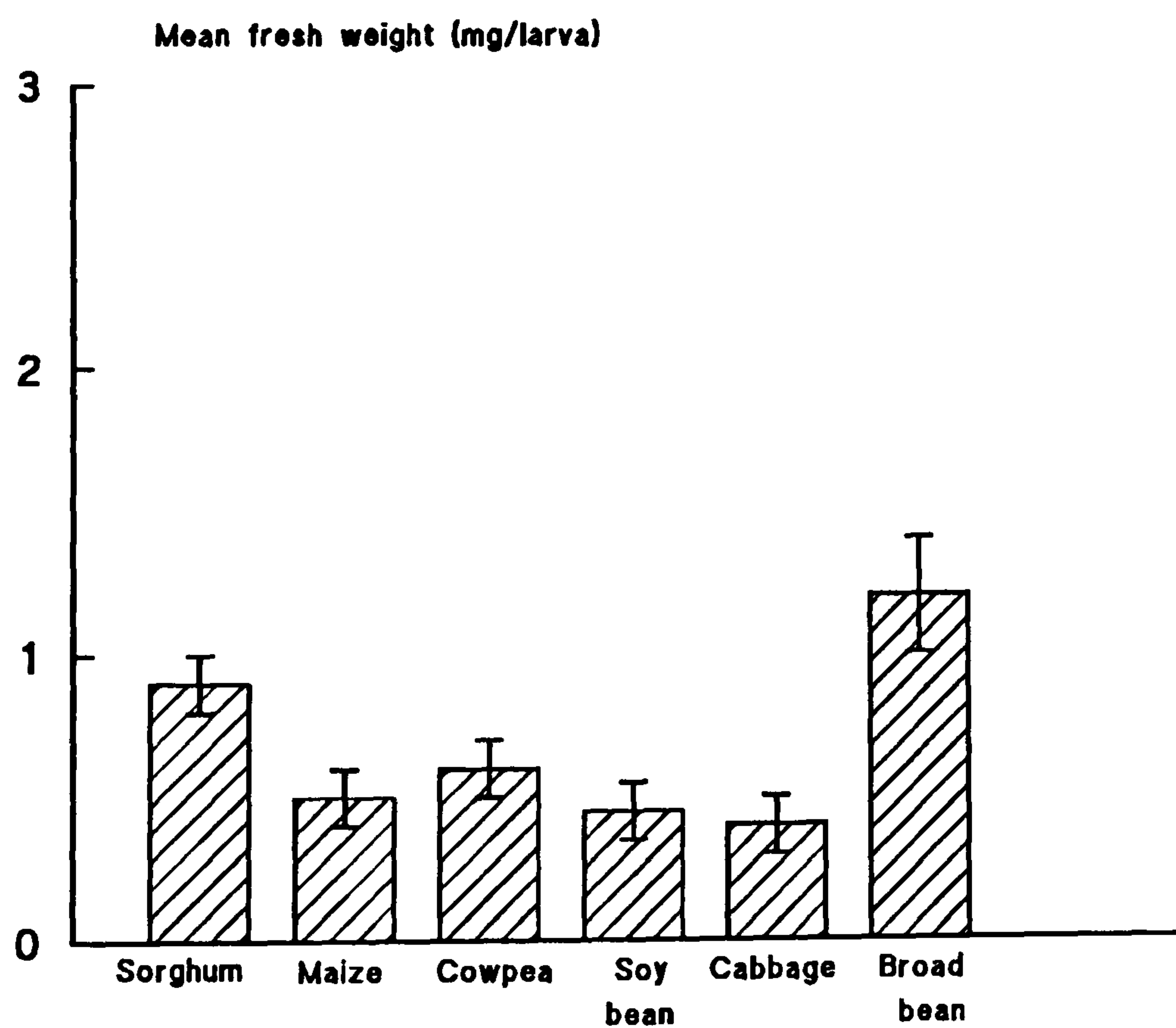


Figure 4.9. Increase in fresh weight (mg) of 6th instar larvae treated with barbitol. Bars represent Standard Errors.

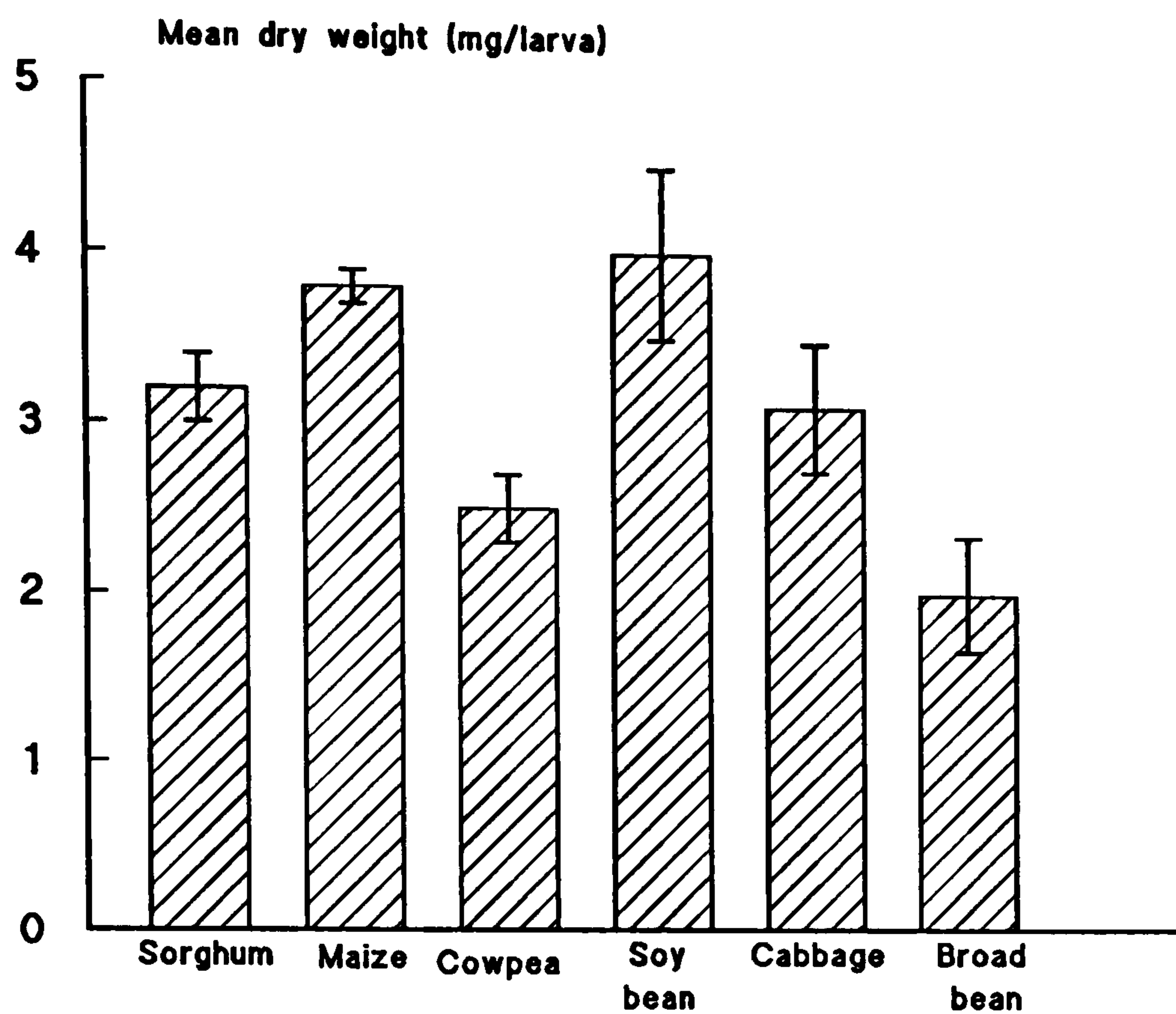


Figure 4.10. Reduction in weight (mg) of foliage ingested by larvae treated with piperonyl butoxide. Bars represent Standard Errors.

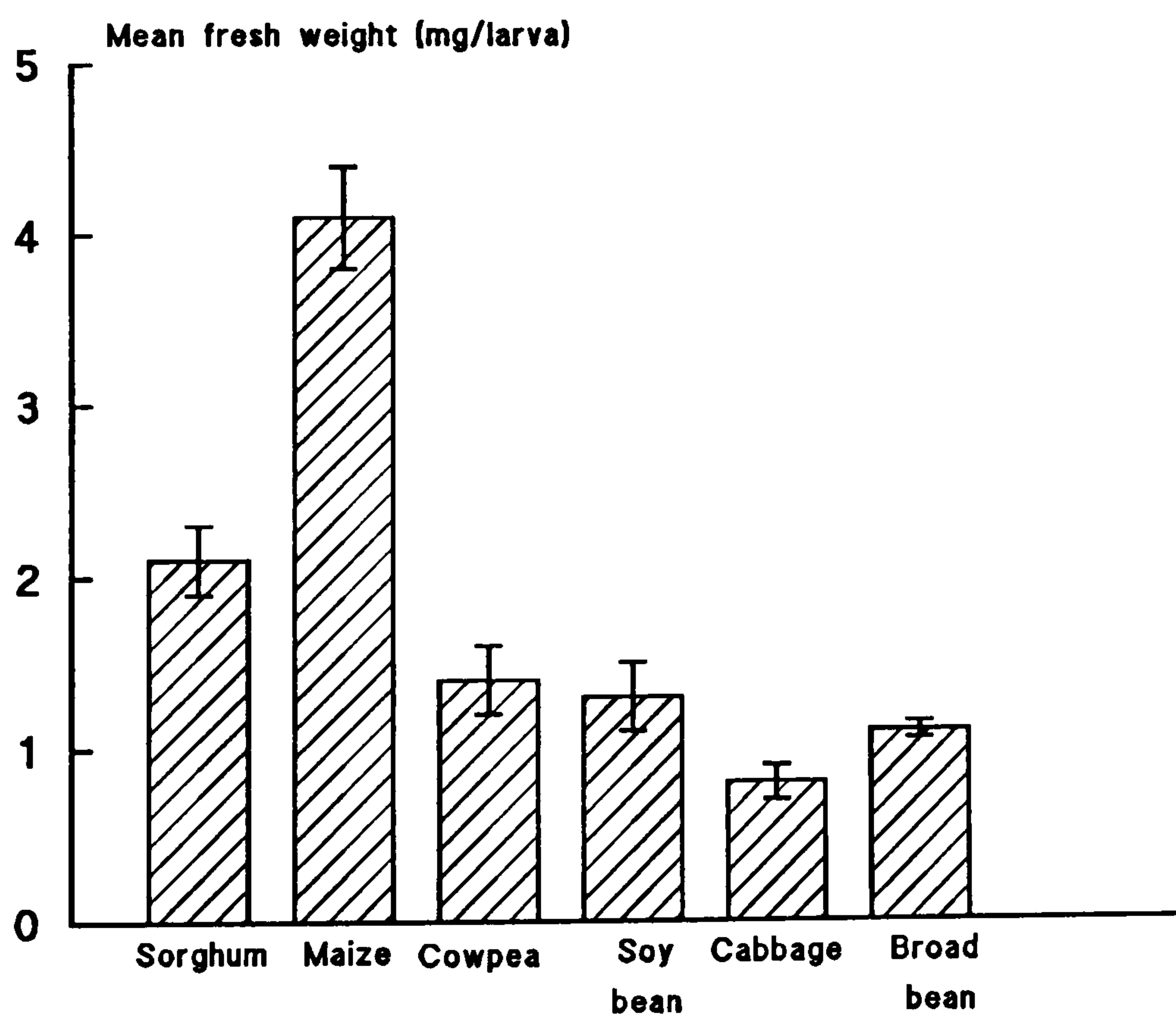


Figure 4.11. Reduction in fresh weight (mg) in larvae treated with piperonyl butoxide. Bars represent Standard Errors.

have been greater on the maize and sorghum on the assumption that the low food intake on these plants was due to the presence of toxins (not tested for in this study) which either inhibited larval food intake or digestion of food by the larvae. Since there was not a greater increase in food consumption of these plants, the variations in the amounts of feed ingested could not be due solely to the effects of the compounds on the concentrations of P-450 in the larval midgut.

Since cytochrome P-450 is involved in the metabolism of other materials such as fatty acids and vitamin D₃ and may be involved in other metabolic activities not yet clearly understood (Gibson and Skett, 1994), it is possible that the variations in food intake may also have been due to the isozymes of P-450 (induced or inhibited by PBO, barbital and β -naphthoflavone) also affecting other metabolic activities related to food intake and digestion.

PBO is an inhibitor of MFO and is routinely applied with insecticides to overcome insecticide resistant *Heliothis armigera* (Forrester et al. 1993) because it inhibits the activity of P-450. When fed orally to *S. frugiperda*, PBO caused a decrease of 38%-74% in the activity of P-450 (Yu and Hsu, 1993).

The implication of the observation in this study on the effects of PBO on larval feeding could be that, in

addition to suppressing the detoxication mechanism of the insect, application of PBO with an insecticide, to overcome insecticide resistance, may also reduce larval food intake.

Yu (1986) showed that in *S. frugiperda* and *Anticarsia gemmatalis*, plant allelochemicals with diverse chemical structures, including terpenoids, alkaloids, glucosinolates, flavonoids and indoles, were metabolised by MFO. It has also been shown that botanical insecticides such as rotenone, nicotine and pyrethrins are metabolised by MFOs (Hodgson and Dauterman, 1980). These observations strongly indicate that MFOs play a role in adaptation to food plants by insects. It would be expected, therefore, that inhibitors or inducers of the MFO would affect larval feeding on plants.

4.3.5 Effect of food plants on larval susceptibility to topically applied insecticides

The results of the study show that food plants influenced the responses of *S. littoralis* to two pyrethroid and an organophosphate insecticides. The larvae were more susceptible to the insecticides when reared on the food plants than when reared on the artificial diet and fed on the food plants for 24 h. On all the food plants tested, larvae were most susceptible to the cypermethrin and least to malathion.

When larvae were fed on the food plants for 24 h, there were variations in the amounts of leaves ingested (Fig. 4.12) and the amounts of faecal pellets (Fig. 4.13).

Whether larvae were fed on the food plants for 24 h or reared on them from the first to the fourth instar, their susceptibilities to cypermethrin, permethrin and malathion showed some variation (Tables 4.6 to 4.11). Although the 90% confidence intervals for the LD50 were wide, consistent trends were observable. Larvae were always more susceptible when fed on sorghum, with maize next and least susceptible when fed on broad bean or cabbage. Soybean and cowpea gave intermediate sensitivities usually similar to the larvae fed on the artificial diet. Insects reared on the artificial diet had higher susceptibility to malathion than those reared on any of the food plants for 24h but the differences among treatments were smaller than in cypermethrin.

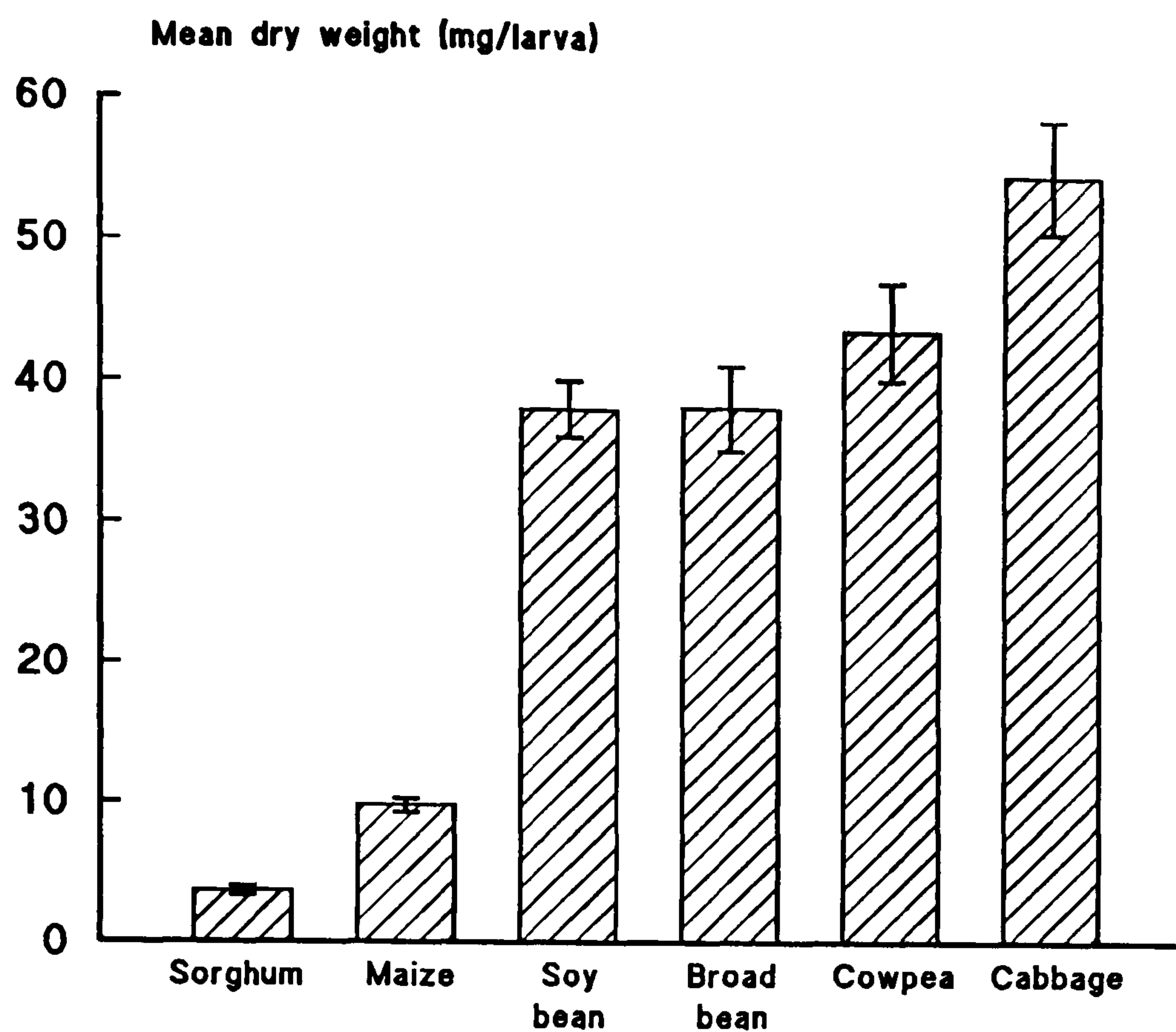


Figure 4.12. Mean dry weight (mg) of foliage ingested by larvae feeding on food plants for 24 h. Bars represent Standard Errors.

