EFFECTIVE USE OF *BACILLUS THURINGIENSIS* BERLINER SUBSP. *KURSTAKI* FOR CONTROL OF DIAMONDBACK MOTH (*PLUTELLA XYLOSTELLA*) (L.) (LEPIDOPTERA: PLUTELLIDAE) ON CABBAGE

by

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EFFECTIVE USE OF *BACILLUS THURINGIENSIS* BERLINER SUBSP. *KURSTAKI* FOR CONTROL OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: PLUTELLIDAE) ON CABBAGE.

DEDICATION

This thesis is dedicated to my wife Christiane.

DECLARATION

I declare that, apart from references, which were rightly cited, this work is the sole effort of mine and has not been presented anywhere else either in whole or in part for the award of a degree.

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ABSTRACT

Two field experiments were carried out at Weija Irrigation Company (Ghana), from February to November 1995 on the effective use of *Bacillus thuringiensis* Berliner subsp. *kurstaki* (Dipel 2X) against the diamondback moth *Plutella xylostella* on cabbage (*Brassica oleracea* var. *capitata* (L.).

In the first experiment, different concentrations (0.25 g/l, 0.5 g/l, 1.0 g/l and 2.0 g/l) of *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.*) were applied at weekly and fortnightly intervals on cabbage plots. In all the treatments, *B.t* applied at weekly intervals was more effective in controlling *Plutella xylostella* larvae than those sprayed every fortnight. The highest harvestable heads of 92.3% was recorded on plots sprayed with 1.0 g/l at weekly intervals. On the control plots only 32.9% of the heads were harvested. Yellow sticky traps used to sample adult diamondback moth and other insects were found to be effective. In all 109 different insect species belonging to 8 orders were collected.

In the second experiment 1.0 g/l of *Bacillus thuringiensis* was applied weekly on cabbage at different stages of the cabbage growth. Cabbage plots treated at 3 and 5 weeks after transplanting did not show any significant difference in yield compared to cabbages that received treatment a week after transplanting. Depending on consumer preference cabbages that were not treated after 5 weeks resulted in yield reduction of about 70% where cabbages with minor damages are accepted and over 95% reduction for premium cabbage.

A weekly spray of Dipel 2X at 1.0 g/l, commencing not later than 5 weeks after transplanting is therefore recommended. It is also suggested that, yellow sticky traps could be used together with *Bacillus thuringiensis* in integrated management of diamondback moth, since *B.t.* does not affect adult *Plutella xylostella.*

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CHAPTER 1

INTRODUCTION

Cabbage *Brassica oleracea* var. *capitata* (L) is a biennial herb (Rice *et al.*, 1993) which as a vegetable is treated as an annual (Sinnadurai, 1992). The crop has a short thickened stem surrounded by a series of overlapping expanded leaves, which form a compact head. Head shape may be pointed or round and leaf colour and shape are variable (Rice *et al.*, 1993).

According to Hommes (1983), there are 23 pest species of cabbage, including 15 Lepidoptera, seven Homoptera and one Hymenoptera. The most serious damage is caused by *Mamestra brassicae* (L.), *Plutella xylostella* (L.), *Artogeia rapae* (L.), *Brevicoryne brassicae* (L.) and *Aleyrodes proletella* (L.) (Hommes, 1983). The diamondback moth *Plutella xylostella* (L.), (Lepidoptera: Plutellidae), is a cosmopolitan and key pest of cruciferous vegetables in numerous countries. The female lays eggs mostly on the lower surface of leaves and the first instar larvae remain as leaf miners. After moulting to the second instar, they become surface feeders and feed on the lower epidermis (Abro *et al.*, 1992). Insecticides have been extensively (excessively) used to control the Diamondback moth and as a result the pest has developed resistance to almost all groups of insecticides available, including *Bacillus thuringiensis* Berliner subsp. *kurstaki* (Sun *et al.*, 1978; Liu *et al.*, 1981; Miyata *et al.*, 1982; Tabashnik *et al.*, 1987; Zhu *et al.*, 1991; Shelton and Wyman, 1992). Resistance occurs because of the activity of different microsomal oxidase enzymes in the diamondback moth larvae (Cheng, 1988) and the physiological and behavioural responses of both *P. xylostella* larvae and adults (Tabashnik *et al.*, 1987; Moore and Tabashnik, 1989). According to Kao *et al.* (1990), two synthetic pyrethroids: deltamethrin and flucythrinate used in insecticide trials had not only lost their effectiveness against *P. xylostella* but treatment resulted in higher

numbers of the pest by reducing both the competition from other pests for the crop and attack by natural enemies.

In Ghana, the resistance of *P. xylostella* to several chemicals is a big problem. The Weija Irrigation Company, which specialises in the cultivation of several vegetables on a large scale, had to suspend the growing of cabbage, their most lucrative crop in 1991, due to their inability to control insect pests, especially *P. xylostella*. The situation is not different from small individual farmers' fields, where various insecticides are sprayed at twice or three times the normal dosage and between 2 to 3 days intervals. The result is the increasing resistance by *P. xylostella* to these insecticides, apart from the environmental hazards. With the rate of development of resistance of *P. xylostella* to insecticides, it is desirable to establish an integrated pest management (IPM) system to reduce the dependence on synthetic insecticides. Some advantages of reducing dosages of chemical insecticides are the subsequent reduction of environmental contamination and their effects on predaceous and parasitic arthropods. Biological agents such as *Bacillus thuringiensis* (*B.t.*) have been shown to have little or no effect on such beneficial arthropods (Jaques, 1965). The safety of *B. thuringiensis* to humans, mammals and other non-target animals permits its use on vegetables immediately before harvest (Roberts *et al.*, 1991). Preparations of the bacterium and the endotoxin can be used near or on streams or ponds used by fish, wildlife or livestock (Roberts *et al.*, 1991). The effectiveness of *B. thuringiensis* against *P. xylostella* has been demonstrated in various studies (Langenbruch, 1984; Kao *et al.*, 1990; Sastrosiswojo and Nuswantara, 1986).

As the use of *B. thuringiensis* product is new in the country, appropriate application techniques are important to ensure their efficacious long-term use. Initial trials of insects susceptibility to *B. thuringiensis* are necessary to establish a baseline information on the sensitivity of different insects to *B. thuringiensis* based products. This information can be

used to develop appropriate resistance management programme against the *P. xylostella* larvae. Information on the critical period of infestation of *P. xylostella* under *B. thuringiensis* and its effect on yield of cabbage is also necessary to enable *B. thuringiensis* to be applied at the right stage of the cabbage growth to reduce waste and cost of treatment. In Ghana, despite the devastating nature of *P. xylostella*, very little published information is available on this pest. Nevertheless, some aspects of its biology have been investigated (Oppong-Boateng, 1993 unpublished) and evaluation of various synthetic insecticides to control the pest has also been carried out (Dankwah, 1991 unpublished).

Information on biological insecticides, together with the critical period for their application, is vital for a possible integration of these methods for the management of *P xylostella*. Unfortunately these pieces of information are lacking in Ghana.

OBJECTIVES

The objectives of this study were therefore to determine:

1. The effect of different dose levels and spray interval of *Bacillus thuringiensis* on the abundance of *Plutella xylostella*.
2. The insect fauna of cabbage under *Bacillus thuringiensis* spray.
3. The critical stage of the growth of cabbage, for the initiation of protection against *Plutella xylostella* under *Bacillus thuringiensis* spray.

CHAPTER 2

LITERATURE REVIEW

2.1 History of cabbage in Ghana

The common cabbage, *Brassica oleracea* var. *capitata* (L.) is believed to have originated from Europe where the wild types are still found in Denmark, north-western France and eastern England (Sinnadurai, 1992). According to Sinnadurai (1992), the British probably introduced the crop into Ghana. There is no record of its introduction but the crop was grown on a small scale around 1940 and production increased during the Second World War (Sinnadurai, 1992). It is not a popular crop in the rural areas but it is popular in urban areas (Sinnadurai, 1992).

2.2 Soil and climatic requirements of cabbage

Soils used for cabbage cultivation should be well provided with organic material and should have good moisture holding properties. A pH of approximately 6.5-6.8 is considered best. Although many cabbage cultivars are tolerant to varying soil conditions, acid soils are not generally suitable (Rice *et al.*, 1993).

Cabbage is a cool season crop. It does well when the climate is cool and moist with an average monthly temperature of 16 to 21°C (Sinnadurai, 1992). However, some cabbage cultivars can withstand temperatures in excess of 30°C but head formation is more likely to occur at temperatures lower than 24°C (Rice *et al.*, 1993). A difference of approximately 5°C between day and night temperatures is necessary for adequate head formation (Rice *et al.*, 1993). Cultivars are generally classified as "early", "medium" or "late", based on the length of time taken to reach maturity (Rice *et al.*, 1993). Cultivars suitable for Ghana are

Copenhagen Market, Drumhead, Suttons Tropical, Japanese hybrid cabbage, Golden acre, Sutton pride of the market, KK cross (Sinnadurai, 1992).

2.3 Insect Pests of Cabbage

Mau and Martin-Kessing (1992), listed the major insect pests of cabbage under the following heading; scientific name, common name and group (Table 1).