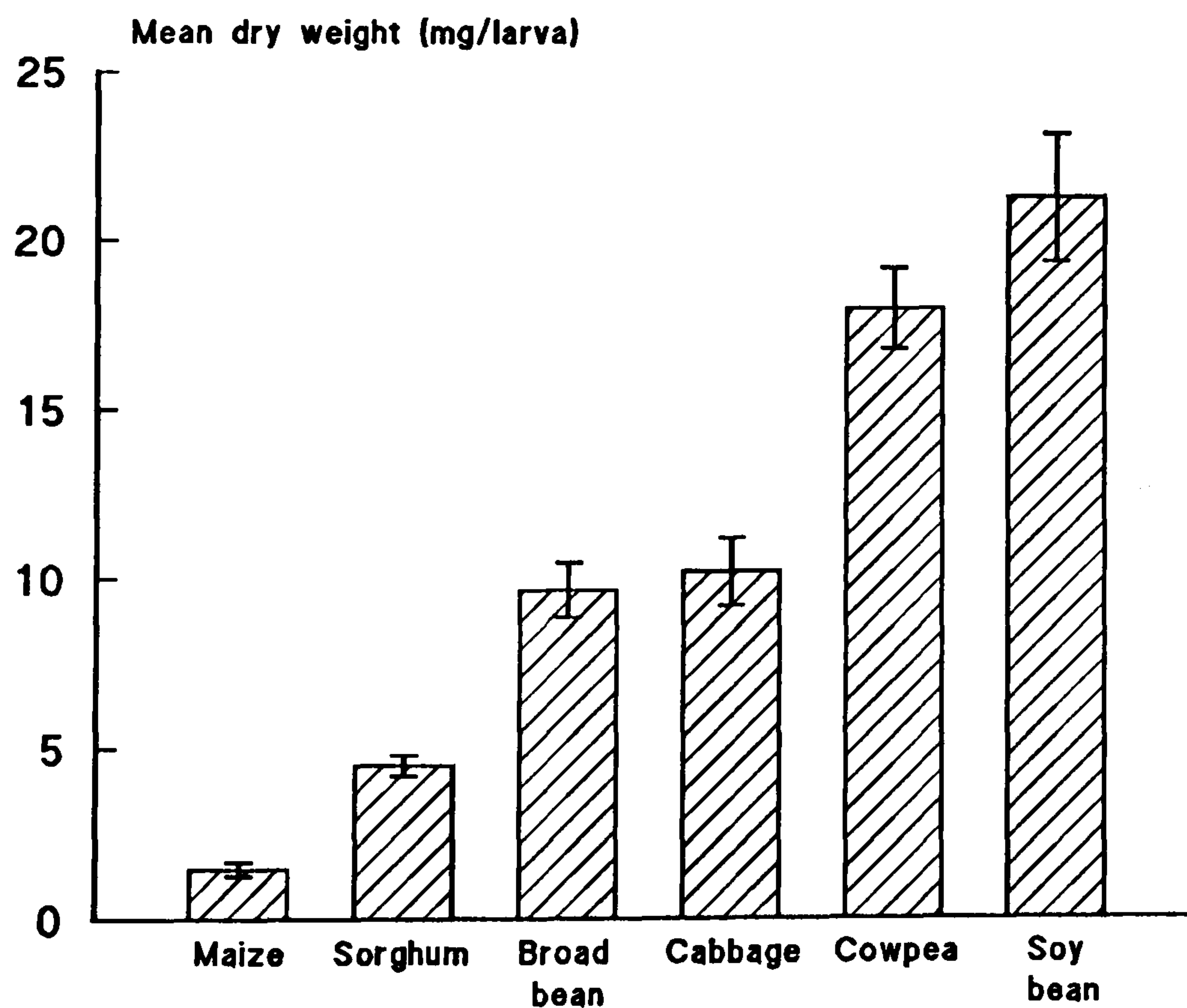


Figure 4.13. Mean dry weight (mg) of faecal pellets of larvae feeding on food plants for 24 h. Bars represent Standard Errors.

Table 4.6. Response of 4th instar larvae fed for 24 h on food plants to topical application of cypermethrin

Food plant	LD ₅₀ µg/larva	95% Confidence Limits
Diet	0.07	0.05 - 0.10
Sorghum	0.04	0.03 - 0.07
Maize	0.06	0.04 - 0.08
Soybean	0.08	0.06 - 0.12
Cowpea	0.09	0.06 - 0.13
Broad bean	0.10	0.07 - 0.14
Cabbage	0.13	0.09 - 0.19

Table 4.7 Response of 4th instar larvae reared on food plants to topical application of cypermethrin

Food plants	LD ₅₀ µg/larva	95% Confidence limits
Sorghum	0.01	0.01 - 0.02
Maize	0.03	0.02 - 0.04
Soybean	0.04	0.03 - 0.07
Cowpea	0.05	0.03 - 0.07
Diet	0.05	0.03 - 0.08
Broad bean	0.05	0.04 - 0.09
Cabbage	0.08	0.05 - 0.12

Table 4.8 Response of 4th instar larvae fed on food plants for 24 h to topical application of permethrin.

Food plants	LD50 µg/larva	95% Confidence limit
Diet	0.08	0.05 - 0.12
Sorghum	0.05	0.04 - 0.08
Maize	0.07	0.05 - 0.09
Soybean	0.11	0.08 - 0.15
Cowpea	0.10	0.07 - 0.15
Broad bean	0.16	0.12 - 0.22
Cabbage	0.18	0.12 - 0.28

Table 4.9. Response of 4th instar larvae reared on food plants to topical application of permethrin

Food plants	LD50 µg/larva	95% Confidence limits
Sorghum	0.02	0.01 - 0.03
Maize	0.04	0.03 - 0.06
Cowpea	0.09	0.06 - 0.12
Diet	0.08	0.06 - 0.11
Soybean	0.09	0.07 - 0.12
Broad bean	0.12	0.09 - 0.19
Cabbage	0.16	0.10 - 0.25

Table 4.10. Response of 4th instar larvae fed on food plants for 24 h to topical application of malathion.

Food plants	LD50 µg/larva	95% Confidence limits
Diet	3.49	2.42 - 4.25
Sorghum	3.96	2.77 - 5.67
Maize	4.24	3.24 - 5.25
Soybean	4.29	3.10 - 5.94
Cowpea	4.32	2.78 - 6.70
Cabbage	4.59	3.07 - 6.86
Broad bean	5.26	3.51 - 7.88

Table 4.11 Response of 4th instar larvae reared on food plants to topical application of malathion.

Food plant	48h LD50 µg/larva	95% confidence Limits
Sorghum	0.31	0.21 - 0.47
Maize	0.59	0.34 - 1.02
Soybean	0.95	0.55 - 1.36
Cowpea	1.12	0.75 - 1.66
Diet	1.23	0.09 - 1.88
Cabbage	1.96	1.29 - 3.01
Broad bean	3.06	2.48 - 4.19

Permethrin and cypermethrin are detoxified mainly by MFO and the susceptibility of the larvae to the two compounds appears to be related to the contents of P-450. The variation in toxicity between permethrin and cypermethrin may be due to differential metabolism of these insecticides by MFO in response to allelochemicals present in the plants (Rose *et al.*, 1992). The monocot fed larvae with low P-450 content had lower LD50 than the larvae fed on the dicot plants in both groups of insects. It is possible that there were inhibitors of P-450 in the monocots and this would have inhibited the activity of P-450 in the larvae that fed on them.

The differences in susceptibility could also be due to a number of other factors including the physiological state of the insect, which in turn would depend on the food plant fed on (Rose *et al.*, 1992). For instance, insect resistant cultivars could affect the toxicity of an insecticide to a pest because of low body weight and stresses in the insect due to the intake of low amounts of food on the resistant plant (van Emden, 1991).

It follows, therefore, that food plants such as maize and sorghum (which reduced larval feeding, retarded growth and development), could have similar effects to resistant crop plants on the larvae feeding on them. In addition, there is the possibility that some naturally occurring synergists may be present in the monocots and these may

have synergised the activities of the detoxication enzymes.

However, whether nutritional or allelochemical factors predominate in the effects of the food plants on the response of *S. littoralis* to insecticides would be difficult to say. Acting in combination, both factors resulted variations in larval weights susceptibility to insecticides.

Secondary plant substances are prevalent in food plants and these can induce or inhibit detoxifying enzymes in larvae feeding on them (Agosin, 1985). The enzymes involved in the degradation of allelochemicals may also be involved in the metabolism of insecticides (Brattsten, 1986). Hung et al. (1990) suggest that considered together, these observations provide an explanation for the differential susceptibility to insecticides of larvae fed on various plants.

Other mechanisms, such as reduced penetration of insecticides caused by diet related changes in cuticular lipids, may also have influenced the larval toxicities of the insecticides. No studies were carried out to test whether the transfer of the insecticides across the cuticle was different in the two groups of insects.

Since the insecticides used in the study could also be detoxified by other enzyme systems that are also induced

by food plants, the variation in the toxicities observed in the study may not wholly be due to the effects of high levels of cytochrome P-450.

4.3.6 Effect of PBO and β -naphthoflavone on larval susceptibility to topically applied insecticides

Studies with various synergists and inducers have shown that their effects on the insect vary (Bagwell and Plapp, 1992). The extent of induction is a function of, among other things, the type and concentration of the inducer used, time of exposure, diet and other unknown factors (Agosin, 1985)

The variations in the susceptibilities of fourth instar larvae when treated with either PBO or β -naphthoflavone prior to the application of the insecticides (Table 4.12) were not significant. The insecticides as well as PBO and β -naphthoflavone were topically applied and it is possible that the penetration of the insecticides could have been affected by the PBO, barbital and β -naphthoflavone.

Table 4.12. Response of 4th instar larvae reared on semi-artificial diet when pretreated with PBO or β -naphthoflavone prior to application of insecticides

Insecticide	LD50 µg/larva	95% confidence limits
Control		
Cypermethrin	0.07	0.03-0.09
Permethrin	0.09	0.06-0.12
Malathion	3.49	2.42-4.25
PBO		
	Ratio 1:1 (Insecticide:PBO)	
Cypermethrin	0.06	0.05-0.09
Permethrin	0.08	0.05-0.11
Malathion	2.44	1.15-4.16
	Ratio 1:5 (Insecticide:PBO)	
Cypermethrin	0.03	0.02-0.04
Permethrin	0.05	0.03-0.06
Malathion	2.22	1.51-3.27
β -naphthoflavone		
Cypermethrin	0.09	0.06-0.12
Permethrin	0.11	0.07-0.18
Malathion	3.71	2.55-5.41

The effects of the compounds on the rate of penetration were not tested. It is possible that PBO may have increased the rate of penetration of the insecticides while β -naphthoflavone would have slowed it down.

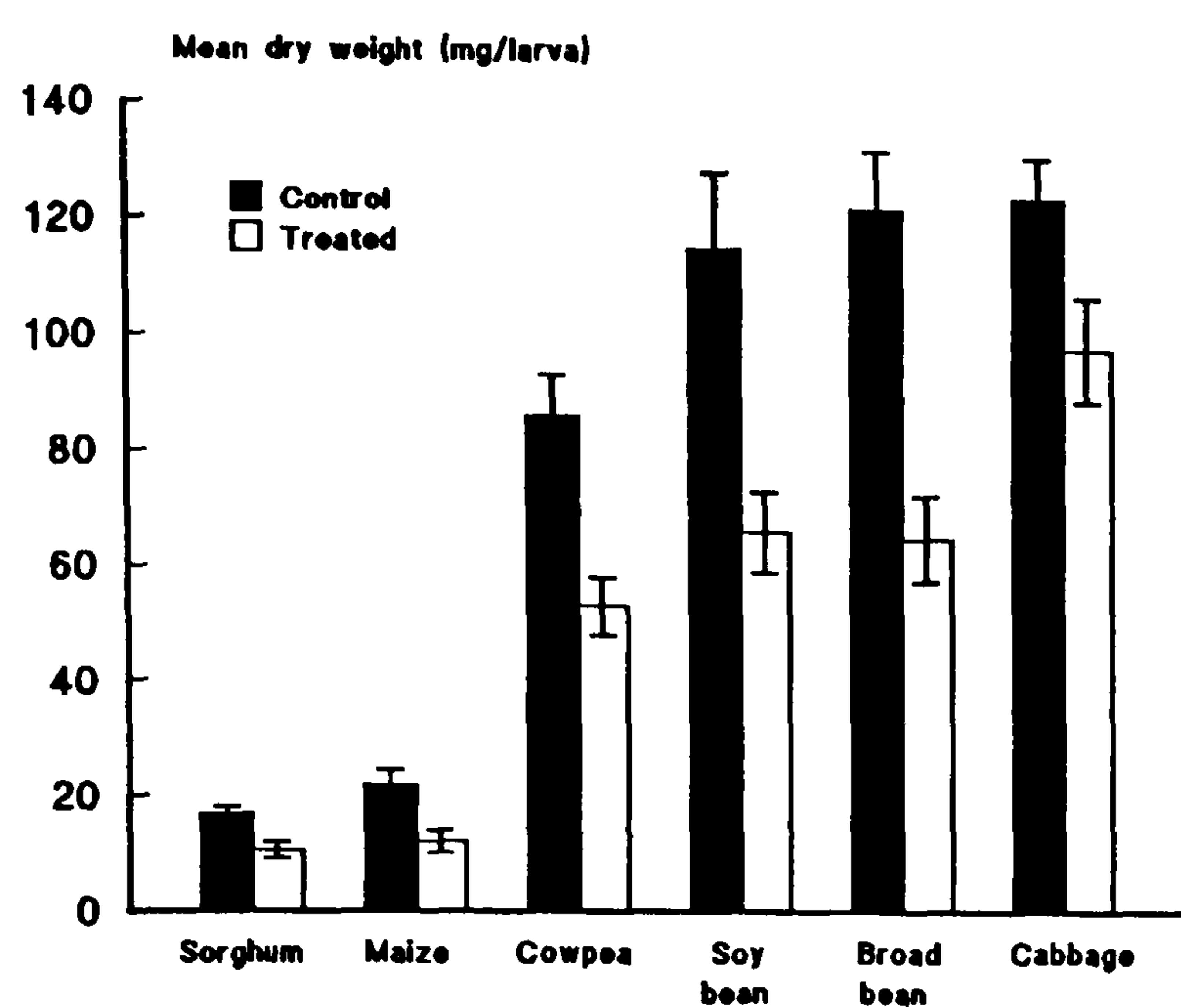
4.3.7 Effect of sublethal dose on larval feeding

Because of all the variables that affect insecticide spraying, it is possible that some insects would come into contact with sublethal doses of insecticides. It is important, therefore, to find out the possible effects of sublethal doses of insecticides on insects' feeding.

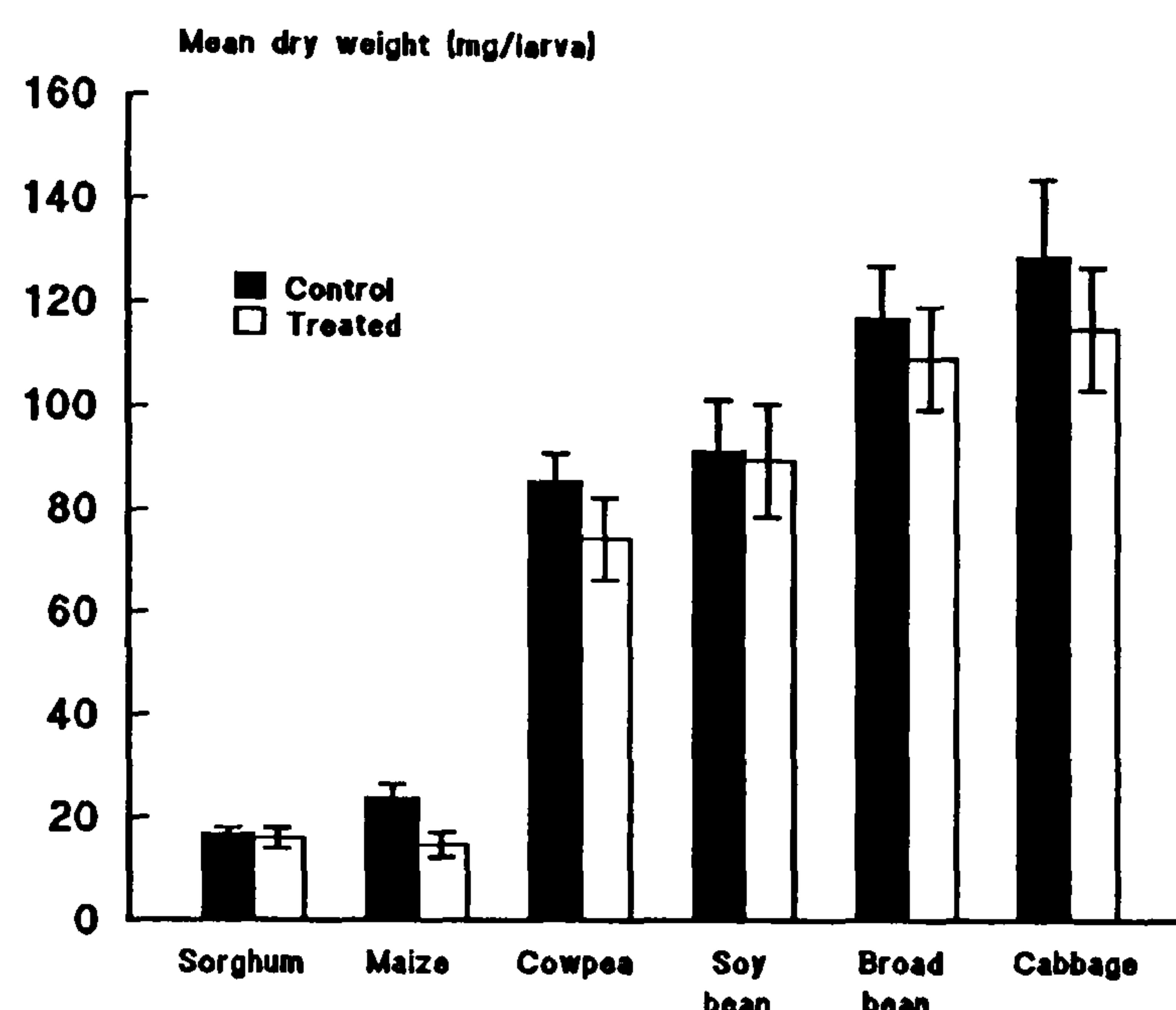
Fig. 4.14 shows the effect of LD₅ of four insecticides on amounts of leaves ingested by treated larvae. Malathion and carbofuran had no effect on larval feeding while permethrin and cypermethrin did.