Table 1 Insect Pests of Cabbage

Scientific Name	Common Name	Group
Brevicoryne brassicae	Cabbage aphid	Aphids
Lipaphis erysimi	Turnip aphid	Aphid
Macrosiphum euphorbiae	Potato aphid	Aphid
Myzus persicae	Green peach aphid	Aphid
Adoretus sinicus	Chinese rose beetle	Beetles
Listroderes difficilis	Vegetable weevil	Beetles
Leucopoecila albofasciata	A fleahopper	Bugs
Nezara viridula	Southern green stink bug	Bugs
Nysius nemorivagus	A lygaeid bug	Bugs
Spanagonicus albofasciatus	Whitemarked fleahopper	Bugs
Pycnoderes quadrimaculatus	Bean capsid	Capsids
Achaea janata	Croton caterpillar	Caterpillars
Agrotis ipsilon	Black cutworm	Caterpillars
Pieris rapae	Imported cabbageworm	Caterpillars
Chrysodeixis eriosoma	Green garden looper	Caterpillars
Helicoverpa zea	Corn earworm	Caterpillars
Hellula undalis	Imported cabbage webworm	Caterpillars
Peridroma saucia	Variegated cutworm	Caterpillars
Plutella xylostella	Diamondback moth	Caterpillars
Spodoptera exigua	Beet armyworm	Caterpillars
Trichoplusia ni	Cabbage looper	Caterpillars
Delia platura	Seedcorn maggot	Flies
Atractomorpha sinensis	Pinkwinged grasshopper	Grasshoppers
Liriomyza brassicae	Serpentine leafminer	Leafminers
Thrips tabaci	Onion thrips	Thrips
Bemisia argentifolii	Silverleaf whitefly	Whiteflies
Bemisia tabaci	Sweetpotato whitefly	Whiteflies

2.4 Insect fauna of cabbage

The insect fauna of cabbage comprised of 220 taxa, representing 186 genera from 92 families in 21 orders (Weires and Chiang, 1973). Weires and Chiang (1973), identified 178 insect species. According to them, of the total taxa, 160 were interacting in the cabbage community. Sixty were apparently transients or of unknown role. They further stated that, the cabbage food web consisted of 11 leaf feeders, 4 root feeders, 21 saprobes, 79 saccharophiles and 85 carnivores. Lepidoptera were the most important pests above ground. *Pieris rapae* (L) was the most abundant of these followed by *Plutella xylostella* (L) and *Tricoplusia ni* (Hb.) (Weires and Chiang, 1973).

2.5 Biology and behaviour of diamondback moth

Diamondback moths are small moths with wings narrowly rounded at the apex hind-wings and about as wide as forewings (Borror and White, 1970). The fore wings of the moth are often brightly patterned with light marks along costal margin of the wings which form diamond-shaped spots when the wings are folded over the abdomen, the basis for its common name (Borror and White, 1970).

The development time of *Plutella xylostella* from egg to adult emergence has been studied by various workers. According to Abro *et al.* (1992), the period varies between 11.93 and 21.1 days in the laboratory and is negatively correlated with temperature. Male pupal period was significantly greater than that of the female. The mean duration of copulation was 36.7 minutes and insects mated on average 2.45 times per night. They further found that, the average number of eggs laid by a female was 194.15 and more than 50% of eggs were laid on the first night of egg laying. Studies carried out by Choi *et al.* (1992) showed that, the population densities of larvae and pupae of *P. xylostella* were greatest from late June to July

in Suwon, Taiwan, where the experiment was carried out. They reported the length of development from egg to adult to be 38.1, 21.7, 16.3 and 12.3 days in females and 38.6, 22.3, 16.5 and 12.5 days in males at 15, 20, 25 and 30°C, respectively. The mean threshold temperature of egg-pupa was 8.1°C. They therefore concluded that *P xylostella* could have 8.4 generations per year in Suwon. However, it had been found that *P. xylostella* could have up to 4 and 17 generations per year in the temperate and tropical countries respectively.

Mating success of diamondback moths can vary greatly; some males may mate with more than 10 females (Yamada, 1979). Mating success of both sexes may be influenced by age (Yamada, 1979), size (Uematsu, 1990), production of sex pheromones (Chow *et al.*,1986) or other attributes that affect mating behaviour. In response to emission of female sex pheromones during the scotophase (Pivinick *et al.*,1990), male diamondback moths walk in front of females while vigorously fanning their wings (Ashihara, 1977). Male response to female sex pheromones varies among populations in the field but did not differ in malathion resistant populations in laboratory bioassays (Maa, 1986). Shirai (1991) studied seasonal changes and effect of temperature on flight ability of the diamondback moth. He measured flight in the laboratory using a flight mill and found that, flight duration and flight distance were longer during winter and spring than during summer. He stated further that flight velocity and the proportion of adults with long-time consecutive flight did not show a distinct seasonal change. However, flight duration and distances showed a significant correlation with fore wing length. The larger adults present during winter and spring had a higher capacity for long distance flight than the small adults present during summer, and the flight ability of the females were almost equal to that of males over the same periods. He estimated the optimum temperature suitable for flight activity of males as 23°C with a range from 18 to 28°C.

2.6 Damage and economic importance of Diamondback Moth

The first instar larvae of diamondback moth feeds in the spongy plant tissue beneath the leaf surface forming shallow mines that appear as numerous white marks (Mitchell *et al.*, 1997). These mines are usually not longer than the length of the body (Mitchell *et al.*, 1997). The larvae are surface feeders in all subsequent stages. These larvae feed on the lower leaf surface 62-78% of the time, chewing irregular patches in the leaves (Harcourt, 1957). All the leaf tissues are consumed except the veins (Mitchell *et al.*, 1997). On some leaves, the larvae feed on all but the upper epidermis creating a "windowing" effect (Mitchell *et al.*, 1997). The last stage larva is a voracious feeder; it causes more injury than the first three larval instars (Mitchell *et al.*, 1997). The Diamondback moth is the most destructive pest of cabbage and other crucifers throughout the world (Mitchell *et al.*, 1997). The annual cost for control of this pest is estimated to be U.S. $ 1 billion (Talekar and Shelton, 1993).

2.7 Resistance and susceptibility of diamondback moth to insecticides

Diamondback moth is now known to have developed resistance to a wide range of insecticides including *Bacillus thuringiensis* (Song, 1991; Perez, *et al.*, 1995; Yu and Nguyen, 1992). A strain of the diamondback moth, collected from cabbage fields in 1991 in North Florida, was examined for insecticide resistance (Yu and Nguyen, 1992). When compared with a laboratory strain, resistance to pyrethroids (permethrin, cypermethrin, fenvalerate, esfenvalerate, cyhalothrin and fluvalinate) ranged from 2132 to 82,475 fold; the highest resistance observed was to fenvalerate. The resistance level observed for organophosphates (chlorpyrifos, methyl parathion, malathion, methamidophos and diazinon) ranged from 20 to 73 fold and was highest to diazinon. Resistance to the carbamates and carbofuran was 409 and 405, respectively (Yu and Nguyen, 1992). Resistance to the cyclodiene endosulfan was 25 fold. Synergist studies show that piperonyl butoxide (microsomal oxidase inhibitor) greatly

reduced the resistance. No resistance to *B. thuriengiensis* subsp. *kurstaki* was observed in the field strain (Yu and Nguyen, 1992). Detoxification enzyme assays revealed that activities of microsomal oxidases (epoxidases, hydroxylase, sulfoxidase, N-demethglase, o-deakylase), glutathione transferases (DCNB and CDNB), esterase (acetylecholinesterase) and reductase (juglone reductase and cytochrome C reductase) were 1.4 to 20.7 fold higher in the field strain than in the susceptible strain. In addition, the bimolecular rate constants for inhibition of acetylcholinesterase by dichlorvos, carbaryl and methomyl ranged from 1.7 to 2.8 fold higher in the susceptible strain than in the field strain. The results indicated that the broad spectrum insecticide resistance observed in the strain was due to multiple resistance mechanisms, including increased detoxification of these insecticides by microbial oxidases, glutathion transferases and reductases and target site insensitivity (Yu and Nguyen, 1992). The susceptibility of *P. xylostella* collected from 3 sites in Osaka prefecture, Japan to *B. thuringiensis* formulation was determined using third instar larvae on watercress (Tanaka and Kimura, 1991). According to the researchers larvae showed high resistance LC 50>280 ppm to the insecticide. They attributed the high resistance to *B. thuringiensis* by *P. xylostella*, to the frequency of spray of *B. thuringiensis* formulation (15-20 times) a year on watercress.

Ferre *et al.* (1991) studied the biochemical mechanism of resistance of *P. xylostella* to *B. thuringiensis*. They used *P. xylostella* originating from an area of the Philippines where the Plutellid had repeatedly been exposed to the microbial insecticides. The field population proved to be >200 fold resistant to Cryla (b) (one of three proteins studied) as against a susceptible laboratory strain. According to the researchers, the crystal protein did not bind to the brush border membrane of the mid-gut epithelial cells of the field population either because of strongly reduced binding affinity or because of the complete absence of the receptor molecule.

To determine whether field-selected resistance of *P. xylostella* to *B. thuringiensis* was based on behavioural or physiological adaptation, Schwarz *et. al.* (1991) measured mortality, food consumption, movement of larvae from susceptible and a resistant colony on untreated and *B. thuringiensis* treated cabbage leaf discs. They found that colonies did not differ in mortality, food consumption or movement on untreated cabbage. However, for a given amount of consumption of treated cabbage, the mortality of resistant larvae was lower than that of the susceptible larvae, which demonstrated clearly that the resistance had a physiological basis. The movement patterns of larvae did not account for the differences between colonies in survival. Resistant larvae did not avoid *B. thuringiensis* treated leaf disc more than susceptible larvae, a response which provided no evidence for behavioural resistance.

2.8 Management / control of *Plutella xylostella*

The three main management or control methods of diamondback moth are:

- The use of trap crops (intercroping)
- Insecticides: synthetic, microbial / biological
- Natural enemies - parasitoids

2.8.1 Trap crops (intercroping)

In field trials in summer in Bangalore, Karnataka, India, it was shown that by growing cabbage intercropped with mustard (*Sinapis alba* L.), the number of insecticide applications against the diamondback moth could be reduced from 25 to 8 with considerable increase in yield (Khan *et al.*, 1991). Srinivasan and Moorthy (1991) also found that Indian mustard is a preferred host for oviposition by *P. xylostella* when compared with cabbage in a laboratory study. In three field trials, different plant patterns of both cabbage and mustard were used to

investigate whether mustard could act as a trap crop. The results revealed that cabbage grown alone supported significantly higher larval populations of the pest in comparison with cabbage intercropped with mustard. They recommended that, a planting pattern of 15 rows of cabbage followed by mustard rows was the most promising for successful management of *P. xylostella*. The effects of non-host plant neighbours on population densities and parasitism rates of *P. xylostella* were studied in Hawaii. According to Bach and Tabashnik (1990), monospecific cabbage plots had greater larval densities and, lower numbers of larvae parasitized by *Cotesia plutellae* (Hymenoptera: Braconidae), compared with plots of cabbage interplanted with tomato, *Lycopersicon esculentum* (Mill.). They subsequently carried out a laboratory experiment, which showed that oviposition females did not discriminate between cabbage grown alone versus cabbage grown with tomato. They suggest that tomato neighbours affect long range host finding or early egg-larval survival or both in the field as well as parasitism rates.

2.8.2 Insecticides

2.8.2.1 Synthetic insecticides

Synthetic insecticides are major tools in diamondback moth management, but their use must be minimised to slow down pesticides resistance development (Idris and Grafius, 1993). Armstrong (1990) reported that the greatest number of marketable heads of cabbage was obtained from plots treated with permethrin at 0.468 l/ha, methamidophos at 2.34 l/ha or fenvalerate at 0.351 l/ha. Cameron (1989) carried out a study on alternative insecticides for control of lepidoptera on cabbages. He reported that the alternative insecticides such as *Bacillus thuringiensis* formulations (Thuricide, Bactospeine) provided significantly less control and generally lower quality cabbages than permethrin (synthetic insecticides). He suggested that due to pattern of insecticide resistance development overseas, it is important to

retain the effectiveness of synthetic pyrethroids and preserve growth regulators for future use in New Zealand. However, as has been pointed out earlier, recent difficulties being experienced with insecticides, such as increasing costs, resistance development, environmental hazards etc. are making synthetic insecticides unpopular in *P. xylostella* management.

2.8.2.2 Microbial insecticides

The main reason for turning to microbial control agents is the current difficulties with chemical insecticides. Among the various difficulties include, environmental hazards, development of resistance or tolerance to pesticides by target insects, withdrawal of registration of insecticides because of newly discovered hazards, lack of new chemicals due to high costs of development. Microbial products, on the other hand, have limited host range, are biodegradable, have much lower registration cost than chemical insecticides and usually fit in well with pest control projects based on the concept of integrated pest management (IPM) (Roberts *et al.,* 1991). Microbial products currently constitute approximately 2 % of the world pesticide market (Payne, 1988). The pathogens currently utilised or those under development as microbial agents are found in four groups: bacterial, nematodes, virus and protozoa (Roberts *et al.,* 1991). The study of bacterial diseases of insects began in the nineteenth century with investigations of diseases of silk worm, *Bombyx mori* (L.) (Pasteur, 1870) and of the honeybee (Cheshire and Cheyne, 1885). These studies were concerned with the eradication of diseases from populations of these domestic insects, but they did draw attention to the mortality that bacteria could cause. The infection of insects by bacteria is influenced by certain physical factors of the environment; temperature affects multiplication of the bacteria and the production and activity of toxins and enzymes (Jaques, 1965). Humidity has little effect on infection by bacterial pathogens (Jaques, 1965).

Studies on the mode of action of the toxin of crystalliferous bacteria (Angus, 1956; Angus and Heimpel, 1959; Heimpel and Angus, 1959); showed that, when cultures of varieties of *B. thuringiensis* are ingested by an insect having alkaline gut contents (pH 9.0 to 10.5); as do most lepidopterous larva. The crystals contained in the sporangia are dissolved and the toxin is released. The gut is paralysed by the toxin, stopping feeding in the insect within an hour after ingestion of bacterial culture or within one to seven hours in few insects, such as *B. mori.* The insect therefore dies from septicaemia in 2 to 4 days. The insecticidal activity of *B. thuringiensis* is related to the rhomboid crystal in the sporangium (Angus, 1954; Hannay, 1954). The crystal endotoxin or delta endotoxin is a high molecular weight protoxin protein, which is transformed to the toxic state in the gut lumen and then causes paralysis in the gut, followed by general paralysis and death by toxaemia or septicaemia. Activity of delta endotoxin is dependent on the ingestion of the crystal by the target insect. Its structure and activity have been studied extensively (Heimpel and Angus, 1960; Fast, 1981; Faust and Bulla, 1982).

Bacillus thuringiensis may produce three toxins in addition to the delta endotoxin: the beta endotoxin or "fly factor" which is heat-stable and water-soluble; the alpha-exotoxin (lecithinase C) which is heat labile and "the louse factor" (Dulmage and Co-operators, 1981). The endotoxins produced by different isolates of *B. thuringiensis* differ in activity spectra. Jaques *et al.*, (1987) suggested that the specificity is dependent on the strain-related origin of the toxin, the degree of solubility of the crystals in the gut and the intrinsic susceptibility of the insect to the toxin. Midgut (brush border) receptor sites specific to delta-endotoxins with different amino acid sequences has been recently reported (Hofmann *et al.*, 1988). Strains of *B. thuringiensis* that produce endotoxins active against certain groups of insects have been identified (Dulmage and Co-operators, 1981; Dunkle and Shasha, 1988). For example, the endotoxin produced by the NRD-12 isolate of *B. thuringiensis* appears to be more effective

than HD-1 (the most commonly used commercial isolate) on gypsy moth, *Lymantria dispar* tobacco, budworm, *Heliothis virescens* and spruce budworm, *Choristoneura fumiferana* but it is similar to HD-1 in potency against the cabbage looper, *Tricoplusia ni* (Dubois 1985 a,b; Dulmage *et al.*, 1985).

One of the best established uses of *B. thuringiensis* in agriculture is to control cabbage looper (*T. ni*), imported cabbage worm (*Pieris rapae*) and diamondback moth (*Plutella xylostella*) on cruciferous crops and to control cabbage looper on several other crops such as celery and tomatoes. Application of *B. thuringiensis* usually protect cabbage, collards and other crops against these pests to the same extent as applications of chemical insecticides (Creighton *et al.*, 1970; Jaques, 1973, and 1977; Tompkins *et al.*, 1986). *Bacillus thuringiensis* and chemical pesticides in a treatment regime for cabbage were found to be a promising method for reduction of the use of chemical pesticides (Jaques 1972, 1973, 1977 and 1988).

The effectiveness of sprays of several bacterial and chemical insecticides in controlling *P. xylostella* and other insect pests on cabbage was evaluated in field plot tests in the Philippines during the dry season of 1972. Weekly applications of Dipel (a preparation of *B. thuringiensis*) at the rate of 0.5 Kg active ingredient /ha from transplanting to harvest gave the best control and resulted in the highest yields of marketable heads (Cadapan and Gabriel, 1972). When each stage of *P. xylostella* was treated with phenthoate 47.5% EC, *B. thuringiensis* 16 (BIV) wp, cartap 50% sp and cypermethrin 5% EC, eggs and pupae were not controlled but mortality of larvae varied among larval instars. Susceptibility of first to second instar larvae was high and that of third to fourth instar larvae was low. Control with *B. thuringiensis* was found to be greatest giving the highest mortality when 4 sprays were applied at 10 days intervals (Choi *et al.*, 1992).

Studies on the compatibility of *B. thuringiensis* and insecticide chemicals have been carried out. Most insecticides were compatible with *B. thuringiensis* having little or no effect on spore germination or cell multiplication (Jaques and Morris, 1981). Low concentrations of some carbamates and organophosphates for example, either did not affect bacterial growth or improved it whereas others, especially the chlorinated hydrocarbons (DDT, aldrin, heptachor), inhibited growth (Jaques and Morris, 1981). Varma and Gill (1980) carried out a study, on the additive effect of pesticides on *B. thuringiensis* formulations for the control of *P. xylostella*. They reported that addition of 0.5 and 1.0 ml malathion and 2.0 and 2.5 g zineb (Dithane) per litre of water in a spray containing *B. thuringiensis* var. *kurstaki* (Thuricide Hpsc) at 1 g/ litre had no synergistic or antagonistic effect on the latter under laboratory conditions. In the field, the toxicity of *B. thuringiensis* var. *kurstaki* (Dipel) decreased when zineb was added; no synergistic or antagonistic effects were observed with any of the other formulations tested. Mortality of 90% or more was only obtained with Thuricide HSPC and its combinations, Dipel and Dipel combinations plus malathion. They reported that even in these treatment effectiveness started to decline on the third day. Krishnaiah *et al.*, (1981), found that weekly sprays of Dipel at 0.5 Kg/ha were fairly effective in controlling *P. xylostella* on cabbage and were comparable with fortnight sprays of methamidophos and quinalphos at 0.5 Kg/ha. They reported that better control was achieved when Dipel was sprayed in combination with chlordimeform, both at 0.25 Kg/ha.

Several formulations of *B. thuringiensis* are now registered in the U.S.A and Canada. Commercial formulations of the HD-1 strains of var. *kurstaki*, including Thuricide, Dipel, Novabac, Futura, Envirobac and Bactospeine are specially designed for use against certain lepidopterous pest of forest, agricultural crops, stored grains, ornamentals or home gardens (Roberts *et al.*, 1991). A noteworthy disadvantage of *B. thuringiensis* as a microbial insecticide for use against leaf-eating pests is that following applications the bacterium

persists on the foliage for only about one week in numbers sufficient to cause toxaemia or septicaemia in significant proportion of the insect population (Jaques and Fox, 1960).

2.8.3 Parasitoids

Historically, diamondback moth was held below economic thresholds in the United States of America by natural enemies (Marsh, 1917). In Southern Ontario, Canada, *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) is a major parasitoid of diamondback moth (Harcourt, 1960, 1963) and parasitizes up to 75% of diamondback moth larvae (Harcourt, 1969, 1986; Bolter and Laing, 1983; Lasota and Kok, 1986). In Indonesia *Diadegma semiclausum* (eucerophage) (Horstmann) kills approximately 85% of diamondback moth larval population (Sastrosiswojo and Sastrodiharjo, 1986). In Thailand, *Cotesia plutella* was reported to parasitize up to 32.4% of diamondback larvae (Keinmeesuke *et al.*, 1990). An egg parasite *Trichogrammatoidea bactrae* (Hymenoptera: Trichogrammatidae) was found for the first time in Thailand, and was reported to parasitize between 16.2-45.2% (Keinmeesuke *et al.*, 1990). *Diadromus subfilicornis* (Gravenhorst) (Hymenoptera: Braconidae) also attack diamondback moth (Idris and Grafus, 1993).