The reduction in larval feeding due to the application of sublethal doses of insecticides could be due to antifeeding or repellent effects (Liu and Wilkins, 1992; Abro et al., 1993). In this study the insecticides were applied to the cuticle, so they are likely to have acted as antifeedants.

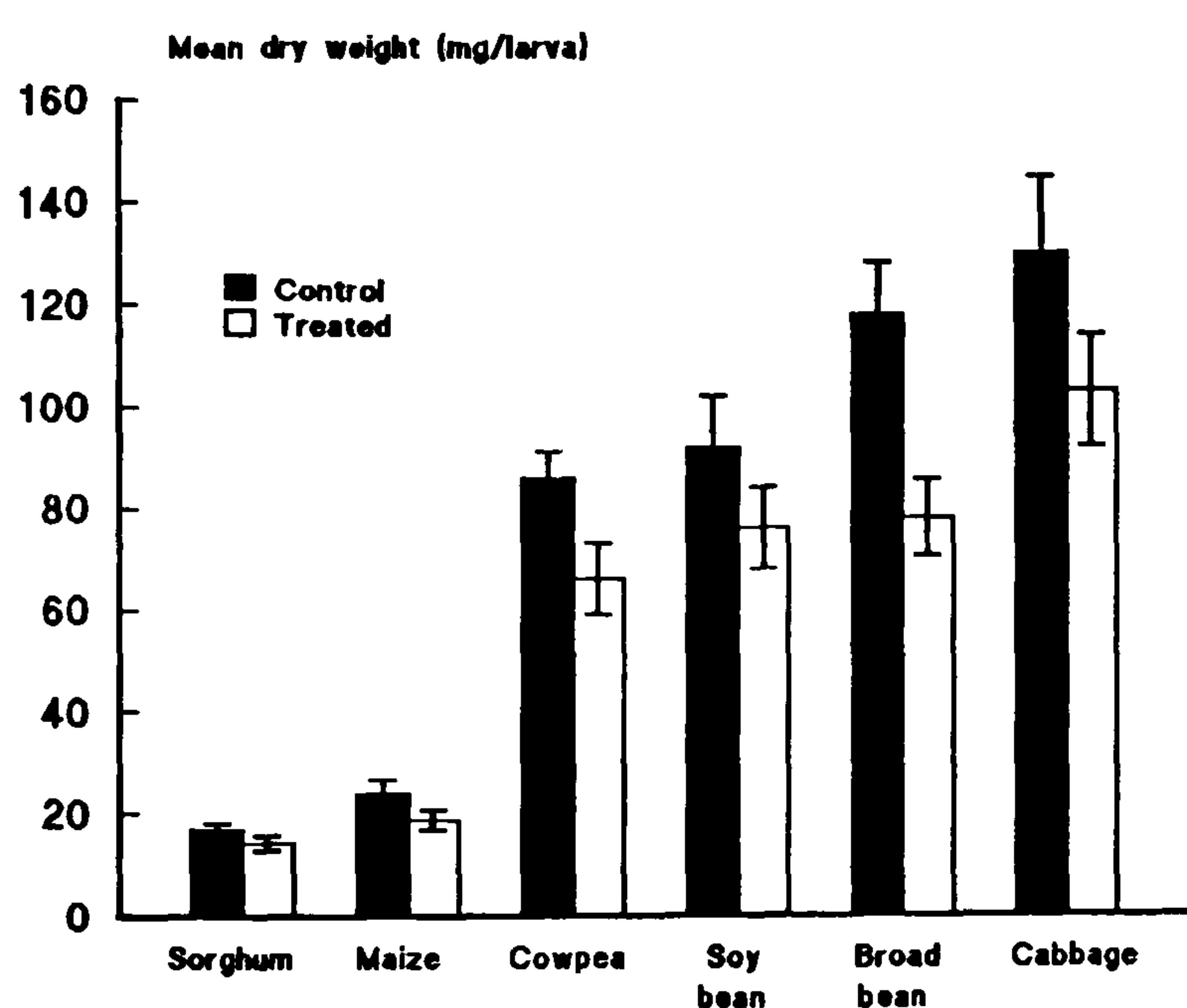
Even though exposure to a sublethal dose of insecticides may not result in death of the insect, the exposure could result in mating disruption, reduced fecundity



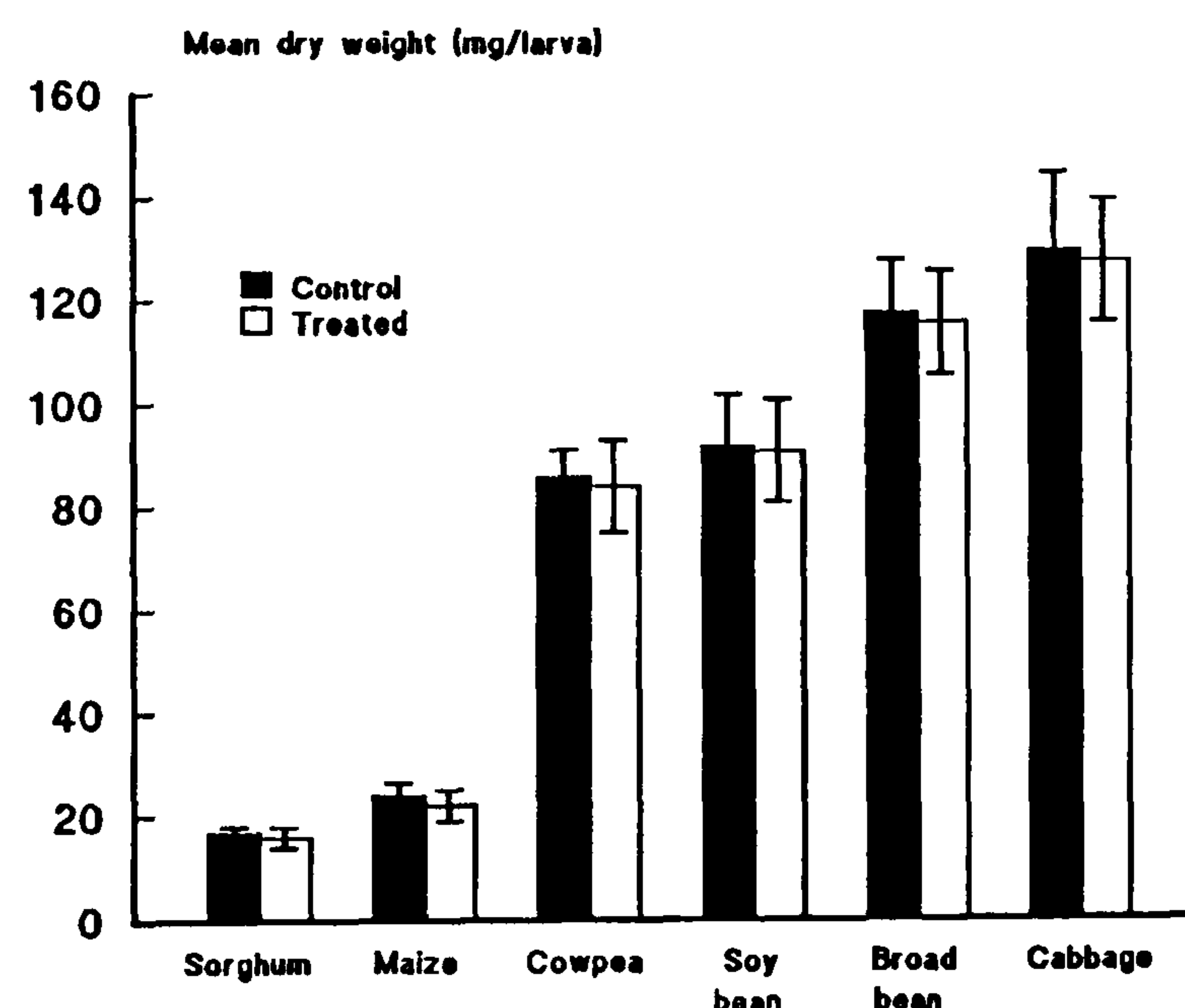
A



C



B



D

Figure 4.14. Effect of sublethal dose LD₅ of cypermethrin (A), permethrin (B), carbofuran (C) and malathion (D) on amounts of leaves ingested by treated larvae.

dynamics of the insect (Jackson and Wilkins, 1985; Clark and Haynes, 1992; Moldenke *et al.*, 1992). The delayed development could result in increased predation and exposure to weather conditions which could further reduce the population of the insects.

Understanding how sub lethal doses affect the behaviour of insects could aid in the search for new behaviour disrupting chemicals that could help reduce the current volume of pesticide use.

4.4 Conclusions

1. Variations in the total cytochrome P-450 content of larval midgut were due to the effects of the plant species that the larvae fed on.
2. The variations in the total P-450 concentration of the larval midgut were associated with variations in the profiles of the peptides of the microsomes.
3. The application of PBO, β -naphthoflavone and barbital influenced the larval food intake.
4. Larval susceptibility to insecticides was influenced by the food plants on which the larvae had been reared.

CHAPTER FIVE

GENERAL DISCUSSION

5.0 GENERAL DISCUSSION

There are indications (Prasad and Bhattacharya 1975) that the monocots, as a group, may not be natural food plants of *S. littoralis* even though other reports (Lal and Naji, 1990) indicate the presence of the insect on some monocots such as maize. There is little information on the basis for the non-preference for the monocots by the insect. Such an understanding of the basis of the interaction could help explain the differences in the infestation of *S. littoralis* on various plants. This thesis was an attempt to relate the growth, development and feeding preferences of *S. littoralis* to some chemical factors of the plants that the insect had been fed on.

The literature, methods and the results of the various experiments on the factors that affect the growth and development of *S. littoralis* have been presented in the previous chapters. This general discussion is aimed at highlighting in a broader context the implications of the observations made in the course of the study for the management of *S. littoralis*.

5.1. Preference for food plants

In a choice test, 1st and 4th instar larvae preferred the dicots to the three monocots. The results imply a lack of preference for the monocots. The observations on the larval preference for dicots would have limited implications for the control of the moth in the field

unless a very wide area were cultivated with monocots in order to reduce the numbers of the insect.

The preference for young leaves as against older ones of the monocots (**Fig. 2.10**) contradicts the observation by Bernays et al. (1974) that seedling grasses become more palatable with age. Long term effects of different ages of leaves on larval feeding were not studied, so it is possible that in the long term the effects on the insect may not correspond to the preference for the younger plants.

5.2 Growth and development of *S.littoralis*

The two most important parameters in the population dynamics of any insect are the survival of the immature stages and the number of eggs that are laid by the female. Collectively, these two factors determine the numbers in the population of insects. The importance of mortality of the immature stages of the pest lies in the fact that any crop protection measure is based on the level of damage to the crop due to the larval feeding activity of the surviving numbers of the pests.

The larvae of *S. littoralis* failed to survive to pupation on millet. In a preliminary experiment, they also failed to survive to the pupal stage on rice, ryegrass and wheat. Only 8% of larvae on sorghum and 32% of larvae on maize survived to the adult stage. The trend of larval

mortality (**Fig. 2.4**) would suggest that the deleterious factors in the monocots had a chronic rather than an acute effect. These observations on mortality on the monocots are consistent with the observation (Ahmed, 1978) that even though *S. littoralis* can be observed on maize plants, they never reach damaging proportions nor cause extensive damage.

The strong antibiotic effects exhibited by the monocots were absent in the dicots tested. In the immature stages, the mortalities were low and the instar durations shorter on these plants. The adults were heavier, lived longer and laid more eggs on these plants. The insect on the dicots would, therefore, have shorter life cycles, more generations in a year and greater numbers of surviving larvae to attack plants in the field.

5.3 Larval feeding on food plants

One possible factor that could affect the growth and survival of the insect is variation in the food intake of the larvae. Larval feeding on the plants followed the same trend as in the preference tests. The total amount of food ingested in a 10 day period by 0-10 day old larvae was low on maize as compared to food intake on cabbage and broad bean (**2.3.21**). The food digestion and utilisation of the 4th and 6th instar larvae (**2.3.20.1**) confirmed the low larval food intake on the monocots.

It might have been expected that, since the amount of

food ingested on the monocots was low, the larvae would exhibit some compensatory feeding behaviour by having a relatively higher efficiencies of conversion of digested and ingested food to body matter. This compensatory behaviour of the larvae on the monocots did not occur (2.3.20.4). The larvae on the monocots, therefore, ingested low amounts of food, had poor digestion of ingested food and had low efficiencies of conversion of food to body materials. These observations indicate that there were factors in the monocots that reduced both the food intake and the digestion and utilisation of the ingested food.

The variations in the amounts of food ingested had some effect on the vigour of the larvae. On the plants that were ingested in low amounts (monocots) the larval weights were lower than on the plants that were ingested in large amounts (dicots). The low larval weights were observed in the entire larval period (**Fig. 2.2**). Consequently, the larvae on the monocots, being small, would be weak and more vulnerable to factors such as biological and chemical control agents.

The greater number of generations on the dicots when taken in association with the greater amounts of food ingested would suggest that control measures would be required at a very early stage for the control of the moth on these plants. However, if the mortality patterns on the

monocots are observed in the field then it is unlikely that chemical control methods would be required for the control of the insect. The development of biotypes of the insect on these plants, if that occurs, would then require a different approach to the control of the pest.

5.4 Possible chemical factors affecting the feeding of the larvae of *S. littoralis*

Attempts were made to determine whether the physical characteristics of the monocots and some chemical factors of both monocots and dicots could have resulted in the variations in food intake of the larvae of *S. littoralis*.

Most of the physical characteristics, such as the presence of trichomes and leaf hardness, were eliminated when the leaves of the monocots were dried, milled and incorporated into an artificial diet, yet the percentage of larvae surviving to the adult stage did not significantly improve when compared with the mortality of the insect feeding on fresh leaves of the monocots. These observations suggest that the mortalities were due more to chemical than physical factors.

In this study water, carbohydrate, free amino acid and C,H,N concentrations were measured in order to determine whether there was a relationship between the performance of the larvae and the concentrations of these factors in the plants. Generally, the total nitrogen, the proportions

of free amino acids and the water contents were lower in the monocots. Glutamic acid, an amino acid implicated in the inhibition of development of other insect species on monocots (Weibull 1988), was present in high levels in maize, millet and sorghum.

Since at the concentrations that they occur in plants carbohydrates are not limiting, the results of this study suggest that it is the level of N, amino acids and the water content of the leaves that may have contributed to the poor growth and development of the larvae on the monocots. Thus larvae on the monocots tested ingested low amounts of food plants which contained low amounts of some important nutrients and high levels of glutamic acid, resulting in low weight of all growth stages.

Other chemical factors of the plant could also have contributed to the variation in the feeding of the larvae of *S. littoralis*. The increase in the consumption of leaves with the surface wax removed and the reduction in ingestion of filter paper treated with wax extracts of the leaves suggest that the surface characteristics of the plants could have influenced the feeding of the larvae. The wax extracts from sorghum were more inhibitory than the extracts from the other plants. However, since the larval food intake on the dicots was not affected when the larvae were fed fresh plants with the surface wax unremoved, the surface wax may have influenced feeding only on the cereal

plants.

The chemical composition of the wax extracts would differ among the various food plants. Woodhead (1983) reported that alkenes are usually present in plant surface waxes and in the Gramineae, C_{29} and C_{31} n-alkenes usually predominate. She observed that the dominant alkenes vary in sorghum varieties. The constituents of the wax extract from the sorghum variety used in this study could, in a further study, be fractionated and the fractions tested for biological activity against the larvae of *S. littoralis*. This could help in the identification of the feeding inhibitory fractions.

Crude leaf extracts of the plants were made and tested for inhibition of larval feeding (3.3.7). It is apparent from the results that different compounds may have contributed to the low food intake in the monocots. The results do not give conclusive evidence that the low food intake on the monocots was due to the presence of feeding deterrents. The extracts were tested individually and it is possible that collectively, the compounds present in the monocots may have a greater inhibitory effect on the larvae than the ones in the dicots.

It could be inferred from these observations that both nutritional and allelochemical factors were implicated in the larval feeding inhibition exhibited by the monocots.

5.5 Larval susceptibility to insecticides

In this study the larvae on the monocots were more susceptible to the insecticides tested than the larvae that fed on the dicots (4.3.4). Two observations could explain the variation in susceptibility to the insecticides.

Firstly, if insecticide toxicity could, though not in all cases, be a function of the body weight of the insect (van Emden, 1991), then the larvae with low body weights would be expected to be more susceptible to insecticides. Variation in the larval body weight of *S. littoralis* was associated with variation in the quality and quantity of food ingested by the larvae. Thus, low food intake on the nutritionally poor Gramineae (due to the feeding deterrents) produced physiologically stressed and weak larvae with low larval weights which, therefore, required low doses of insecticides to produce high mortalities.

Secondly, variation in larval susceptibility could also have been due to variation in the contents of P-450 in the larval gut (4.3.2). The cytochrome P-450 content of the larval gut was lower in the larvae that fed on the monocots. Variation in the concentration of the P-450 was associated with variation in the LD50 of the insecticides that were tested.

Generally, the LD50's of the three insecticides tested were lower for the larvae reared on the food plants from

the 1st to the 6th instar than for the larvae that were initially reared on the artificial diet. This suggests that some compounds in the leaves may have acted to increase the activity of enzymes that detoxify the insecticides.

5.6. Role of P-450 in larval feeding and susceptibility to insecticides

There is indirect evidence that cytochrome P-450 plays a role in the feeding of larvae of herbivorous insects (Yu, 1983). Treatment of larvae with the P-450 inducers β -naphthoflavone and barbital affected the food intake of the treated larvae (and produced further indirect evidence of the involvement of P-450 in larval feeding) and reduced the susceptibility of the larvae to insecticides. Since the observed effects were due to the treatment of the larvae with barbital and β -naphthoflavone, the variations in the food intake and the LD50 suggest the involvement of MFO.