According to Harcourt (1969, 1986), high synchronisation of *D. insulare* with the developmental stages coupled with its excellent searching capability make it more suitable for use as an alternative and supplemental method in integrated diamondback moth management. In a field study in Taiwan where cabbage planting was confined to a large net house, the rate of parasitism of *P. xylostella* by *D. eucerophage* increased from 13.1% one month after planting to 65.4% just before harvest (Talekar and Yang, 1990). Talekar and Yang (1991), studied the characteristics of parasitism of diamondback moth by two larval parasitoids. They reported that parasitism was high at 15-25°C in *D. eucerophaga* and at 20-35°C in *C. plutellae*. Both parasitoids searched actively for host and oviposited only in the light. They

further stated that, while *A. plutellae* parasited all four instars of larvae, *D. eucerophage* attacked only the first three instars. *D. eurcerophaga* parasitism was greater when larvae were feeding on common cabbage than when they fed on cauliflower, broccoli or Chinese cabbage. Detoxifying enzymes and susceptibility to three insecticides by *Cotesia plutella* and *Diadegma semiclausum*, have been determined (Chiang and Sun, 1991). They reported that both insects were susceptible to malathion and methyl parathion and were considerably tolerant to fenvalerate. According to Talekar and Yang (1991), deltamethrin, a non-selective insecticide was toxic to adults of both parasitoids, but the selective teflubenzuron, pirimicarb and *Bacillus thuringiensis* were not.

2.9 The critical period of protection against *Plutella xylostella*

The critical period of infestation of *P. xylostella* and its effect on the yield of cabbage were determined in Costa Rica in 1988-89. According to Carballo and Hruska (1989), plots where no insecticide treatment was applied in the first and second stage (pre-formation of heads) gave yields which were not significantly different from those obtained in plots which received treatment during the whole cycle. When no protection was applied in the third stage of the crop (formation of heads), a reduction of over 73% in the yield was observed during the first and the second stages (0.35 and 7.3 larvae and 5.2 and 49.0 perforations per plant, respectively) did not reduce the yield. The third stage, when levels of infestation were 0.3-5.2 larvae and 6.0-14.2 perforations per plant, was the critical stage when insecticides should be applied. The economic threshold for the third stage was 0.05-0.4 larvae and 0.2-1.28 perforations per plant, depending on the phenology and prices. Lumaban and Raros (1973), evaluated cabbage yields in the Philippines when sprays of *B. thuringiensis* had been applied during different growth periods of the crop. They revealed that damage by *P xylostella* (L) was most critical four to five weeks after transplanting and recommended that insecticide

should be applied to give protection during the critical periods of crop growth but not necessary for the entire growth period.

2.10 Yellow sticky traps

Experiments on the development of a sticky trap monitoring system for *Plutella xylostella*, were carried out in cabbage fields in North Sulawesi, Indonesia (Hallett *et al.*,1995). They reported that although the relationship between trap catches and larval populations for individual fields was weak, the relationship between catches in all fields pooled and larval populations two weeks later was strong, suggesting that a region-wide predictive system would be feasible. An increase in larval numbers as distance from traps increased further suggests that sticky traps may have potential for use in mass trapping (Hallett *et al.*, 1995).

CHAPTER 3

MATERIALS AND METHODS

EXPERIMENT 1

3.1 Site

The project was carried out from February 1995 to November 1995 at the Weija Irrigation Company (WEICO) at Tubaman, Weija near Accra. Two basic reasons guided the choice of site.

1. WEICO is a vegetable project (company) and large hectares of land are under cultivation. Cabbage is one of the major vegetables grown and over the years, synthetic insecticides have been used extensively and intensively to control insect pests including the diamondback moth. This pest is known to have developed resistance to those insecticides that are in use there (Mr. N. Gyadu, Officer-in-charge and farmers, personal communications).
2. Availability of regular water supply which is necessary for cabbage growth.

3.2 Nursery

3.2.1 Soil sterilisation

Soil collected under a tree from Sinna's garden at the Crop Science Department of the University of Ghana was put into sacks and sent to the Soil Science Department for sterilisation. This was done using an autoclave set at 125°C for 1 hour.

3.2.2 Nursing of seeds

K.K. cross cabbage seeds were nursed in ridges made in three seed boxes of dimensions 58 cm by 36 cm containing sterilised soil. The seeds were sown on the 2nd of March 1995 between 3 p.m. and 3.30 p.m. The seed boxes were covered with black net

immediately after sowing to reduce the direct rays of the sun from getting into contact with the seeds and also to prevent birds from scratching the surface to remove seeds.

Germination began after three days. The black net was then raised to allow more sunlight to penetrate and for free movement of air around the seedlings.

3.2.3 Pricking out

Seedlings were pricked out after a week into a bigger container of dimensions 112 cm by 84 cm containing sterilised soil. A starter solution containing 10 g of NPK dissolved in 34 litres of water was applied. The pricked plants were covered for two days with black net to reduce the intensity of sunrays to the seedlings. The black net was reused to fence the container of the pricked seedlings. This was done to prevent animals from destroying the seedlings.

3.3 Fieldwork

3.3.1 Land preparation

The land was ploughed twice. After the second ploughing the land was pegged and 36 beds of size 4.2 by 2.3 m were raised. The beds were then raked to break the big clods and to level them.

3.3.2 Transplanting

Seedlings were transplanted when they were four weeks old. The plot was irrigated in the morning and transplanting begun at 2.30 p.m. till 6.15 p.m. The transplanting could not be completed on the same day. It was therefore continued the following day.

3.3.3 Weeding

Weeding was carried out once a week with hoe after transplanting.

3.3.4 Fertiliser application

Sulphate of ammonia (NH_4SO_4) and 15-15-15 nitrogen-phosphorus-potassium (NPK) fertiliser were applied according to Sinnadurai (1992) recommendations. 24 g of NPK was applied in two split doses to each plant; the first dose, a week after transplanting and the second dose 20 days after application of the first one. A week after the second application, 12 g of NH_4SO_4 was applied to each plant.

3.3.5 Irrigation

For the first two weeks after transplanting, the crop was irrigated everyday using sprinklers. Thereafter they were irrigated every other day.

3.4 Field plan

Randomised complete block design (RCBD) was used.

Plot size	4.2 m by 2.3 m
Number of spray treatments	8
Number of replications per treatment	4
Total number of plots sprayed	32
Number of plots in one replicate	8
Number of control (unsprayed) plots	4

The control plots were sited 7 m away from the other plots to prevent spray drift.

Distance between plots in each replicate 1 m

Planting distance	60 cm within rows and 75 cm between rows
Plant arrangement	7 plants per row, 3 rows per plot.
Total number of plants per plot	21
Total number of plants per treatment	84
Record plants per plot	5 central plants
Total number of record plants	180
Total number of plants in experiment	756

3.5 Treatments applied

The biopesticide used was Dipel 2X [Abbot laboratories, North Chicago, Il], a wettable powder which contains *Bacillus thuringiensis* subsp. *kurstaki*. This organism occurs naturally in soils and other common environmental situations. It contains 32,000 I.U. (International unit) of potency per mg.

Four different concentrations of Dipel 2X were prepared with two spray intervals to give eight treatments and a control as follows:

1. Control (no spray)
2. 0.25 g/l every 7 days
3. 0.25 g/l every 14 days
4. 0.5 g/l every 7 days
5. 0.5 g/l every 14days
6. 1.0 g/l every 7 days
7. 1.0 g/l every 14 days
8. 2.0 g/l every 7 days
9. 2.0 g/l every 14 days

A label with the treatment inscriptions was placed at the middle portion in front of each plot as shown on Plate 1.

The quantities of Dipel 2X per litre of water to make up the desired concentrations were weighed at the Crop Science Department with an electronic scale. The weighed Dipel 2X were put into small white polythene sheets with dimensions 30 cm by 10 cm. The concentrations and the time interval of sprays were indicated on the polythene sheets with a permanent marker. The polythene sheets containing the Dipel 2X were brought to Weija for use. This was done every 2 weeks. Spraying was done using a CP 15 knapsack sprayer fitted with a cone nozzle with a flow rate of 1.83 min./l and application rate of 806.42 l/ha. An adhesive (Adral) at 2 ml / 4 litres of water was added to the Dipel spray. This was done to prevent the washing away of the *B. thuringiensis*, should there be any rainfall immediately after spraying. Watering was suspended for 24 hours after spraying. The first damage was observed two weeks after transplanting. The first spray was carried out a week after.

3.6 Data collection

3.6.1 Sampling of larvae and pupae

Sampling of larvae and pupae began four days after the first spray, thereafter it was done every week. Diamondback moth larvae and pupae and other larvae and pupae were collected from all the leaves of five central plants on each plot into collecting jars. Each plot had a collecting jar. 36 bottles were used all together and labelled appropriately, with a black permanent marker. Collection of insect larvae always began at 6 a.m. The collected larvae and pupae were brought to the entomology laboratory of WEICO where they were sorted out, counted and recorded. They were stored in 70% alcohol and coded for reference. Some larvae and all pupae were reared to adult stage to aid in identification.

PLATE 1: MODE OF PLACEMENT OF LABELS

PLATE 2: YELOW STICKY TRAP FRESHLY COATED WITH SPECIAL STICKY GLUE

3.6.2 Sampling with yellow sticky traps

Yellow sticky traps were used to sample for adult diamondback moth and other adult insects. The traps, which measured 30 cm by 20 cm, were nailed to sticks of length 58 cm. One trap was fixed in the middle row of each plot between plant number four and five. White transparent polythene sheets (45 cm by 24 cm) coated with special glue were put as jackets on the yellow sticky traps (Plate 2). New sheets were used every week.

The number of diamondback moths and other lepidoptera were noted before the removal of jackets of sticky traps. This was to keep track of exact numbers in case of damage to fragile lepidoptera adult. The polythene sheets were removed by turning the sheets inside out. The replicate and plot numbers were noted on polythene sheets with black permanent marker. The polythene sheets with the insects were then sent to the entomology laboratory and each was turned inside out again. A pair of forceps was used to pick the insects from the traps. The large insects were pinned directly whilst the small ones were mounted on cards. The insects were coded with alphabets and numbers.

3.7 Harvesting

The cabbage heads were harvested nine weeks after transplanting. The heads were turned to one side and the cut with a sharp knife. The five central plants in each plot were harvested. In certain plots where some of the central plants were missing, the next plant on the same row was harvested. In some plots heads were harvested from other rows and in others especially the control plots, all available heads were harvested. The harvested heads per plot were put into separate black polythene bags. The replicate and plot number were written on a white paper with a black permanent marker and placed in each polythene bag. The polythene bags were sent to the entomology laboratory of the Crop Science Department, where the outer

wrappers were all removed and the stalk cut. Each head was then weighed on an electronic scale and the weight recorded.