A probable mechanism for the effect on food intake could be suggested. The higher levels of MFO in the body of the insect, due to the effects of barbital and β -naphthoflavone, could have resulted in the detoxication of some of the feeding inhibiting (antifeeding) factors in the ingested plants and, therefore, have resulted in the increase in food intake. The variation in food intake would then be related to the differential effects of the applied compounds on the P-450 in the larvae. The reduction in food intake on the application of PBO also suggests the

involvement of MFO in larval feeding.

However, though the effects on larval feeding were obtained only when the compounds were applied to the larvae, the effects of barbital, PBO and β -naphthoflavone do not offer conclusive evidence of the involvement of P-450 in larval feeding, for a number of reasons. No studies were made to determine how much of the PBO, barbital or β -naphthoflavone applied to the cuticle of the larvae had been absorbed by the insect. Secondly no studies were carried out to determine the effects of the compounds on the digestion and utilisation of food.

Finally, recent studies (Kennaugh *et al.*, 1993) suggest that increase in insecticide detoxication due to PBO may not necessarily be due to its effects on detoxication by MFO. A direct link between the compounds and larval feeding therefore cannot be made, based on the data obtained in this study.

5.7 SUGGESTIONS FOR FUTURE WORK

Even though all polyphagous insects do have food preferences and there are plants that the insects will not feed on, most studies on insect-plant interactions have focused on variation of insect responses due to effects of varieties of plants of the same species. If, as may be the case with *S. littoralis*, species of a plant taxonomic group may not be preferred, then it may be appropriate to

determine whether factors common to these members in a taxonomic group could be identified as the causes for the insects' response. Such information would improve our understanding of insect-plant interactions.

Even though the data obtained in this study are limited, there were clear indications that the Gramineae would not support large populations of the insect. Further studies in other monocots on the role of nutritional and allelochemical factors in the feeding of *S. littoralis* could be carried out.

Further research on the deterrent leaf extracts could also result in the identification of the naturally occurring synergists of P-450 in the monocots. If further studies could establish a direct link between an inhibitor of P-450 and a reduction in larval feeding, then it may be possible to reduce the application of insecticides in the field. The genetic basis of the differential responses to the nutritional factors could then be determined and transgenic methods could be used to transfer such genes to other susceptible crops in order to make them more resistant to larval feeding.

If some of these factors could be linked to the sex ratio of the insect, it may be possible to reduce the pest population by producing male biased insects. It is a speculation worth considering.

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APPENDIX ONE

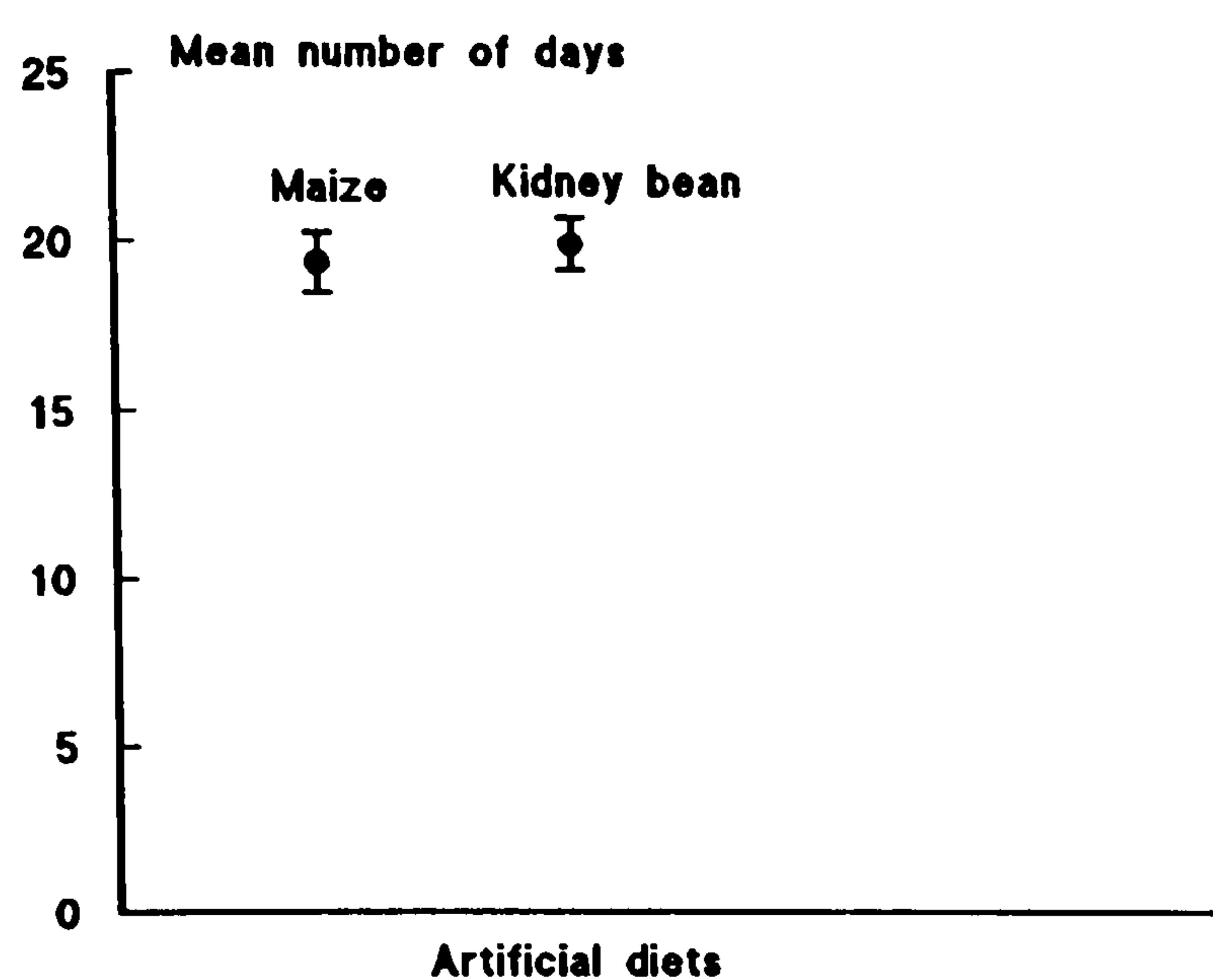


Figure 1. Mean larval periods on artificial diets.