3.8 Data analysis

The counts of *P. xylostella* larvae, pupae and adults were first transformed by the square root (X + 0.5) (Steel and Torrie, 1980) prior to analysis to accommodate zero values. All data were analysed by analyses of variance (ANOVA) for randomised complete block design. Analyses were performed using the factor; calc and range programmes of mstat-c (Eisensmith and Russels, 1989). Means were separated using protected LSD ($\alpha = 0.05$). The number of larvae and pupae collected from 20 central plants of each treatment were converted to per plot basis for graphical representations.

For the percentage harvestable heads, the number of plants at harvest per treatment was counted and recorded. The number of normal and multiple heads per plot were counted and recorded before harvest. The percent harvestable heads were calculated as the number of normal heads divided by the total number of plants for each treatment at harvest multiplied by 100.

EXPERIMENT 2

The agronomic practices were carried out as in experiment 1.

3.9 Field plan

Randomised complete block design (RCBD) was used.

Plot size	4.2 m by 2.3 m
Number of replications per treatment	4
Number of treatments	4
Total number of plots	16
Number of plots in one replicate	4
Distance between replicates	2 m
Distance between plots in one replicate	1 m
Planting distance	60 cm within rows and 75 cm between rows
Plant arrangement	7 plants per row, 3 rows per plot.
Total number of plants per plot	21
Total number of plants per treatment	84
Record plants per plot	5 central plants
Total number of record plants	80
Total number of plants in experiment	336

3.10 Treatments applied

1.0 g/l of Dipel 2X sprayed at weekly interval (selected from the first experiment) was applied at 3 different stages of the cabbage growth in the field as follows:

Stage 1 a week after transplanting and received a total of 7 sprays before harvest

Stage 2	3 weeks after transplanting and received a total of 5 sprays before harvest
Stage 3	5 weeks after transplanting and received a total of 3 sprays before harvest
Stage 4	no spray

3.11 Data collection

3.11.1 Collection of larvae and pupae

Larvae and pupae were sampled weekly as in experiment 1.

3.11.2 Harvest

Harvesting was carried out as in experiment 1. At the entomology laboratory of the Crop Science Department, the outer (horizontal) wrapper leaves were discarded, as the vertical portion is the preferred part of the cabbage. The inner (vertical) wrapper leaves were assigned a damage score from 1 = minor to 3 = major damage; the heart was scored from 1 = perfect to 6 = little retrieval. The sum of the 'inner' plus 'heart' scores gave a total damage score which were used to describe cabbage as 'acceptable quality', that is damage less than 6, or 'premium quality' that is damage less than 4. Five cabbage heads from each plot, were weighed individually.

3.12 Data analysis

Counts of *P. xylostella* larvae and pupae were analysed as in experiment 1.

Percentage yield reduction of acceptable and premium cabbages were calculated by adding the number of cabbage heads whose inner and outer score damage were more than 6, in the case of acceptable heads, and more than 4 in the case of premium cabbages. These numbers were divided by the total number of cabbage heads sampled in each treatment and multiplied by 100.

CHAPTER 4

RESULTS

EXPERIMENT 1

4.1 Insects

4.1.1 Effect of treatments on *Plutella xylostella*

The *Bacillus thuringiensis* spray had an effect on the larvae of diamondback moth as the mean number per plant on the control plots were significantly higher than those of the sprayed plots (Table 2). However, within the sprayed plots although differences were observed they were not significant at 5% alpha level (Table 2). The lowest count of *P xylostella* larvae was observed on plots treated with 1.0 and 2.0 g/l sprayed weekly (Table 2).

Table 2 Mean number (± s.e) of diamondback moth larvae and pupae for each treatment

Treatments (Dipel 2X)	Larvae	Pupae ±
Control	2.47 (± 2.11) [a]	1.30 (± 0.48) [a]
0.25 g/L 7days	1.31 (± 0.82) [b]	0.84 (± 0.15) [bc]
0.25 g/L 14days	1.21 (± 0.31) [b]	0.74 (± 0.08) [bc]
0.5 g/L 7days	1.17 (± 0.88) [b]	0.71 (± 0.01) [c]
0.5 g/L 14days	1.28 (± 0.41) [b]	0.87 (± 0.17) [b]
1.0 g/L 7days	0.93 (± 0.36) [b]	0.75 (± 0.06) [bc]
1.0 g/L 14days	1.10 (± 0.32) [b]	0.84 (± 0.13) [bc]
2.0 g/L 7days	0.91 (± 0.30) [b]	0.71 (± 0.02) [c]
2.0 g/L 14days	1.29 (± 0.43) [b]	0.81 (± 0.10) [bc]

Within a column means (± s.e) followed by the same letter are not significantly different from each other by LSD (P>0.05)

More diamondback moth larvae developed into pupae on the control plots compared to those of the sprayed plots. Within the treated plots relatively more pupae were collected from plots sprayed fortnightly than plots sprayed weekly except that of 0.25 g/l sprayed weekly as shown also in Table 2.

Adult diamondback moth numbers did not follow any specific pattern as shown in Table 3. A significant difference was observed between the unsprayed plots and those of the 0.5 g/l and 1.0 g/l sprayed weekly plots. However, no difference was observed among the other treatments.

Table 3 Mean densities (± s.e.) of diamondback moth adult for each treatment sampled with yellow sticky traps.

Treatments (Dipel 2X)	Mean densities (± s.e.) of adults moths
Control	1.74 (± 0.89) [a]
0.25 g/l 7days	1.41 (± 0.86) [ab]
0.25 g/l 14days	1.39 (± 0.33) [ab]
0.5 g/l 7days	1.34 (± 0.28) [b]
0.5 g/l 14days	1.52 (± 0.8.2) [ab]
1.0 g/l 7days	1.32 (± 0.41) [b]
1.0 g/l 14days	1.60 (± 0.60) [ab]
2.0 g/l 7days	1.44 (± 0.23) [ab]
2.0 g/l 14days	1.53 (± 0.42) [ab]

Within a column means (± s.e.) followed by the same letter are not significantly different from each other by LSD (P>0.05)

Figure 1 shows the population of diamondback moth larvae, collected each week after the spray. On the first collection week, more larvae were collected on the weekly spray plots of 0.25 g/l, 0.5g/l and 1.0 g/l with 13, 24 and 11 larvae per plot, respectively. The trend changed in the second collection where more larvae were collected on the control and fortnight spray plots of 0.5 g/l and 2.0 g/l with 7, 13 and 10 per plot, respectively. The subsequent collection showed more larvae on the control plots compared with the sprayed plots, with the highest number of single collection of 115 per plot, sampled on the fifth week. The number of diamondback moth larvae collected on the control plots dropped from 115 to 29 on the sixth week. Throughout the collection, more larvae were collected on the fortnight sprays compared to the weekly sprays of *Bacillus thuringiensis* apart from the first and the

last collections where more larvae were collected from some weekly spray treatments (Figure 1).

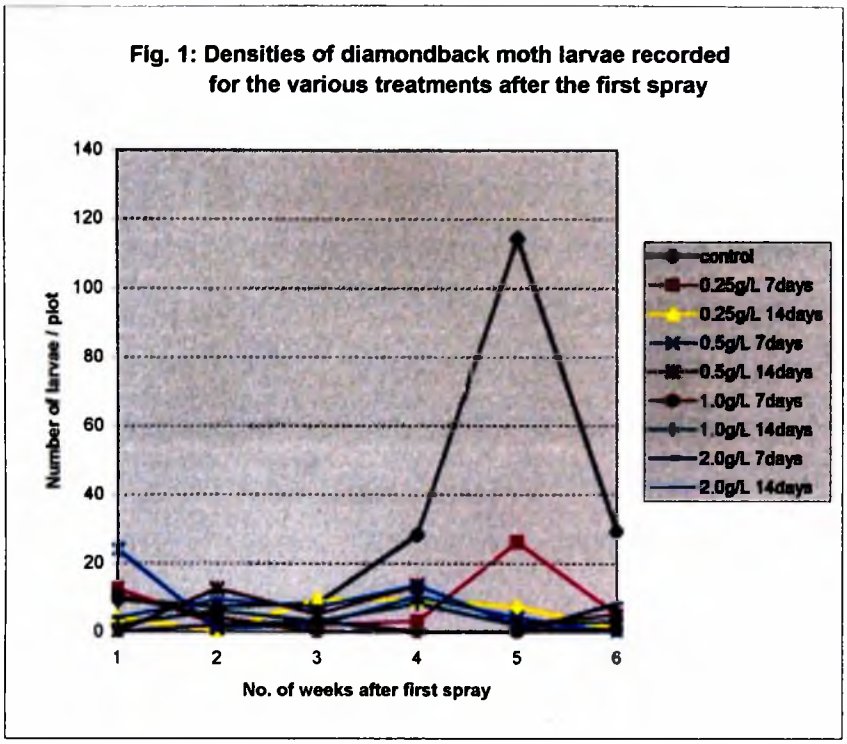


Figure 2 shows the total number of larvae collected from each treatment. It shows that most of the larvae were collected on the control plots and for the treated plots, weekly sprays of 1.0 g/l and 2.0 g/l had the least number of diamondback moth larvae. However, the fortnight sprays of 1.0 g/l and 2.0 g/l were less effective as the number of diamondback moth larvae collected from such plots were almost the same as plots treated with 0.25 g/l and 0.5 g/l fortnightly. More *P. xylostella* larvae were collected on plots sprayed weekly with 0.25 g/l and 0.5 g/l than on plots of same concentrations sprayed fortnightly, suggesting low efficacy of these concentrations as increased frequency of spray did not reduce the density of *P. xylostella* larvae.

Fig. 2 : Total no. of diamondback moth larvae collected from each treatment

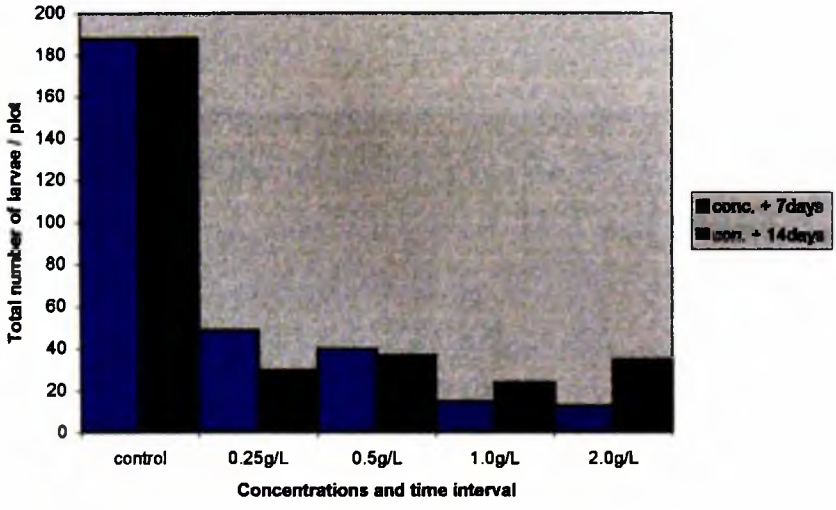
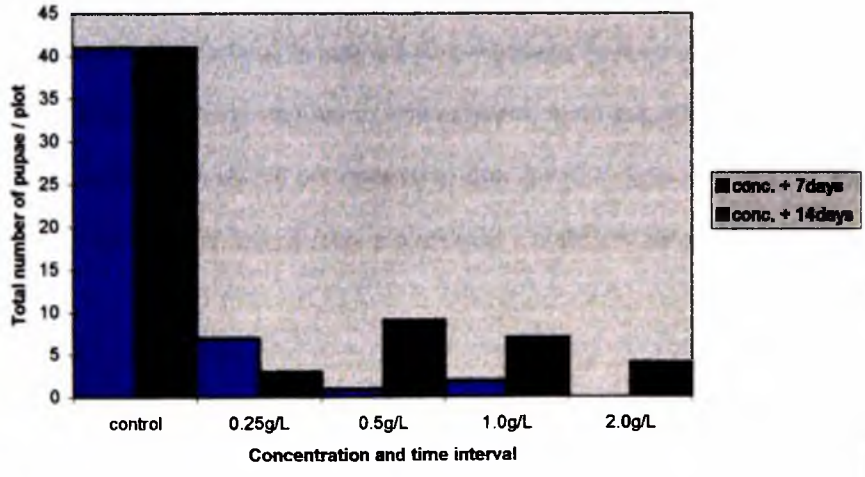


Fig. 3 : Total no. of diamondback moth pupae collected from each treatment



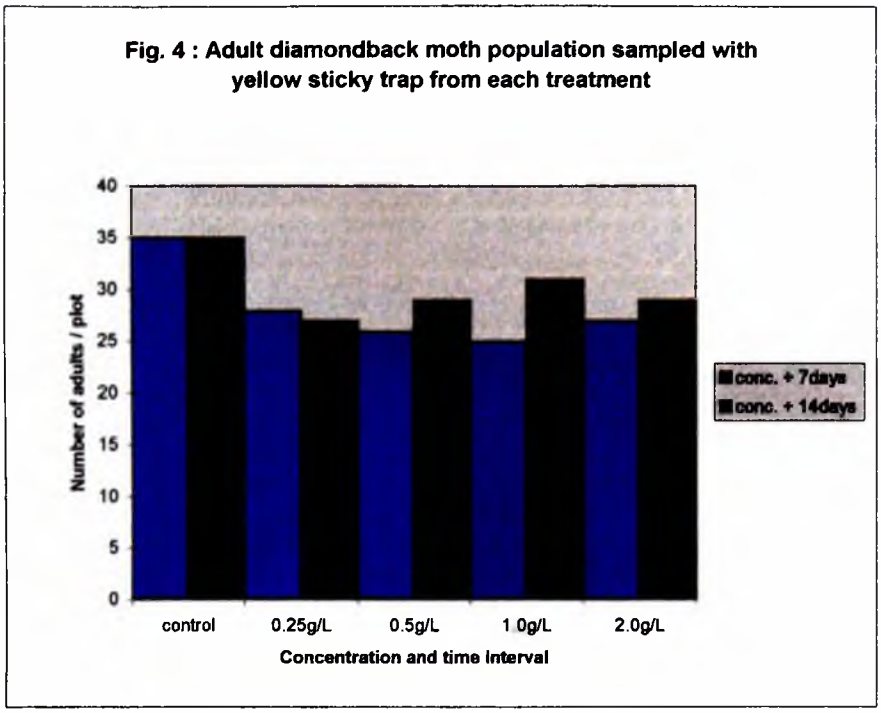


Figure 3 shows the total number of pupae collected per treatment. More pupae were collected on the control plots and none on the weekly sprays of 2.0 g/l. In all the treatments more larvae pupated on the fortnightly sprayed plots compared to the weekly-sprayed plots.

Figure 4 shows the total number of diamondback moth sampled with yellow sticky traps. Adult diamondback moth did not respond to *Bacillus thuringiensis* spray, as increased concentration did not result in considerable reduction in population numbers (Figure 4).

4.1.2 Insect fauna of cabbage sampled with yellow sticky traps

Table 4: Insect fauna of cabbage sampled by yellow sticky traps on cabbage plots of all treatments.

Order	Family	Genus	Species
Lepidoptera	Plutellidae	*Plutella*	*xylostella*
Lepidoptera	Noctuidae	*Trichoplusia*	*ni*
Lepidoptera	Noctuidae	*Helicoverpa*	*armigera*
Lepidoptera	Noctuidae	*Spodoptera*	*littoralis*
Coleoptera	Curculionidae	*Alcidodes*	*sp.*
Coleoptera	Lagriidae	*Lagria*	*sp*
Coleoptera	Coccinellidae	*Cheilomenes*	*lunata*
Coleoptera	Galerucidae	*Asbecesta*	*cyanipennis*
Coleoptera	Coccinellidae	*Cydonia*	*vicinia*
Coleoptera	Coccinellidae	*Epilachna*	*chrysomelina*
Coleoptera	Galerucidae	*Ootheca*	*mutabilis*
Coleoptera	Curculionidae		
Coleoptera	Curculionidae		
Coleoptera	Buprestidae		
Coleoptera	Carabidae		
Coleoptera	Coccinelidae	*Epilachna*	*semilis*
Hymenoptera	Ichneumonidae		
Hymenoptera	Ichneumonidae		
Hymenoptera	Apidae		
Hymenoptera	Formicidae		
Heteroptera	Flatidae		
Heteroptera	Jassidae		
Heteroptera	Flatidae		
Heteroptera	Flatidae		
Heteroptera	Jassidae		
Heteroptera	Pentatomidae	*Aspavia*	*sp.*
Heteroptera	Cercopidae		
Heteroptera	Vellidae		
Heteroptera	Vellidae		
Heteroptera	Cercopidae		
Heteroptera	Lygaenidae		
Heteroptera	Lygaenidae		
Heteroptera	Dictyopharidae		
Heteroptera	Reduviidae		
Heteroptera	Alydidae	*Tupulus*	*sp*
Orthoptera	Pyrgomorphidae	*Zonocerus*	*variegatus*
Orthoptera	Tettigoniedae	*Cognatus*	*krauss*
Orthoptera	Gryllidae		
Diptera	Diopsidae		
Isoptera	Termitidae		
Hemiptera	Pentatomidae	*Nezara*	*viridula*
Dictyoptera	Blattelidae		

The insect fauna of cabbage sampled with yellow sticky traps are shown in Table 4. Yellow sticky traps were effective in sampling adult diamondback moth and other insects as can be seen in Plate 3. In all 109 insects species were collected from eight orders. Out of these, 10 were Lepidoptera, 13 Hymenoptera, two Dictyoptera, 46 Coleoptera, 24 Heteroptera, 10 Orthoptera, two Diptera, one hemiptera and one Isoptera. Of these, 42 were identified to family level, 17 to genus level and 13 to species level.

4.2 Effect of treatments on yield

At harvest, clear visual differences in level of damage were observed among the various treatments. Plates 5 and 6 show the state of cabbages on a control plot and a plot treated weekly with a concentration of 1.0 g/l respectively. Cabbages from the control plots (Plate 5) showed greater foliar damage with either small or multiple head formation and sometimes no head formation (Plate 4). In contrast plots treated with 1.0 g/l of *B. thuringiensis* had fewer holes on foliage with larger and better developed heads (Plate 6).

PLATE 3: INSECTS STUCK TO A YELLOW STICKY TRAP

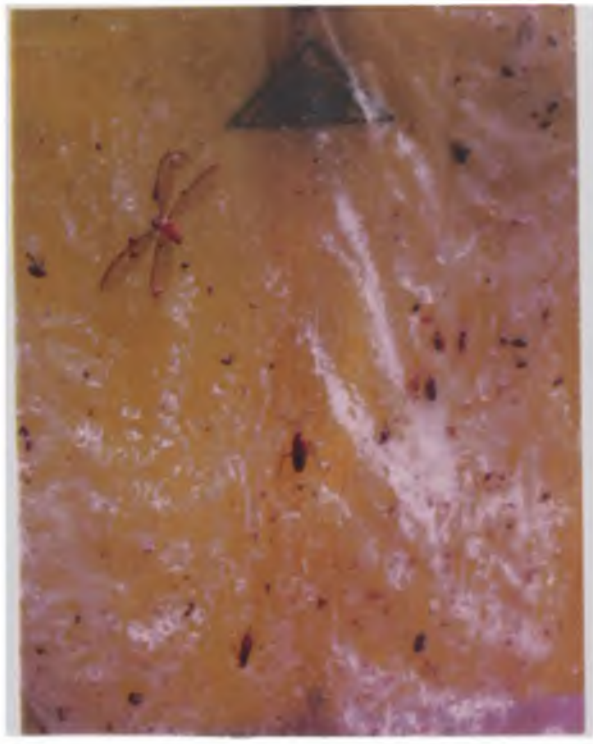


PLATE 4: CABBAGE WITH DESTROYED HEAD, A TYPICAL SITUATION OBSERVED ON CONTROL PLOTS

PLATE 5: STATE OF CABBAGE PLANTS JUST BEFORE HARVEST ON A CONTROL PLOT.

PLATE 6: STATE OF CABBAGE PLANTS JUST BEFORE HARVEST ON A PLOT SPRAYED WEEKLY AT A CONCENTRATION OF 1.0 g/l.

Table 5 Mean yield (± s.e) per plant and percentage of harvestable heads for each treatment.