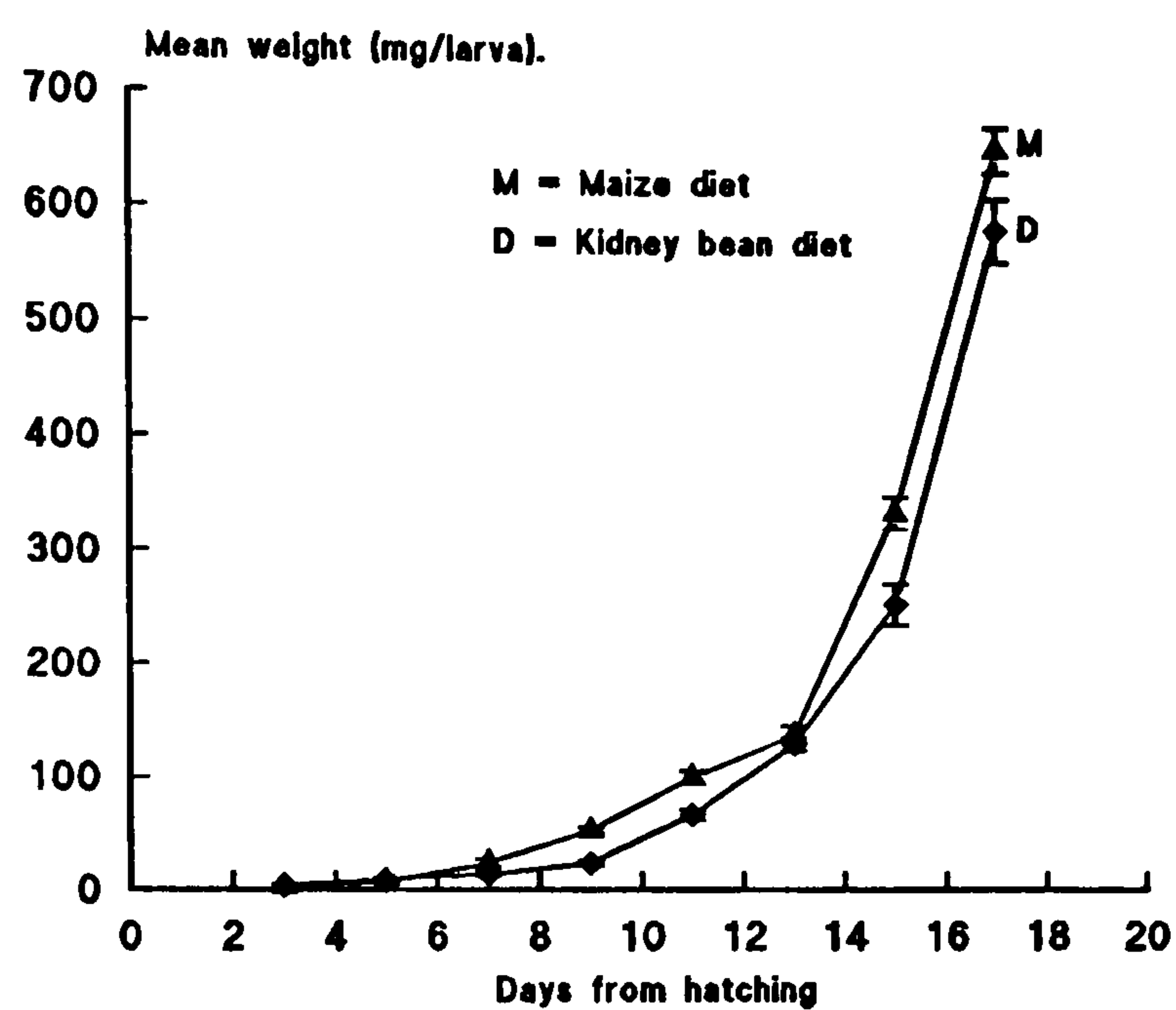


Figure 3. Mean larval fresh weight on semi-artificial diets

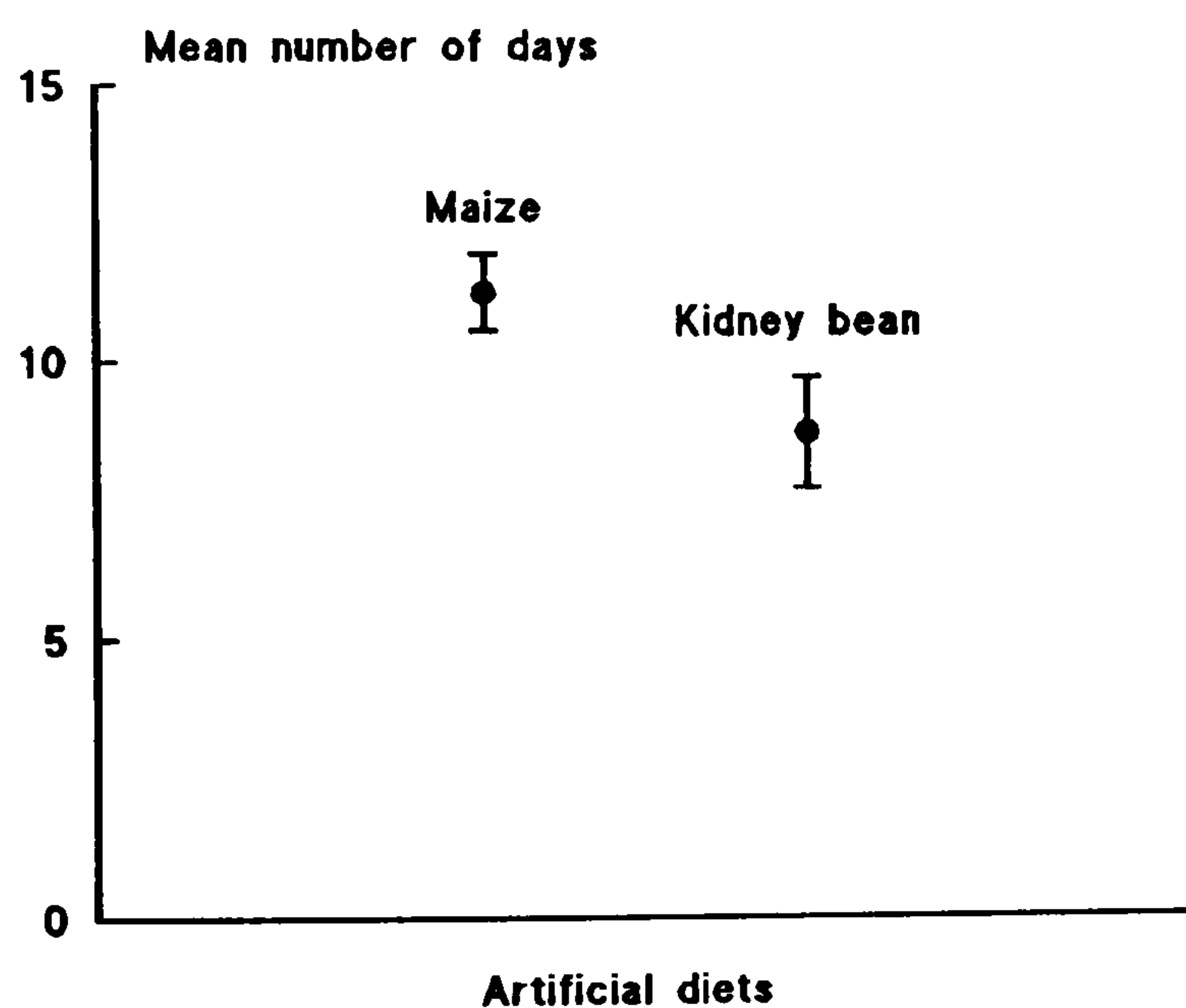


Figure 2 Mean pupal (mixed sexes) period on artificial diets

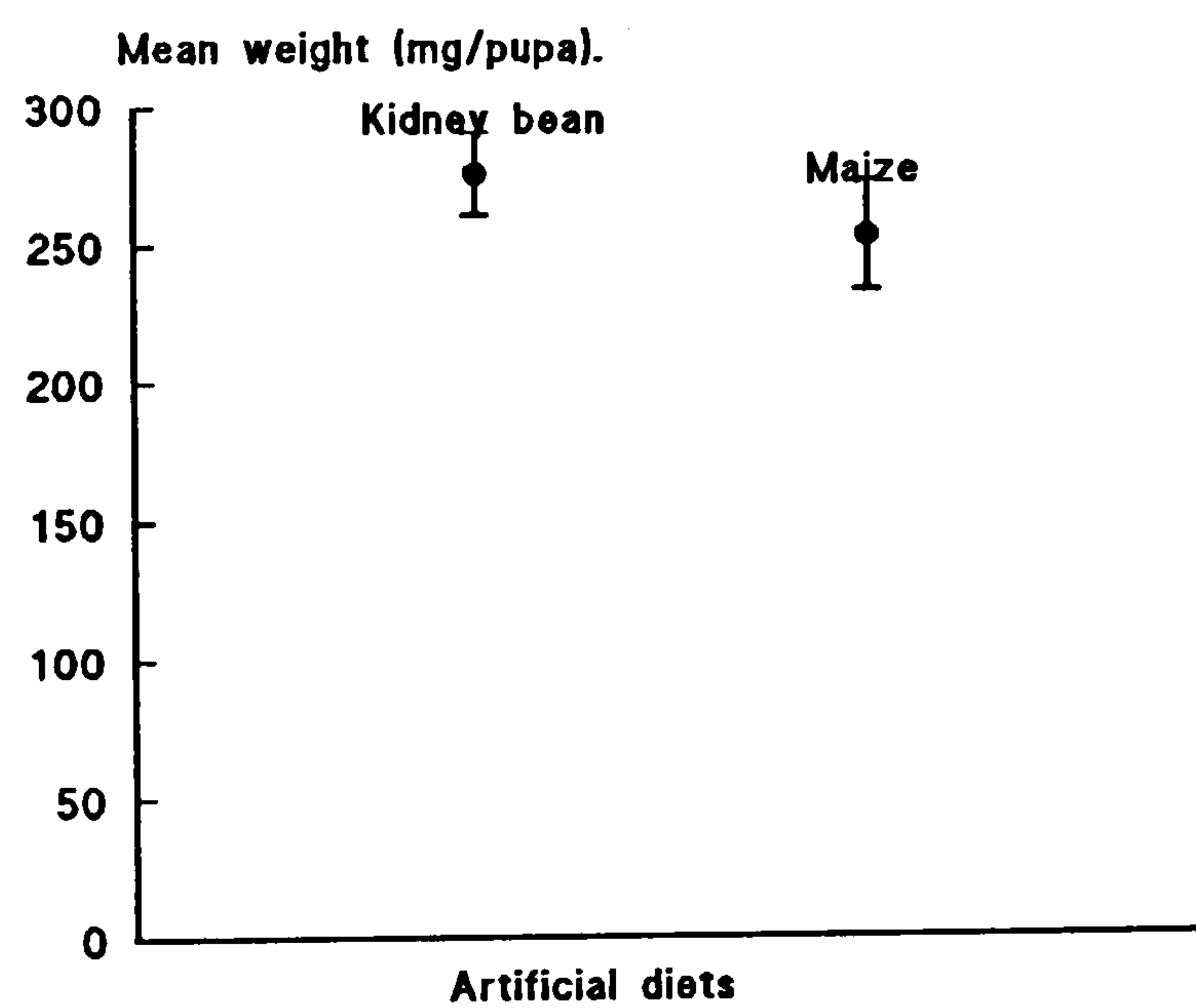


Figure 4. Mean pupal (male) weights on diets

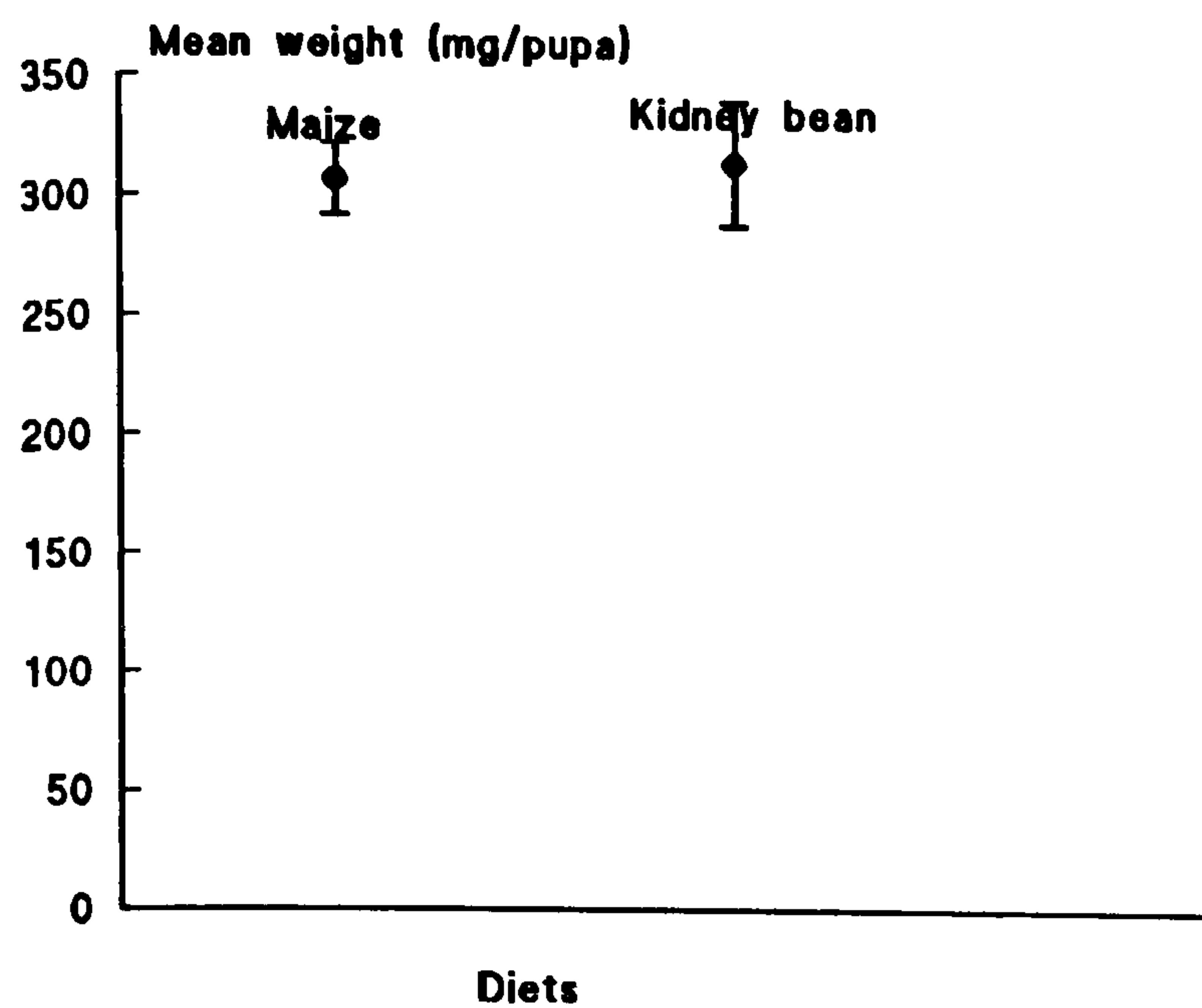


Figure 5. Mean pupal (female) weights on artificial diets

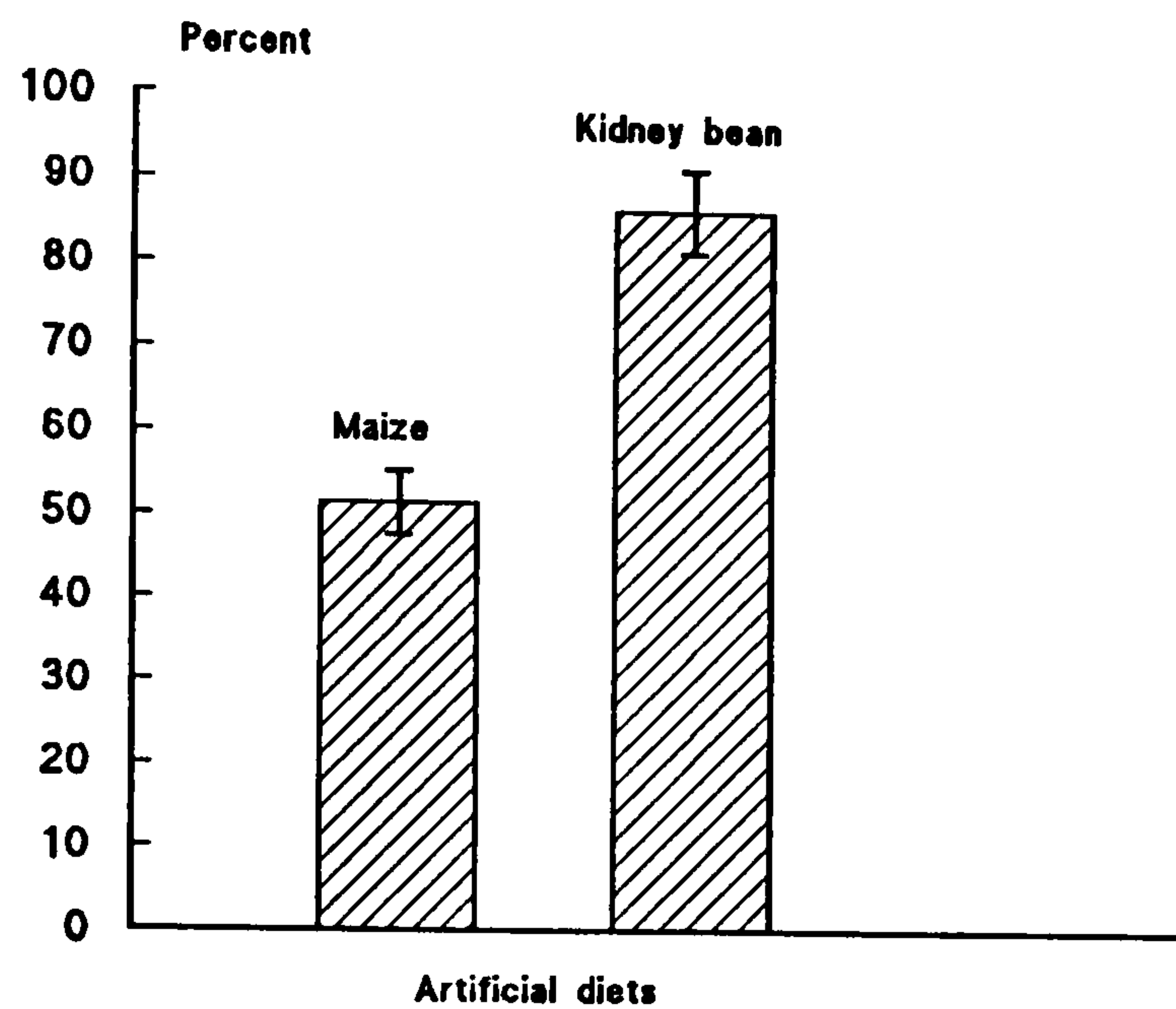


Figure 8. Mean percentage of larvae surviving to the adult stage

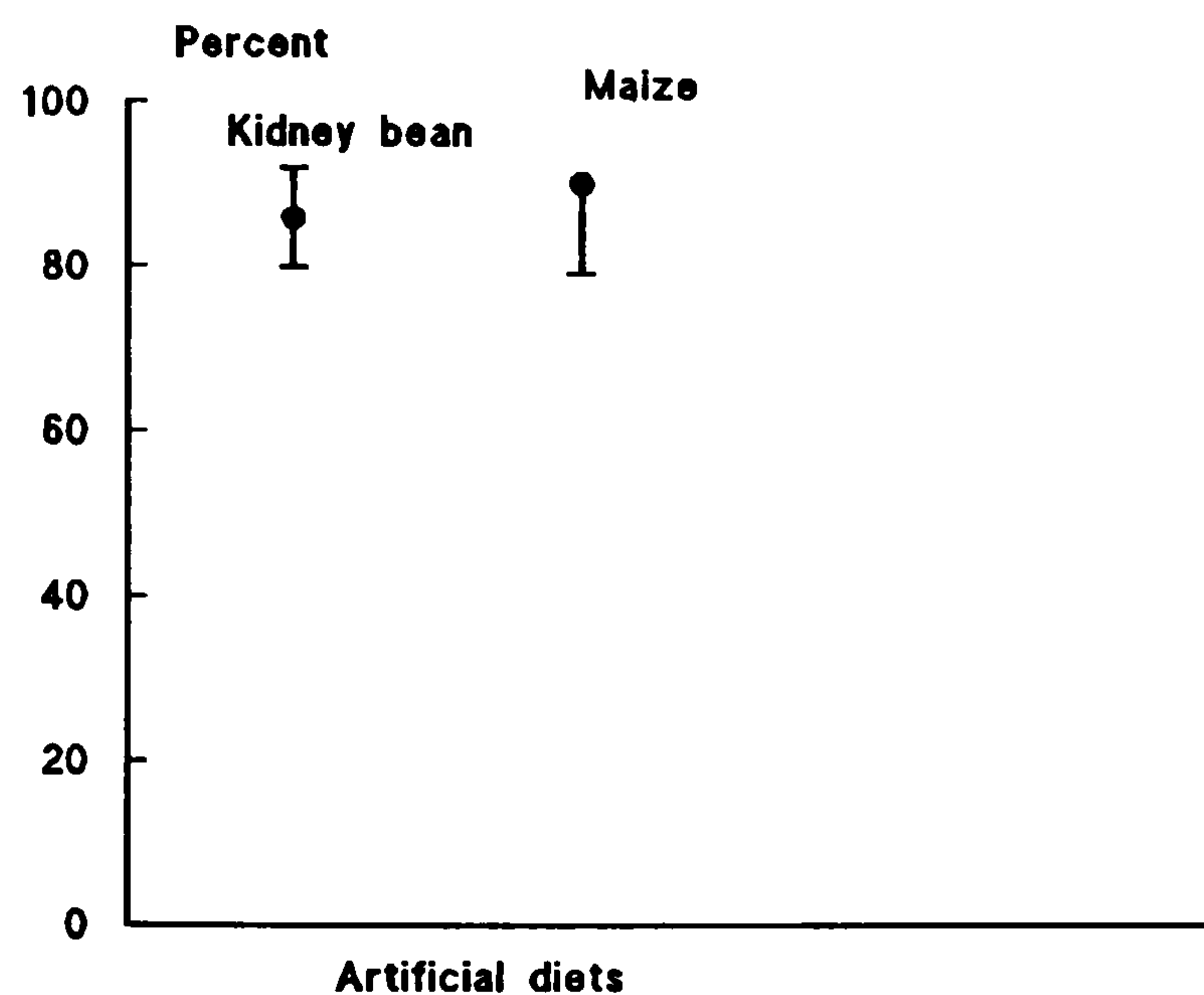


Figure 6. Mean percentage pupal (mixed sexes) formations. formation on diets

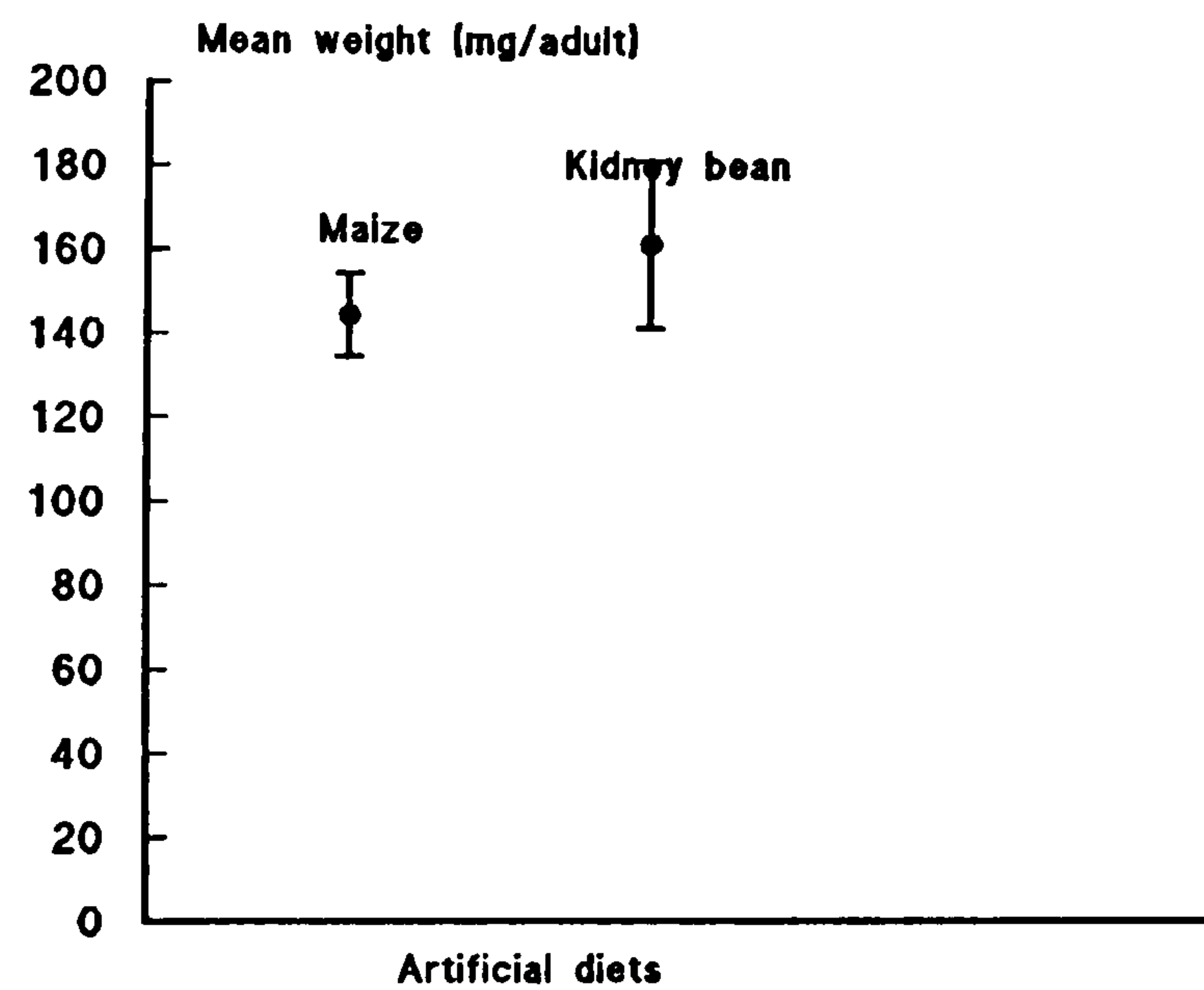


Figure 9. Mean adult (mixed sexes) weights on artificial diets

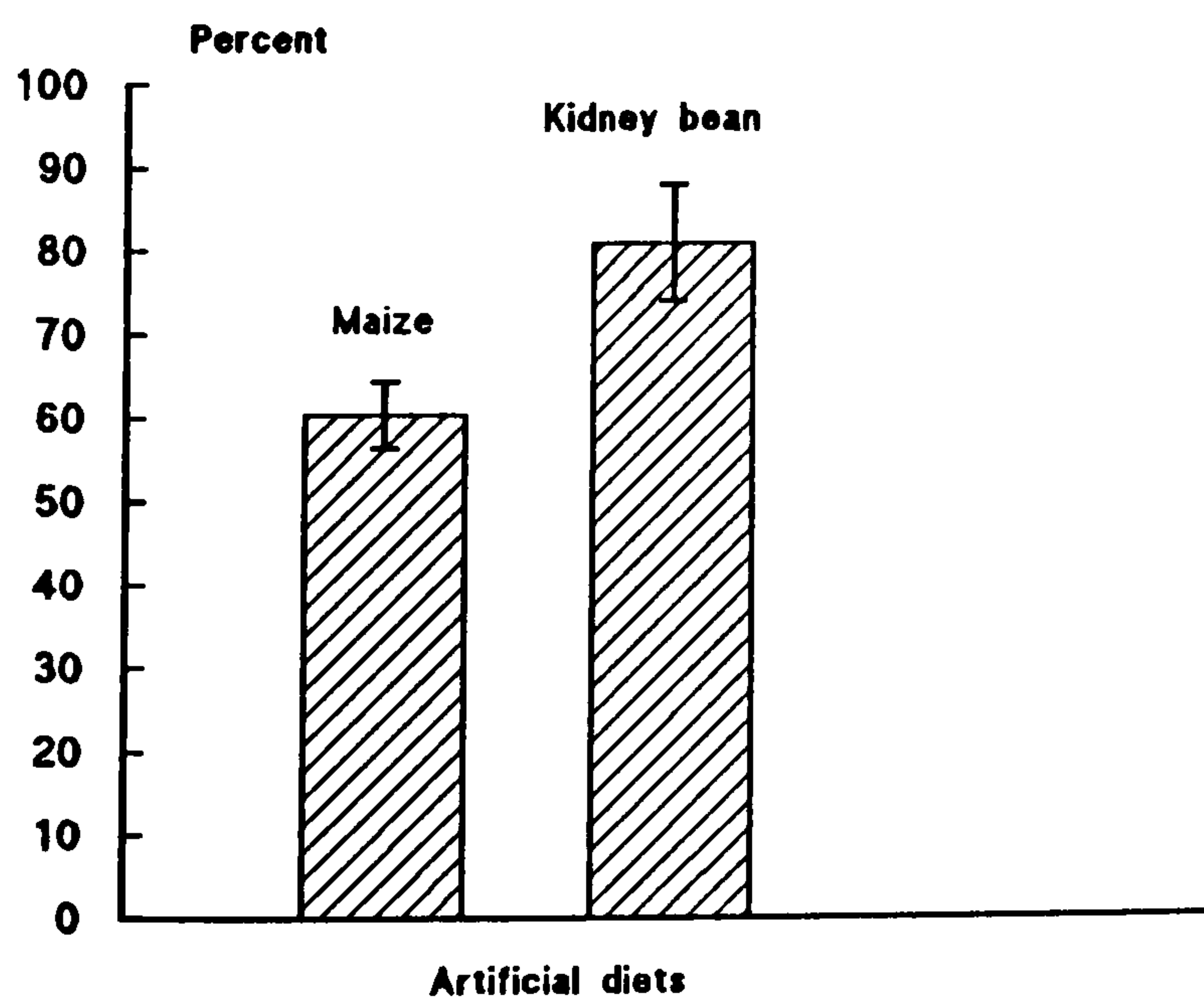