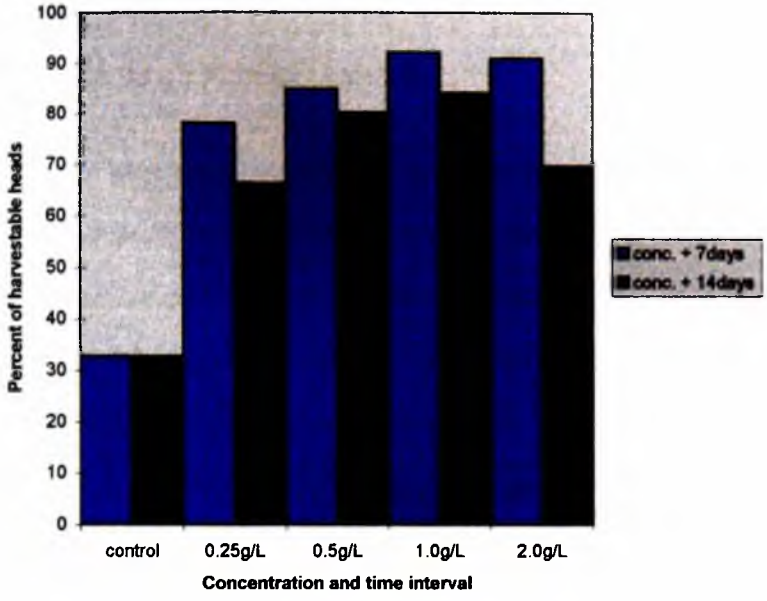
Treatments (Dipel 2X)	Mean yield (± s.e.) per plant (g)	% Harvestable heads
Control	571 (± 4.93) [h]	32.9
0.25 g/l 7days	590 (± 1.20) [f]	78.3
0.25 g/l 14days	621 (± 2.45) [d]	66.7
0.5 g/l 7days	676 (± 1.13) [a]	85.1
0.5 g/l 14days	580 (± 1.21) [g]	80.6
1.0 g/l 7days	644 (± 0.34) [b]	92.3
1.0 g/ 14days	628 (± 1.11) [c]	84.4
2.0 g/l 7days	613 (± 0.58) [e]	91.1
2.0 g/l 14days	613 (± 1.19) [e]	70.1

Within a column means (± s.e.) followed by the same letter are not significantly different from each other by LSD (P>0.05)

Table 5 shows the mean yield per plant (g) and the percentage of harvestable heads. All the mean yields for each treatment were significantly different from each other at (P<0.05). The highest yield was recorded on the weekly spray of 0.5 g/l with an average weight of 676 g, followed by the weekly sprays of 1.0 g/l with an average weight of 644 g. The lowest weight of 571 g was recorded from the control plots. High percentages of harvestable heads were recorded on the weekly spray plots of 1.0 g/l, 2.0 g/l and 0.5 g/l with 92.3%, 91.1% and 85.1% respectively. The lowest percent harvestable heads of 32.9% was recorded on the control plots with most of heads being damaged resulting in tiny multiple heads (Plate 5). Figure 5 shows the percentage of harvestable heads under each treatment.

Since differences in weight could also be attributed to soil factors and other agronomic practices and also considering the economic gains to the farmer by producing more harvestable heads, the 1.0 g/l sprayed at weekly intervals is selected to be the best concentration and spray interval.

Fig. 5: Percentage of harvestable heads obtain from each treatment



EXPERIMENT 2

4.3 Effect of treatments on *Plutella xylostella*

Cabbage plants that were treated a week after transplanting (Stage 1) gave the lowest mean number of diamondback moth larvae per plant. However, this density was not significantly different from plants treated three weeks after transplanting (Stage 2) at alpha 5% (Table 6). Significant differences in densities of diamondback moth larvae were, however, observed among plants sprayed at Stages 1 and 2 on one hand and those treated five weeks after transplanting (Stage 3) and plots without spray (Stage 4). Stage 4 had the highest density of diamondback moth larvae (Table 6).

The highest density of pupae were collected on Stage 4 plots, although this density was not significantly different from plots treated at Stage 3. Pupae collected on Stages 3 plots were also not significantly different from plots treated at Stage 2. Stage 1 plots recorded the lowest density of diamondback moth pupae, however this density was also not significantly different from plots treated at Stage 2 (Table 6).

Table 6 Mean number (± s.e) of diamondback moth larvae and pupae per plant treated with Dipel 2X at different stages

Treatments	No. of larvae	No. of pupae
Stage 1	1.21 (± 0.30) ^{c}	0.7 (± 0.15) ^{c}
Stage 2	1.47 (± 0.45) ^{c}	0.9 (± 0.30) ^{bc}
Stage 3	2.02 (± 1.01) ^{b}	1.0 (± 0.72) ^{ab}
Stage 4 (Control)	2.93 (± 1.85) ^{a}	1.2 (± 1.02) ^{a}

Within a column means (± s.e) followed by the same letter are not significantly different from each other by LSD (P>0.05).

There was a gradual increase in the number of larvae collected each week on all the plots (Figure 6), except the fourth week where there was a decrease in the number of larvae

collected on stage 3 plots (although treatment had not yet begun on these plots). The point at which treatment was applied for Stages 1 and 4 are not indicated (Figure 6), because the first week of collection represent two weeks after transplanting and no treatment was applied on stage 4 plots. The highest number of larvae for all stages of spray was recorded during the fifth week corresponding to the sixth week after transplanting since larvae were collected a week after treatment (Figure 6). Generally, fewer numbers of larvae were collected on the treated plots (Figure 6) compared to the untreated plots.

Figure 7 shows that most of the larvae (252) were found on plots without spray (Stage 4). The number being more than eight times (29) the number of larvae collected on plots treated a week after transplanting (Stage1), five times (50) more than that of plots treated three weeks after transplanting (Stage 2) and twice (123) that of plots treated five weeks after transplanting (Stage 3). Only one larva was able to pupate on plots treated a week after transplanting (Figure 8). Most of the pupae (28) were collected on plots that received no treatment.

Fig. 6 : Densities of diamondback moth larvae recorded weekly for the various stages of spray

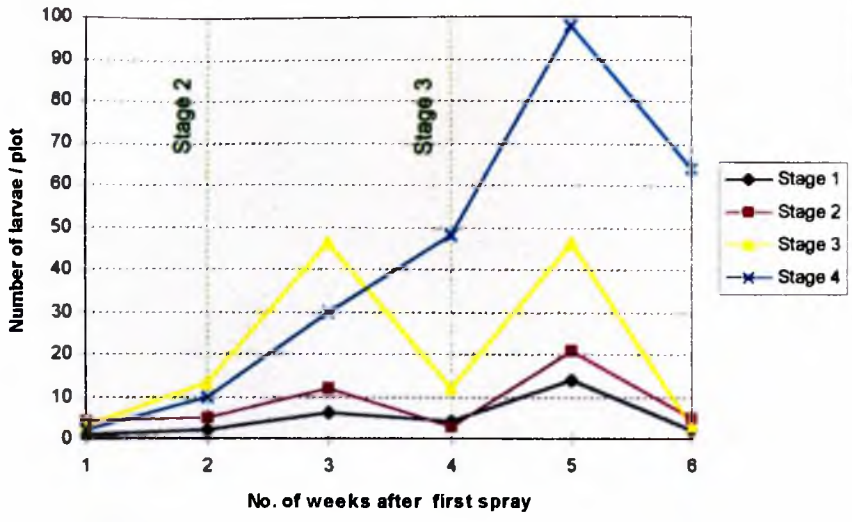
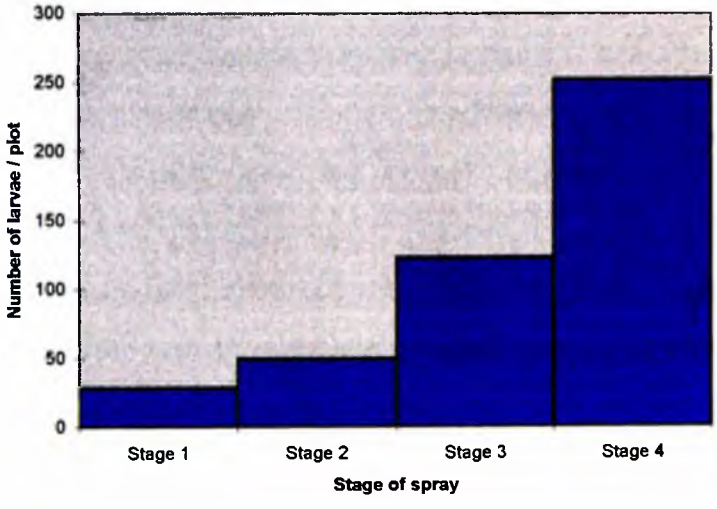
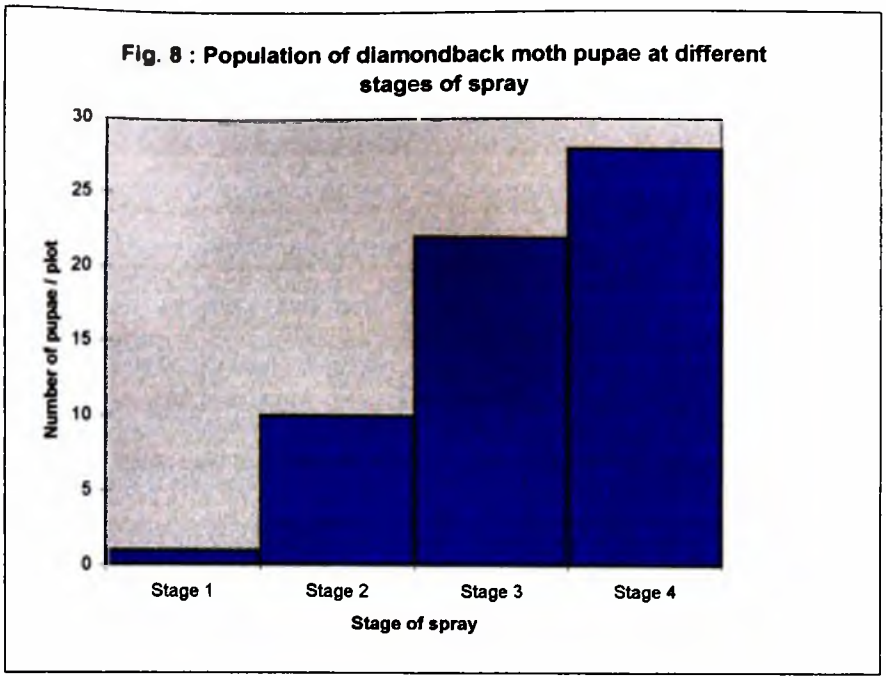


Fig. 7 Diamondback moth larvae population at the different stages of spray





4.4 Effect of treatments on yield

Table 7 Mean yield, leaf damage and yield reduction per plant for each treated plant

Treatment (dipel 2X)	Yield (kg)	Leaf Score		Yield Reduction (%)	
		Inner	Outer	Acceptable Cabbages	Premium Cabbages
Stage 1	1.8 (± 0.06) [a]	1.1 (± 0.05) [b]	1.0 (± 0) [b]	0	0
Stage 2	2.1 (± 0.07) [a]	1.1 (± 0.07) [b]	1.2 (± 0.09) [b]	0	0
Stage 3	1.8 (± 0.07) [a]	1.2 (± 0.08) [b]	1.2 (± 0.09) [b]	0	0
Stage 4	1.3 (± 0.08) [b]	4.1 (± 0.25) [a]	1.1 (± 0.07) [b]	70	95

Within a column means (± s.e) followed by the same letter are not significantly different by from each other by LSD (P>0.05).