Figure 7. Mean percentage adult (mixed sexes) emergence on diets

APPENDIX TWO

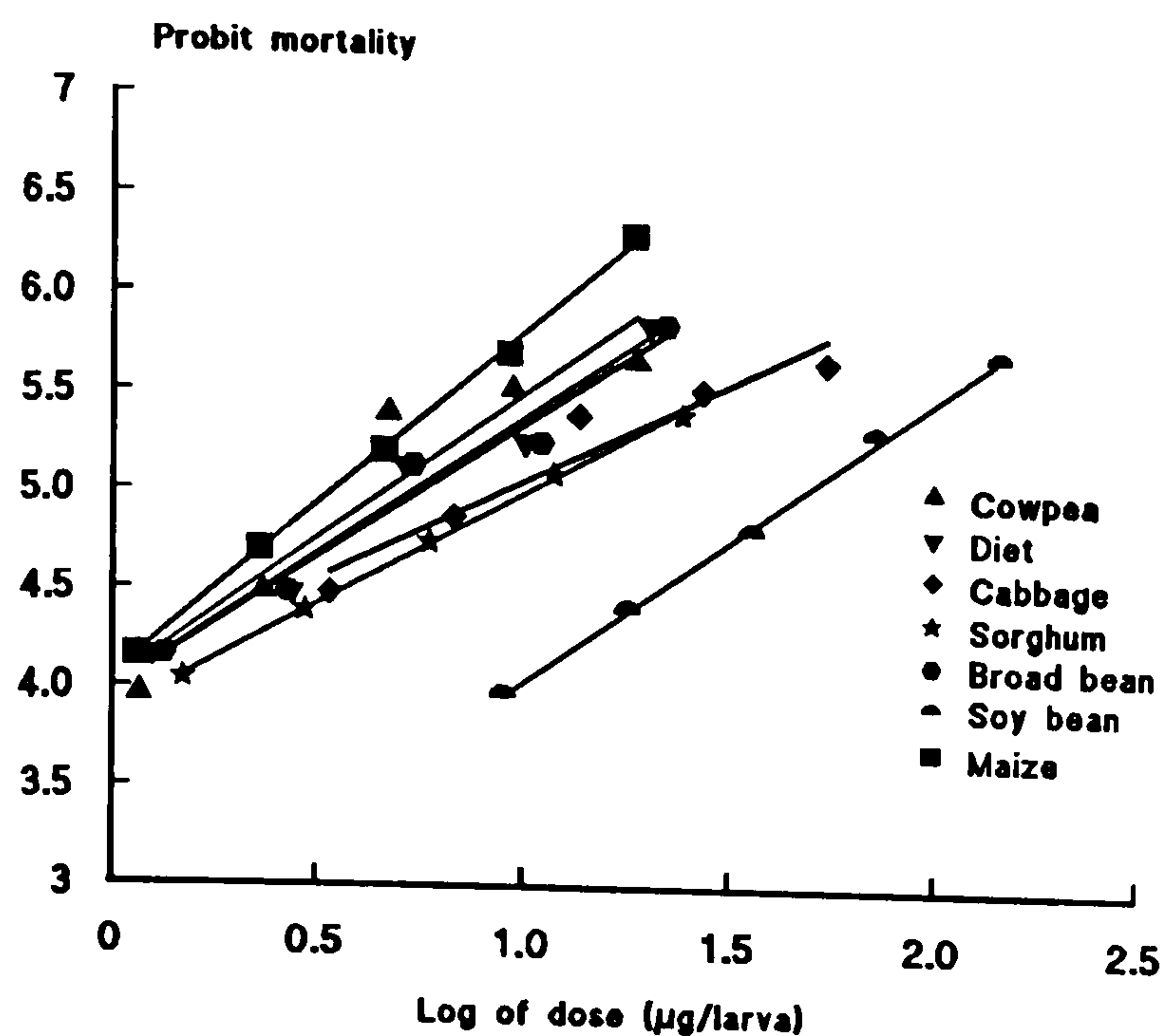


Figure 1. Responses of 4th instar larvae reared on food plants to topically applied cypermethrin.

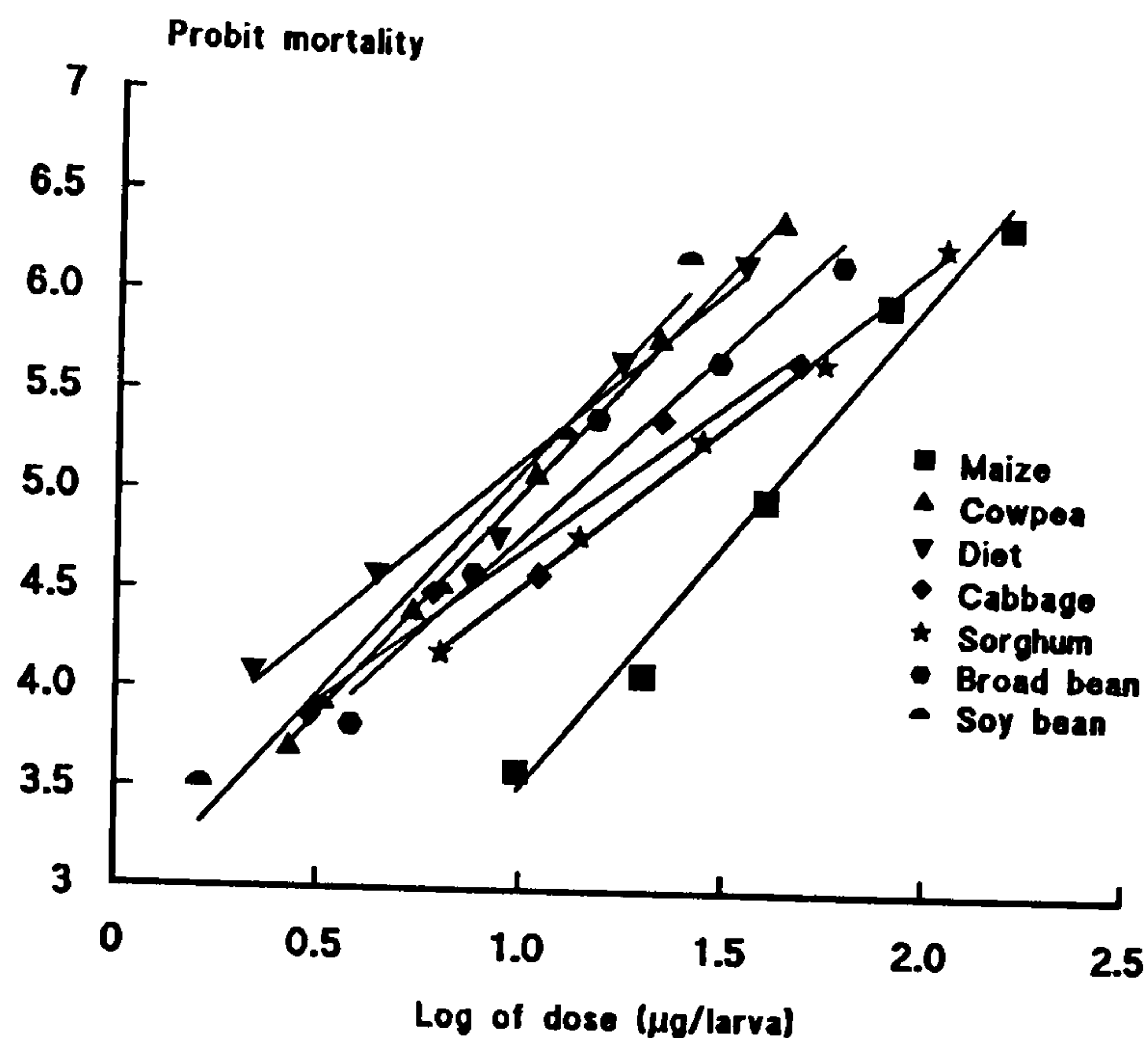


Figure 4. Responses of 4th instar larvae reared on food plants to topically applied permethrin.

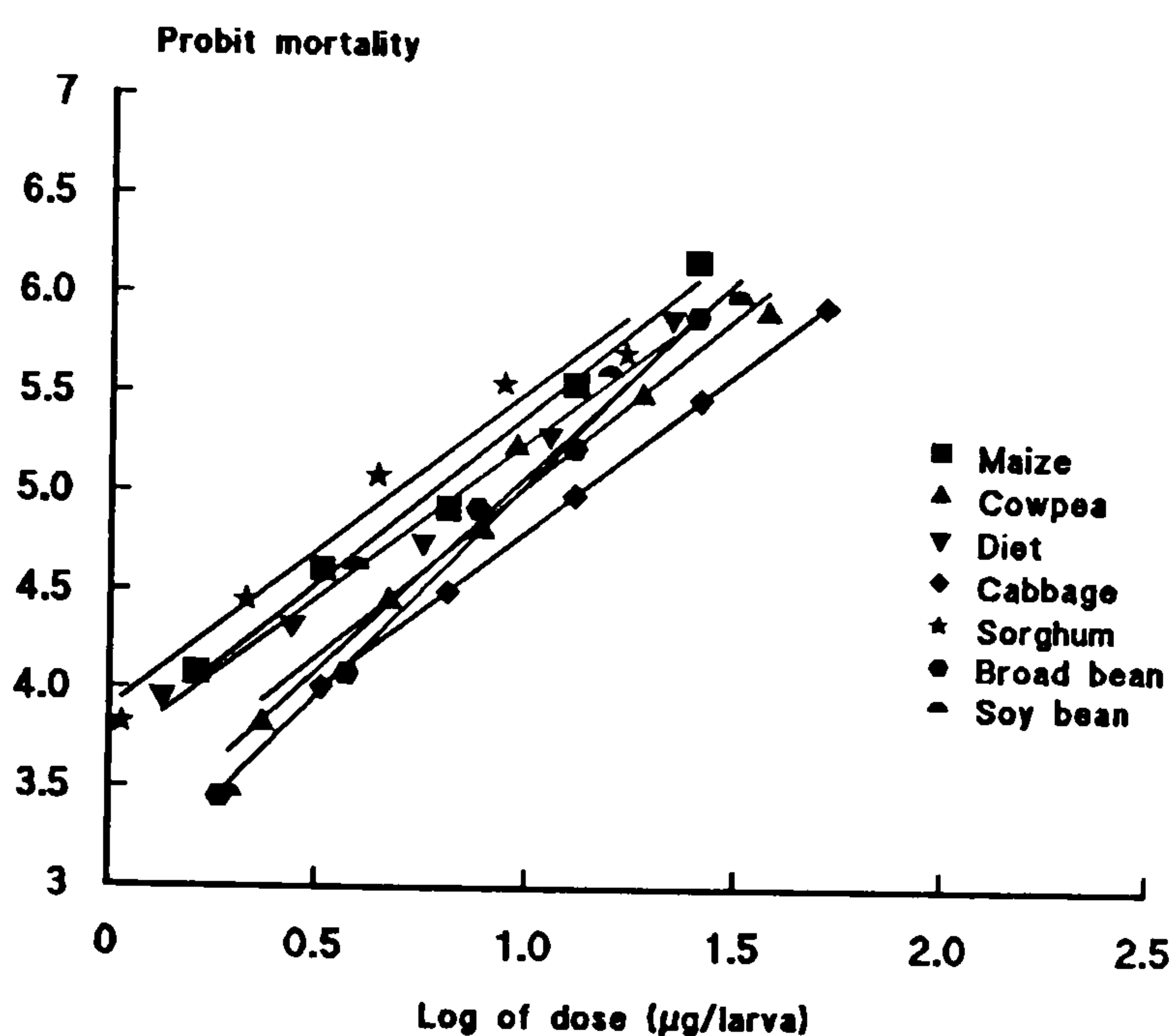


Figure 2. Responses of 4th instar larvae fed on food plants for 24h to topically applied cypermethrin.

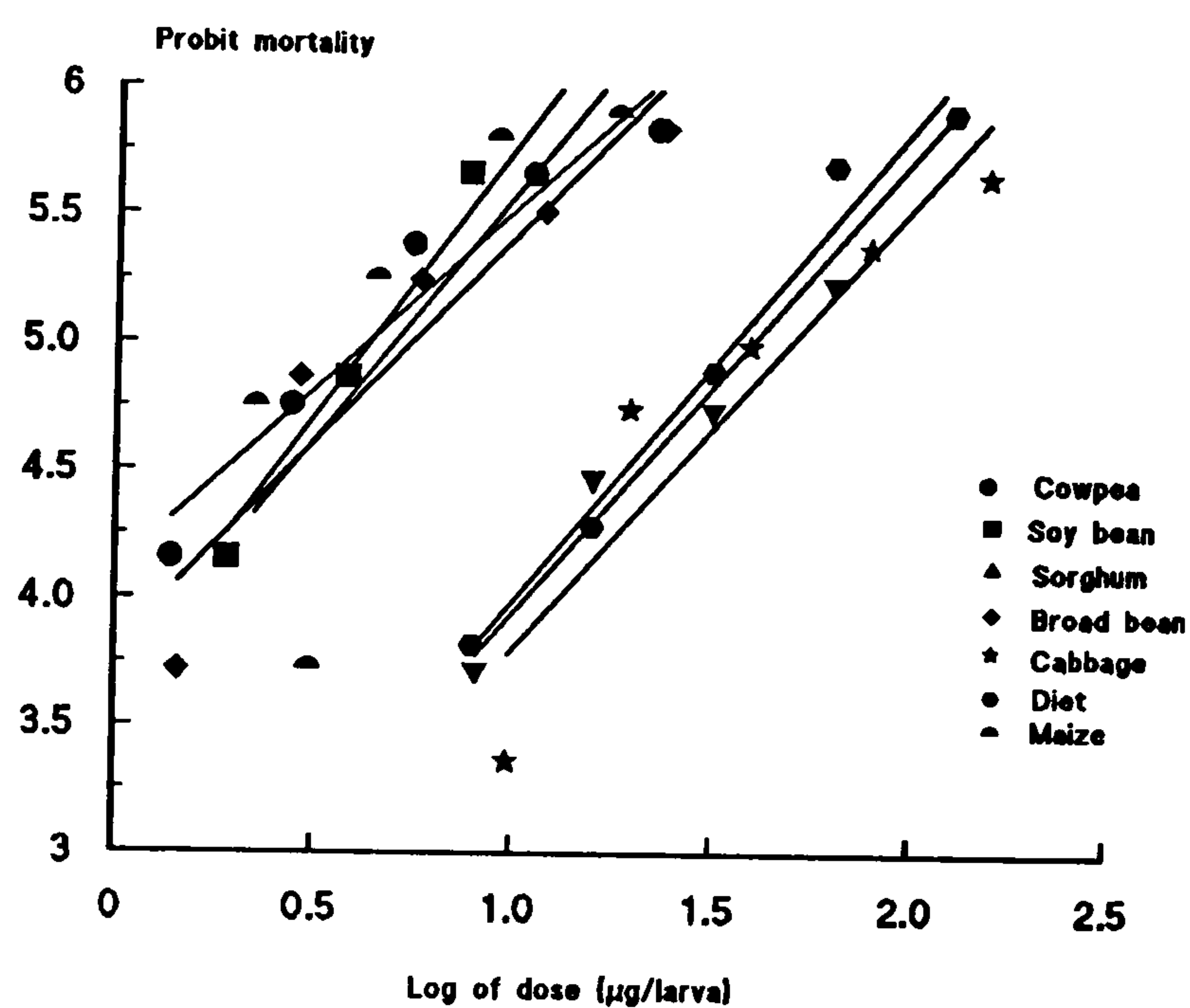


Figure 3.5. Responses of 4th instar larvae fed on food plants for 24 to topically applied malathion.

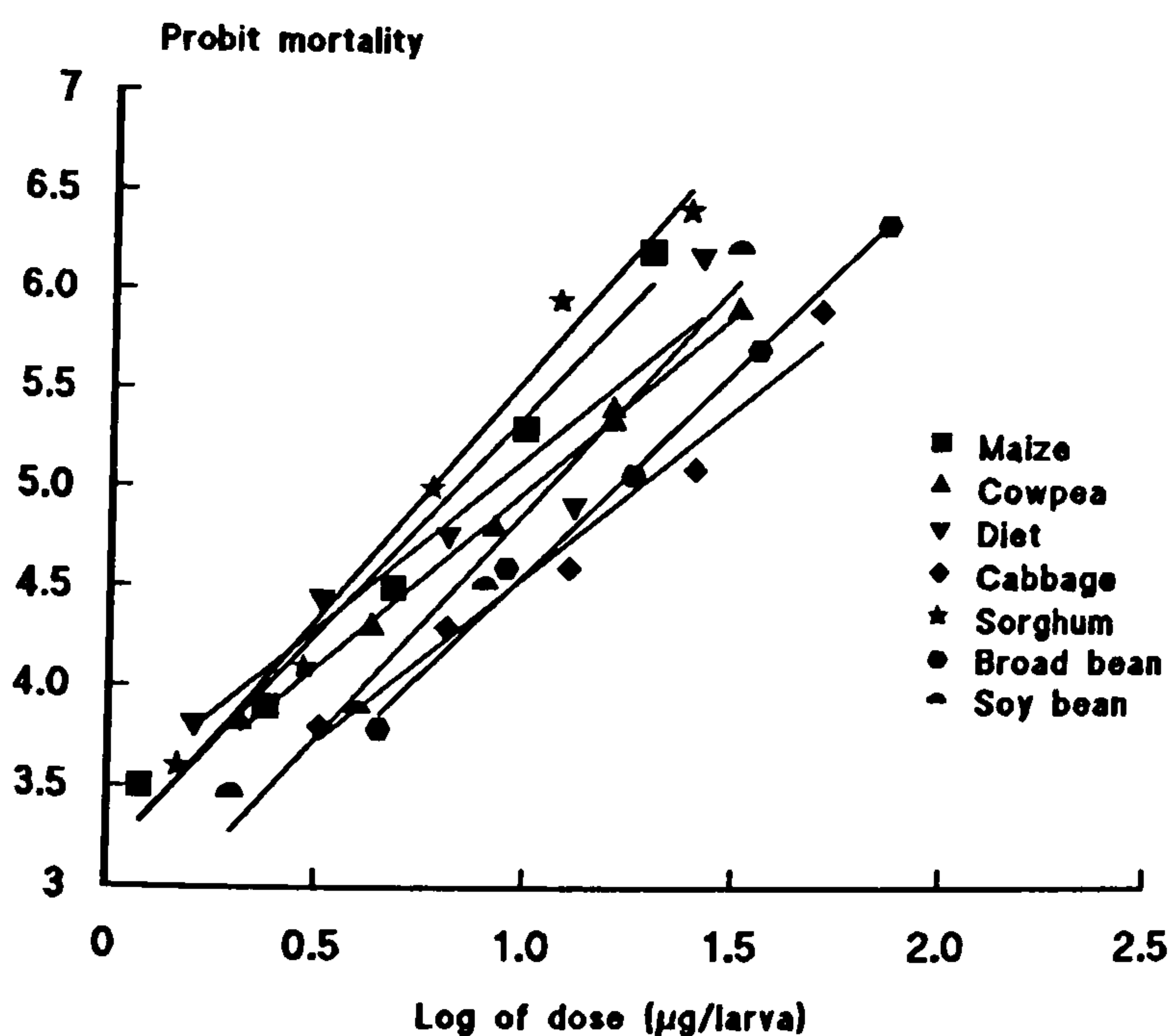


Figure 3. Responses of 4th instar larvae fed on food plants for 24h to topically applied permethrin.

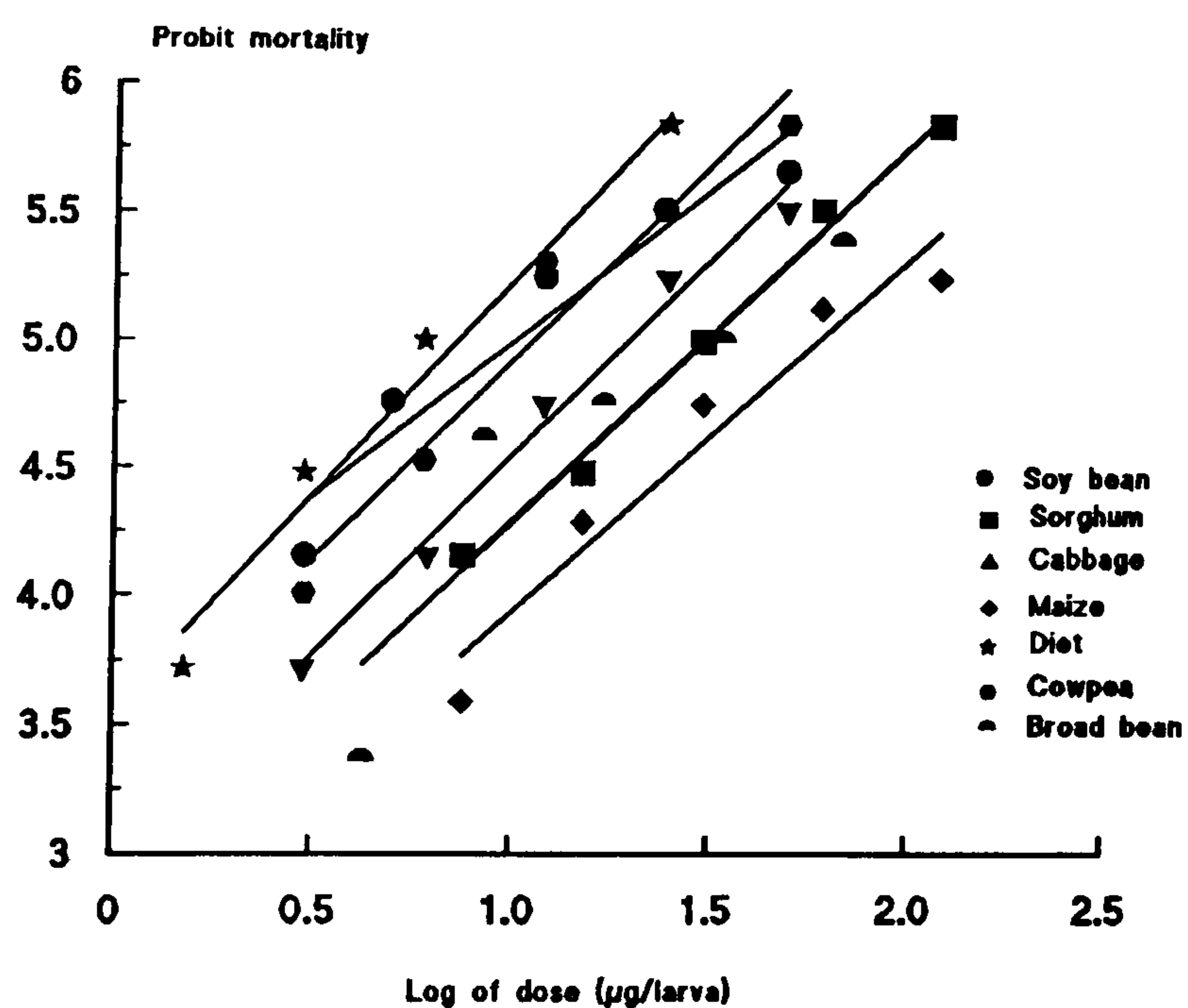


Figure 6. Responses of 4th instar larvae reared on food plants to topically applied malathion.

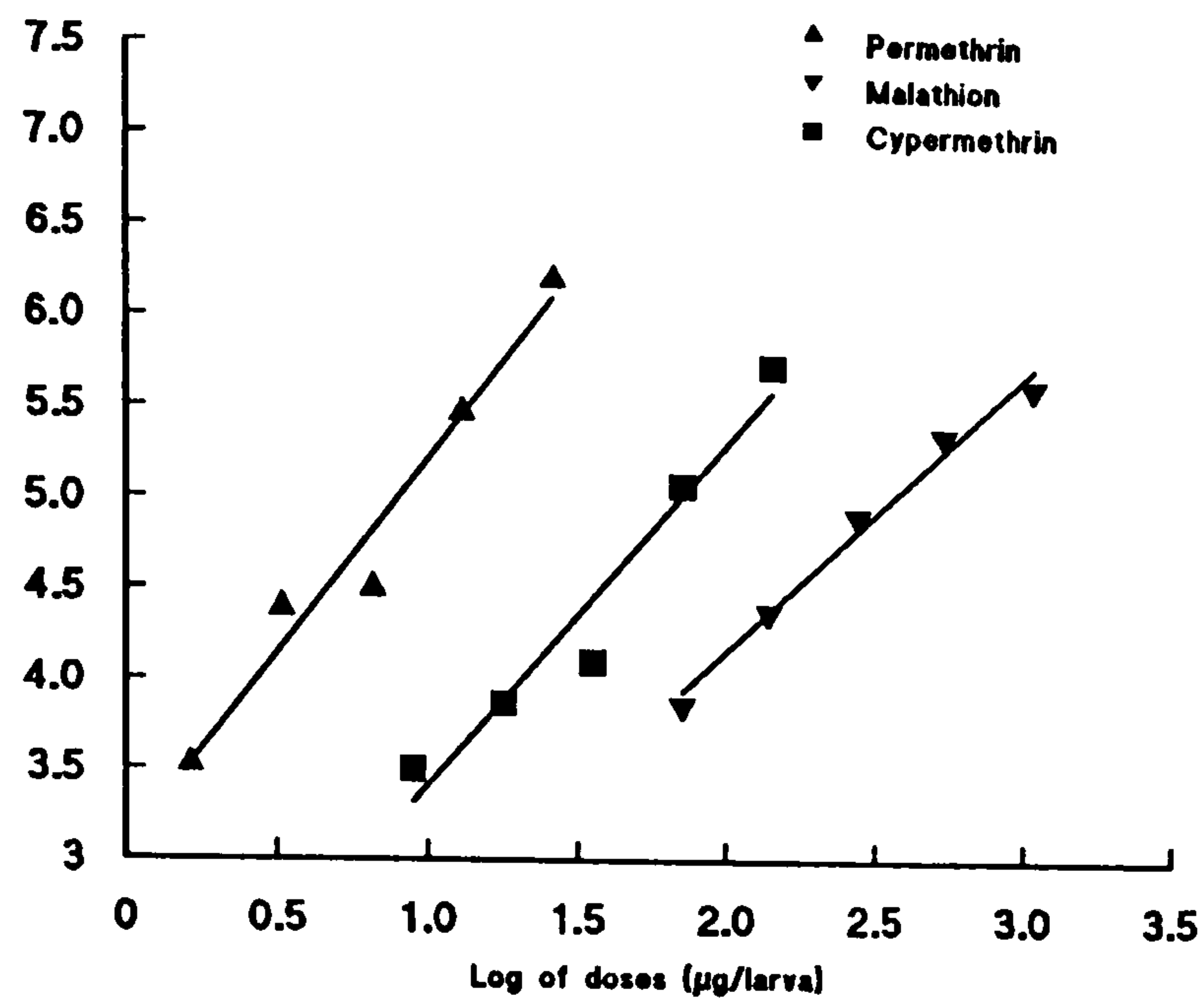


Figure 9. Responses of 4th instar larvae reared on artificial diet to topically applied PBO and insecticide (ratio 1:1)

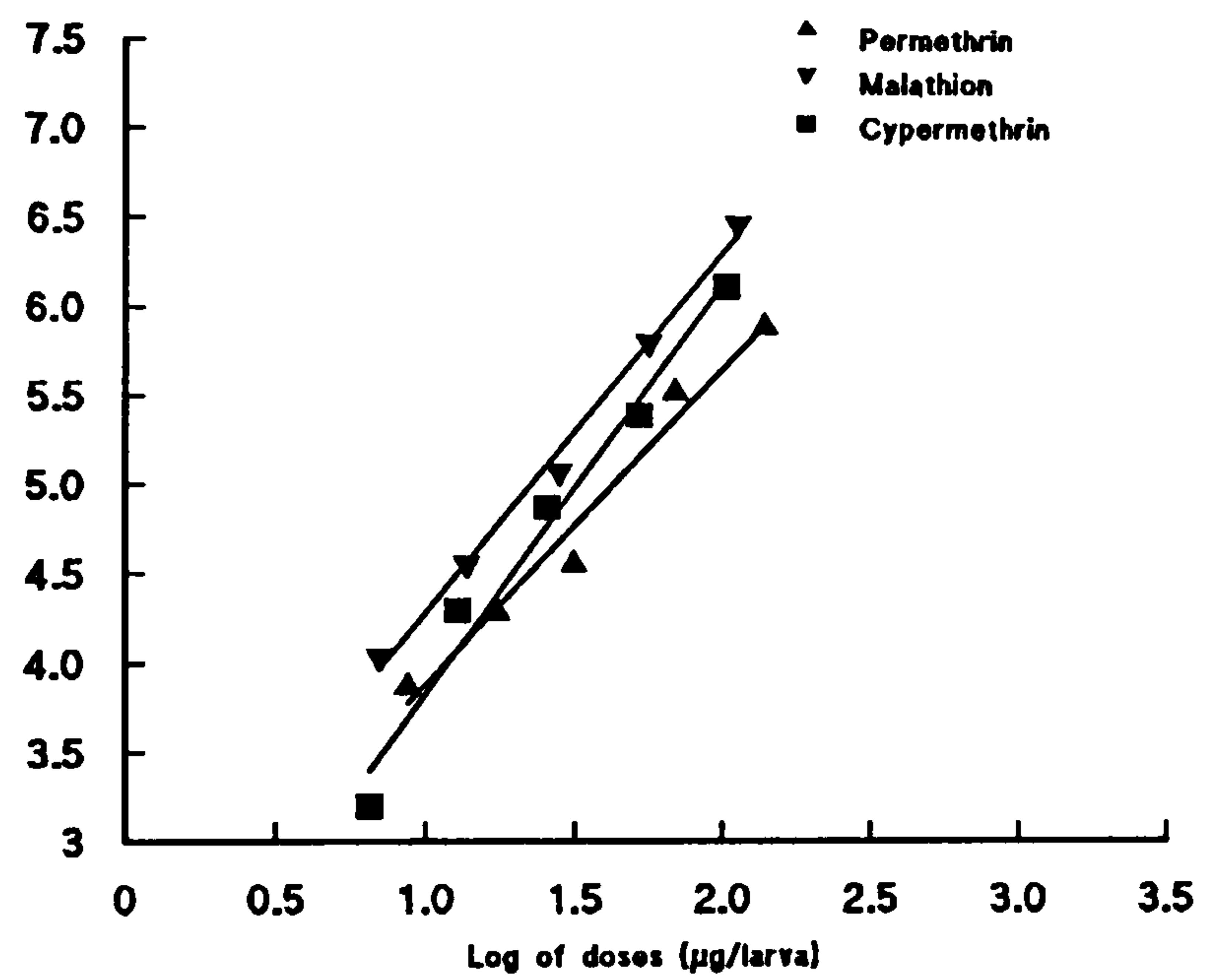


Figure 8. Responses of 4th instar larvae reared on artificial diet to topically applied PBO and insecticide (ratio 1:5)

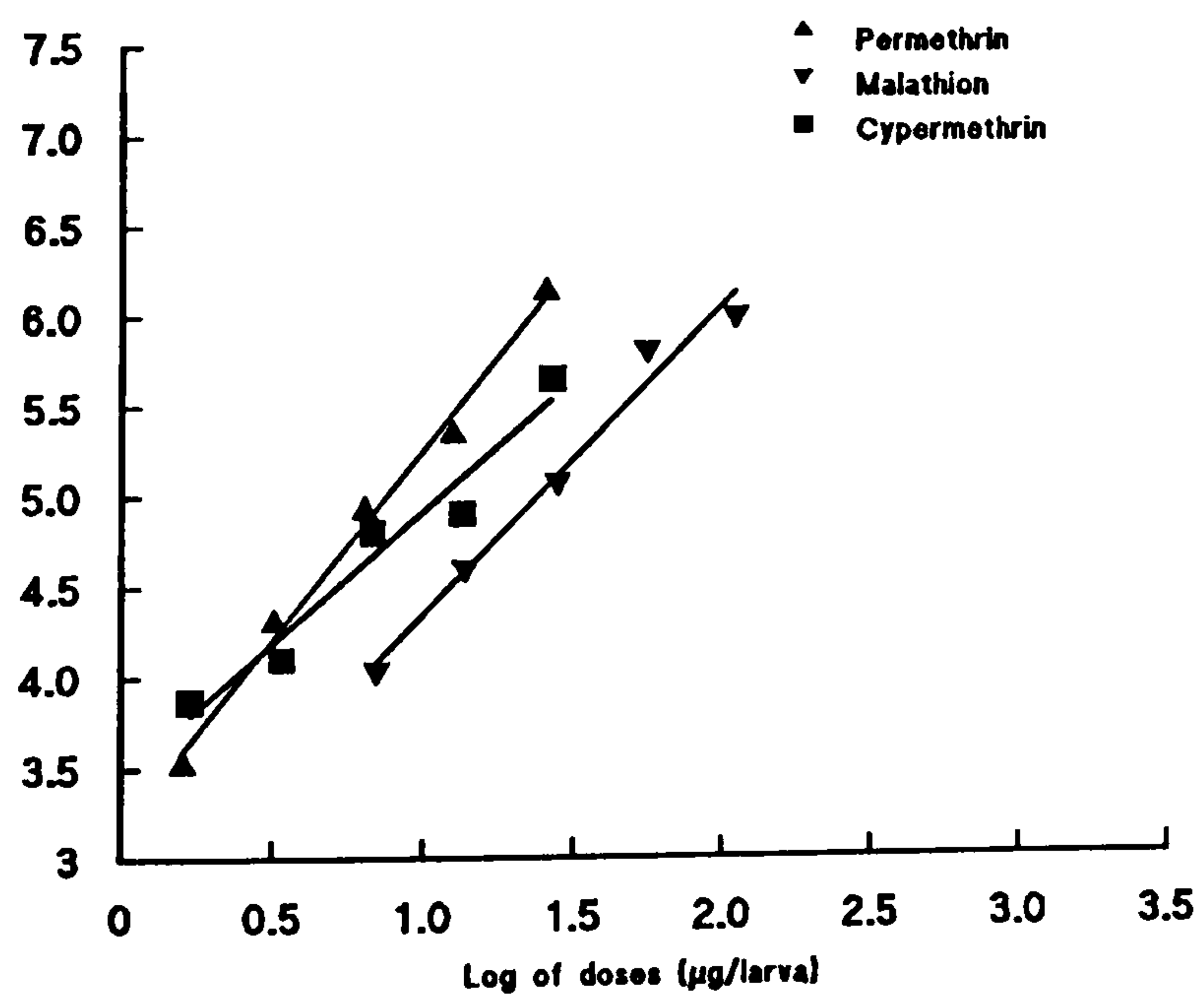


Figure 7. Responses of 4th instar larvae reared on artificial diet to topically applied β -naphthylflavone and insecticide.

Table 1. Regression equations of probit mortality curve of larvae treated with cypermethrin

Food plant	Equations	
	Larvae reared on plants	Larvae fed on plants for 24h
Diet	$Y = 4.01 + 1.76X$	$Y = 3.66 + 1.60X$
Cowpea	$Y = 4.05 + 1.76X$	$Y = 3.29 + 1.75X$
Cabbage	$Y = 3.98 + 1.39X$	$Y = 3.18 + 1.63X$
Sorghum	$Y = 4.01 + 1.01X$	$Y = 3.89 + 1.62X$
Broad bean	$Y = 3.85 + 1.14X$	$Y = 2.88 + 2.17X$
Soy bean	$Y = 3.96 + 1.37X$	$Y = 3.09 + 2.00X$
Maize	$Y = 2.56 + 1.46X$	$Y = 3.68 + 1.72X$

Table 2. Regression equations of probit mortality curve of larvae treated with permethrin

Food plants	Equations	
	Larvae reared on plants	Larvae fed on plants for 24h
Diet	$3.42 + 1.77X$	$3.43 + 1.73X$
Maize	$1.04 + 2.49X$	$3.14 + 2.25X$
Cowpea	$2.74 + 2.27X$	$3.20 + 1.79X$
Cabbage	$3.17 + 3.17X$	$2.88 + 1.68X$
Sorghum	$2.88 + 1.66X$	$3.10 + 2.47X$
Broad bean	$2.85 + 1.95X$	$2.53 + 2.05X$
Soy bean	$2.84 + 2.29X$	$2.59 + 2.31X$

Table 3. Regression equations of probit mortality curve of larvae treated with malathion

Food plants	Equations	
	Larvae reared on plants	Larvae fed on plants for 24h
Diet	3.55 + 1.76	2.11 + 1.87X
Maize	2.55 + 1.39X	3.65 + 1.94X
Cowpea	3.37 + 1.55X	4.11 + 1.41X
Cabbage	2.99 + 1.55X	2.05 + 1.75X
Sorghum	2.82 + 1.47X	2.13 + 1.81X
Broad bean	2.79 + 1.48X	3.80 + 1.62X
Soy bean	3.79 + 1.21X	3.65 + 2.12X

Table Regression equations of probit mortality curve of larvae treated with PBO, barbital and β -naphthoflavone

PBO + INSECTICIDE (1:1)	
PBO + cypermethrin	1.52 + 1.90X
PBO + permethrin	3.07 + 2.15X
PBO + malathion	1.10 + 1.53X
PBO + INSECTICIDE (1:5)	
PBO + cypermethrin	1.54 + 2.32X
PBO + permethrin	2.11 + 1.78X
PBO + malathion	2.25 + 2.03X
β -NAPHTHOFLAVONE + INSECTICIDE	
β -naphthoflavone	
+	
cypermethrin	3.46 + 1.45X
β -naphthoflavone	
+	
permethrin	3.17 + 2.08X
β -naphthoflavone	
+	
malathion	2.64 + 1.70X

APPENDIX THREE

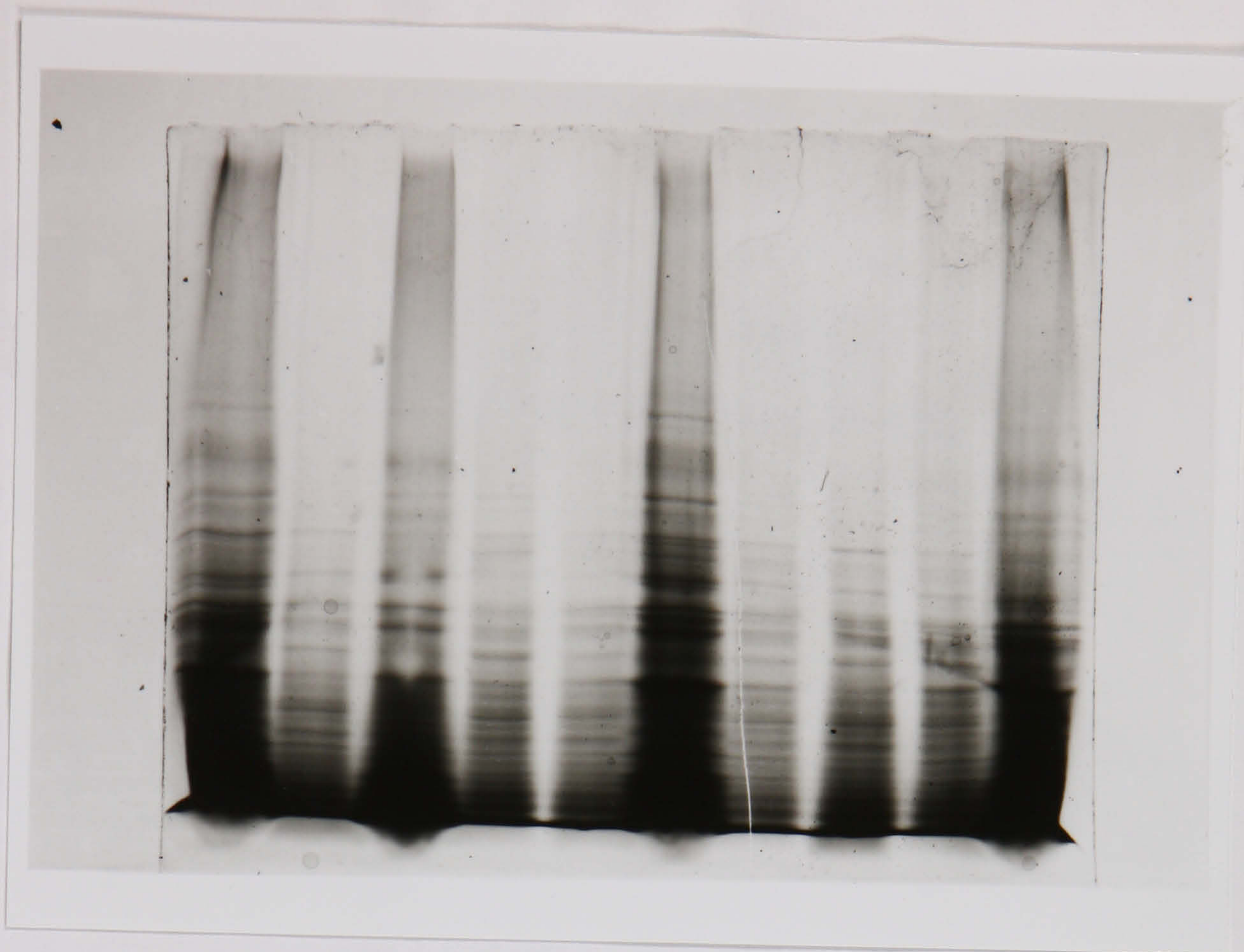
Table 1. Durations of larval instars on food plants

		Durations (days) Mean (SE)						
		INSTARS						
		1st	2nd	3rd	4th	5th	6th	7th
Bb		1.2 (0.2)	2.4 (0.3)	2.3 (0.3)	2.7 (0.6)	3.3 (0.5)	2.5 (0.5)	
Cab		2.2 (0.3)	3.1 (0.4)	4.2 (0.4)	4.1 (0.3)	3.4 (0.6)	4.4 (0.7)	
Cow		2.8 (0.3)	2.2 (0.5)	3.5 (0.4)	3.6 (0.5)	3.2 (0.3)	3.7 (0.3)	
So		2.4 (0.3)	2.2 (0.5)	3.5 (0.4)	3.6 (0.5)	3.2 (0.5)	3.7 (0.3)	
Ma		2.7 (0.4)	2.9 (0.4)	3.6 (0.6)	3.2 (0.4)	4.1 (0.3)	4.1 (0.6)	
		4.2 (0.9)						
S		3.2 (0.4)	3.2 (0.5)	2.7 (0.4)	4.8 (0.7)	3.9 (0.5)	4.6 (0.6)	
		5.2 (1.0)						

Durations were determined by presence of shed headcapsules.

BB (broad bean); Cab (cabbage); Cow (Cowpea); So (Soy bean);
Ma (Maize); S (sorghum)

A



B

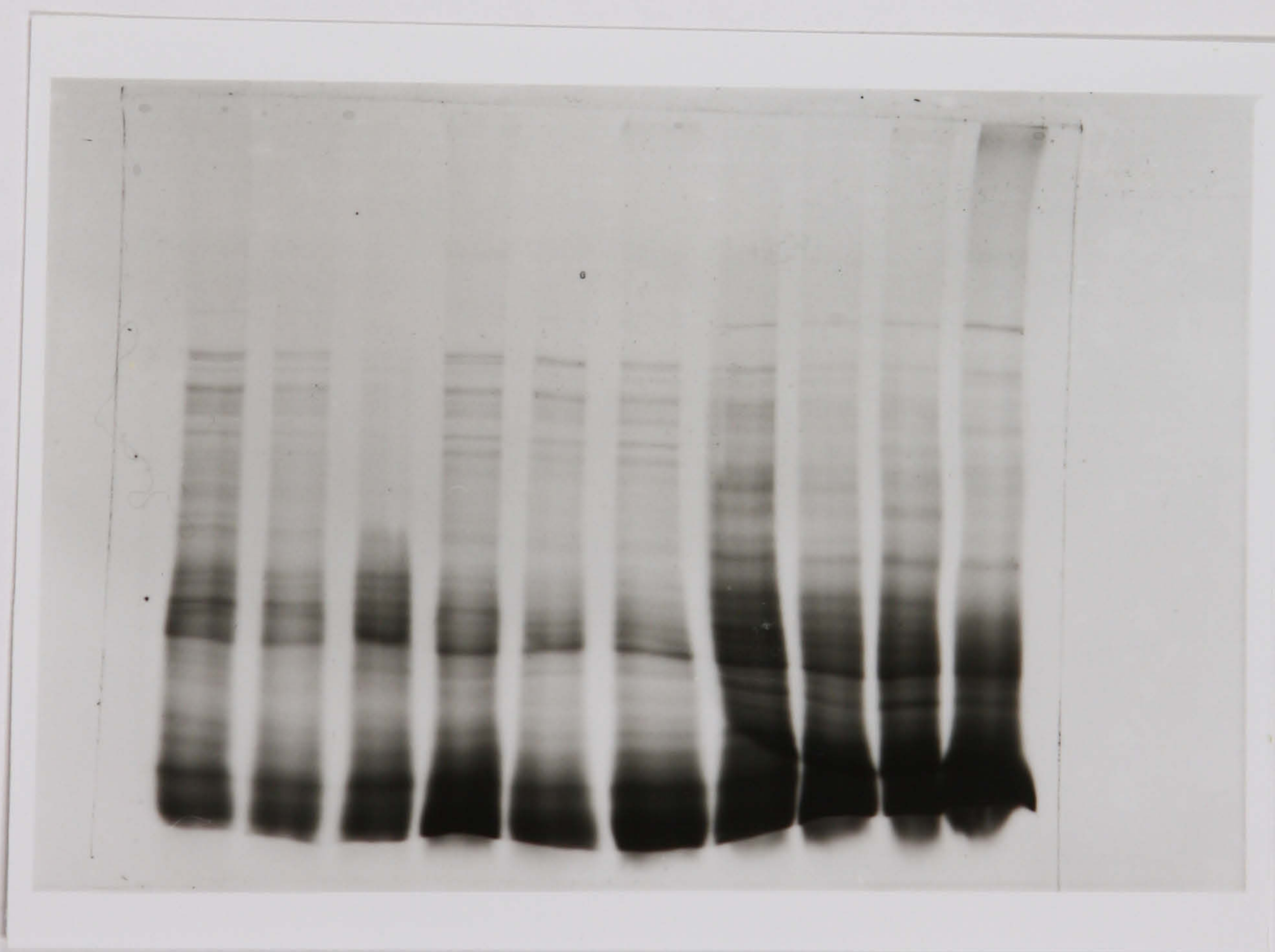
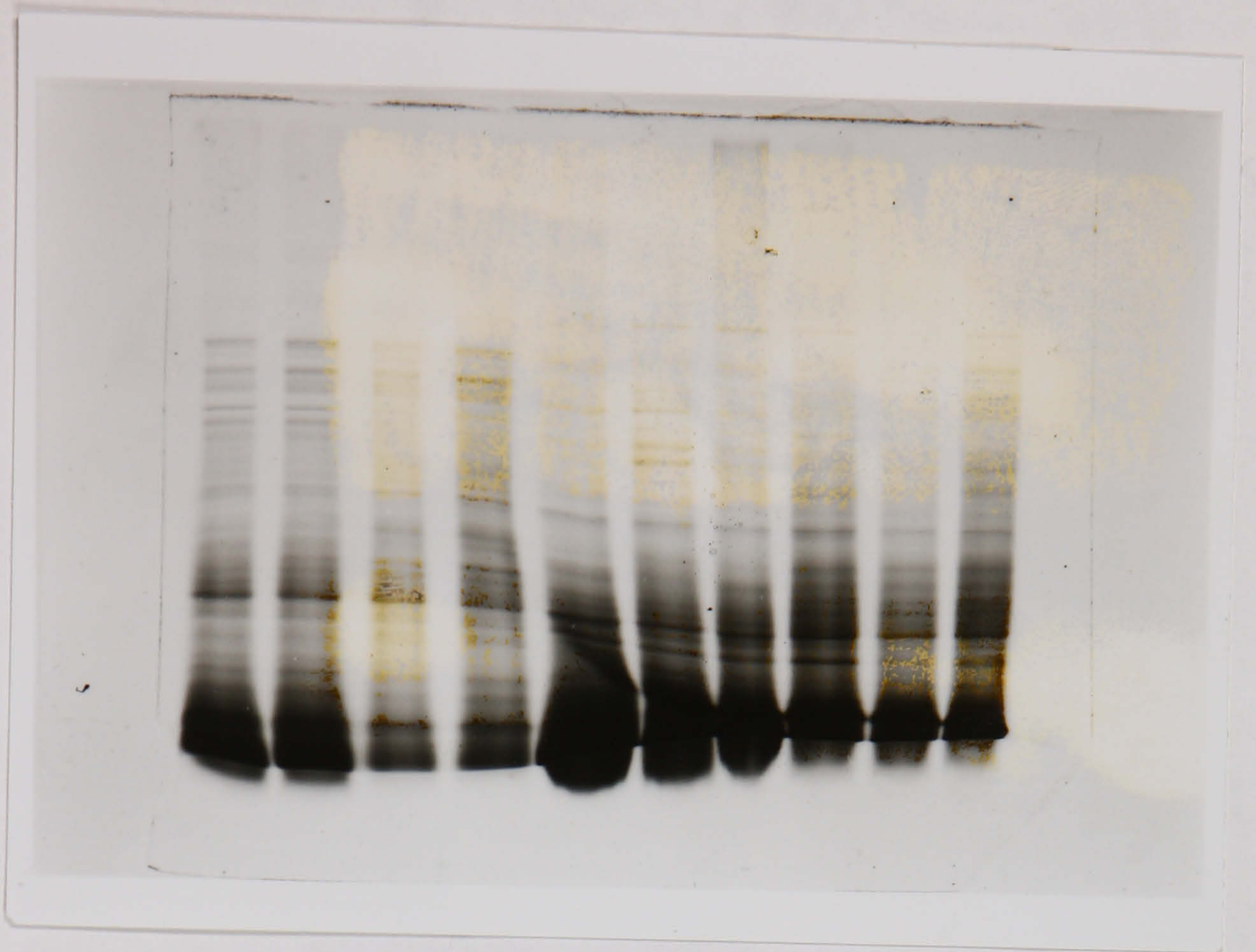


Figure 1. Representative peptide profiles of microsomal preparation from larvae reared entirely on food plants (A) or fed for 48 h on the plants (B).

A



B

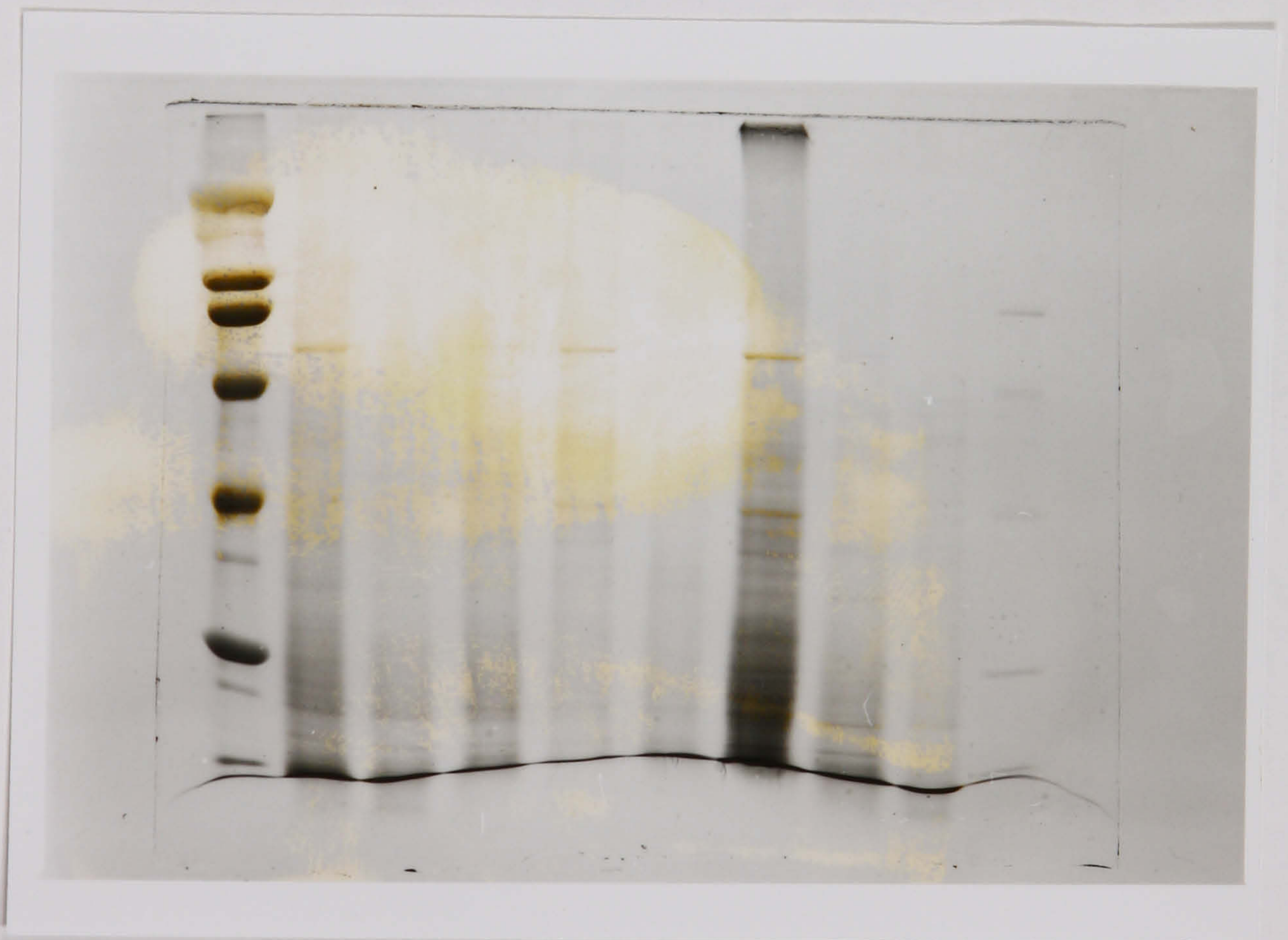


Figure 2. Representative peptide profiles of microsomal preparation from larvae reared entirely on food plants (A) or treated with various inducers and inhibitors of P-450 (B).