The mean yield, leaf damages and yield reduction per plant for each treated plant are shown in Table 7. The highest mean yield was recorded on plots treated three weeks after transplant (Stage 2). This was, however, not significantly different (P<0.05) from those treated a week (Stages 1) and five weeks (Stage 3) after transplanting. Yield differences were observed between cabbages sprayed at Stages 1, 2 and 3 and that of Stage 4.

More damage was done to the outer and inner foliage of the cabbage heads in the non-treated plots (Stage 4) as compared to cabbage heads of stages 1, 2 and 3 (Table 7). Most of the damage (holes) to the outer foliage were recorded on Stage 4 plots. Stage 1 had the lowest damage to the outer foliage. Mean damage to the outer foliage of Stage 2 plants was however, not significantly different ($P<0.05$) from that of stage 3 (Table 7).

There was no yield reduction in cabbage heads harvested from plots treated a week, three weeks and five weeks after transplanting (stages 1, 2 and 3. respectively, Table 7). Cabbages harvested from Stage1 plots gave minimal damage on wrapper leaves and perfect cabbage hearts; and almost perfect hearts in the case of Stages 2. Only 30% of the cabbage heads harvested from stage 4 plots were classified as acceptable and among these only 5% were of premium quality

CHAPTER 5

DISCUSSION AND CONCLUSION

The drastic yield reduction obtained from the two experiments, in plots that were not treated, suggests the importance of protecting cabbage plants by spraying with Dipel 2X (*B. thuringiensis*). In the first experiment there was a yield reduction 67.1% (unharvestable heads), this percentage is close to the 70% yield reduction obtain in the second experiment. A farmer who produces at such a loss would surely be out of business in a short time. Although the cost and benefit ratio of Dipel 2X was not determined, the farmer would make more benefit by reducing the yield loss to 7.7% {(experiment 1), (1 g/l 7, days)} and no loss (experiment 2; Stage 3) by protecting his crops with *B. thuringiensis* at these recommendations.

The developmental period of diamondback moth from egg to adult at a field temperature of 30°C is approximately 12 days (Choi *et al.*, 1992). At Weija where the prevailing average temperature is about 30°C, it implies that a new generation of diamondback moth may be produced before two weeks. Eggs that hatch into larvae after a week of spray on plots treated fortnightly will have more chance of survival and cause more damage to the cabbage plant than weekly spray cabbage plants. On the other hand, eggs laid on weekly-sprayed plots are more likely to hatch into larvae when *B. thuringiensis* toxins are still active. These larvae would have less chance of survival to cause damage to the cabbage plants. This could explain why treatments of various concentrations carried out weekly had more effect on the diamondback moth larvae populations compared to the fortnightly sprays. The high number of larvae, recorded on the sixth week from plots sprayed weekly with 2.0 g/l consisted of freshly hatched larvae from one plot and on one plant. This observation was an exception. First instar larvae are leaf miners (Abro *et al.*, 1992). As miners, they have greater

chance of escaping the *B. thuringiensis* spray, because *B. thuringiensis* is not a systemic insecticide as most inorganic insecticides. This could partially explain the occurrence of this observation.

Sprays of *B. thuringiensis* do not deter oviposition by *P. xylostella* (Groeters *et al.*, 1992). The laying of eggs on cabbage plants that were sprayed weekly with high concentration of *B. thuringiensis* (2.0 g/l) shows that the adult female diamondback moth is not selective on choice of oviposition sites. This could explain why adult diamondback moth population did not follow any particular pattern. The increase in the number of diamondback moth larvae per plot from 10 on the first collection to 115 on the fifth sampling occasion on the control plot could be attributed to the short life cycle and the high reproductive rate of diamondback moth adults. The average number of eggs laid by a female is about 194.15 and more than 50% are laid on the first night of oviposition (Abro *et al.*, 1992). The sudden drop in the number of diamondback moth sample from 115 to 29 per plot on the sixth week in the control plots is not well understood, however it may be attributed to other factors such as parasitism. Diamondback moth has been known historically to be held below economic threshold in the United States of America by natural enemies (Marsh, 1917). A parasitoid, *Diadegma insulare* has been reported to parasitize up to 75% of diamondback moth larvae in the Southern Ontario state of Canada (Harcourt, 1969, 1986; Bolter and Liang, 1983; Lasota and Kok, 1986). Two species of ichneumonidae identified among the fauna sampled with the yellow sticky traps could have played a row in this. It could be that there was functional response of these parasitoids to the population of diamondback moth larvae.

The effective trapping of adult diamondback moths with the yellow sticky traps indicates its importance to reduce adult diamondback moth populations. In all 257 adults were sampled, 35 from the control plots, 28, 26, 25, 27 from plots sprayed weekly with 0.25 g/l, 0.5 g/l, 1.0 g/l and 2.0 g/l respectively. From fortnight sprayed plots, 27, 29, 31, and 29 were

trapped from plots treated with a concentration of 0.25 g/l, 0.5 g/l, 1.0 g/l and 2.0 g/l respectively. Together with *B. thurigiensis,* yellow sticky traps could play a useful role in integrated diamondback moth control since *B. thurigiensis* does not infect the adult diamondback moth. The great diversity of insect fauna sampled supports the fact that *B. thurigiensis* has little or no harmful effects on non-targets insects (Jaques, 1965). It would therefore be compatible with the integration of natural enemies in the control of diamondback moth. Apart from the damage done by the population of other lepidoptera sampled, the functions of the other insects were not determined in this study. They may be minor pests, predators, parasites or incidental transients.

The high mean number of *Plutella xylostella* sampled from plots without treatment (Stage 4) in the second experiment was because the plots were not protected by *B. thuringiensis* spray as compared to the other plots where *B. thuringiensis* was applied. The mean population of diamondback moth collected from Stages 1 and 2 did not differ because Stage 2 plots missed just two weeks of spray. Within this period the increase in the population of diamondback moth was insignificant and can best be described as population which was beginning to build up (Lumaban and Raros, 1973). Mean yield from plants that were treated from Stages 2 and 3 did not differ from that of Stage 1 which received spray of *B. thuringiensis* throughout the growing period. The population of diamondback moth larvae per plant reached a peak six weeks after transplanting for all the treatments. The increase in population of diamondback moth larvae per plant from two (first collection) to 98, six weeks after transplanting on plots without treatment (control) had serious effect on cabbage quality. This subsequently resulted in only 30% of acceptable cabbage. However, there was a high recovery rate from damage caused by diamondback moth to cabbage plants that were treated within five weeks after transplanting. It is clear from this study that treatment later than a period of five weeks after transplanting can not reverse the damage caused by diamondback

moth larvae to cabbage. Cabbage head formation within this period could explain why damage caused at this stage is irreversible, since the economic part of the cabbage is the head and once destroyed renders the cabbage unmarketable. Based on consumer preferences, cabbage plants that were not treated after five weeks resulted in yield reductions of about 70%, for acceptable cabbages (those with minor damages). On the other hand if premium cabbage heads are preferred, yield reduction can go as high as 95%. The critical stage to apply *B. thuringiensis* spray to cabbages was therefore found to be five weeks after transplanting. This finding supports the claim by Carballo and Hruska (1989). Cabbage yields obtained from Stages 2 and 3 (pre-formation of head) of spray did not differ from yields that were realised from plots treated throughout the whole cycle that is, stage 1 (Carballo and Hruska, 1989). When no treatment was applied (Stage 4) a reduction of over 73% was observed (Carballo and Hruska, 1989). These findings have also revealed that farmers do not have to go in to spray with the first sight of a diamondback moth on their cabbage plants. Farmers could reduce waste by spraying at the appropriate time to reduce the frequency of spray from 7 to 3 (experiment 2). It would also help to avoid or delay the development of resistance by diamondback moth, since abuse of the *B. thuringiensis* spray can result in the development of resistance to *B. thuringiensis* by diamondback moth as has been reported in some countries (Song, 1991; Perez *et al.*, 1995).

One of the major disadvantages of *B. thuringiensis* spray for leaf-eating pests is that following applications, the bacterium persists on the foliage for only about a week in numbers sufficient to cause toxaemia or septicaemia in significant insect populations (Jaques and Fox, 1960). Since the *B. thuringiensis* spray has no effect on the eggs and adults of diamondback moth, (Angus, 1956; Angus and Heimpel, 1959) eggs laid before and after a week of spray can escape the effect of the *B. thuringiensis* toxin. Diamondback moth larvae and other

lepidopterous larvae can only be infected when they hatch within the time that the *B. thuringiensis* are still effective and can cause toxaemia.

RECOMMENDATIONS

From the results of the experiment carried out, it is clear that in order to safely manage *Plutella xylostella* with *Bacillus thurigiensis* and to maximise grower's yield and profit. it is recommended that:

1. There should be weekly spray of Dipel 2X at 1.0 g/l.
2. Commencement of spray should not be later than five weeks after transplanting.
3. Yellow stick traps can be incorporated in integrated management of diamondback moth.

For the purpose of further research it is recommended that, the biology of the suspected parasitoids should be studied.

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Appendix

Appendix 1: Analysis of variance tables for experiment 1

Appendix 1a:

Analysis of variance table for larvae collected

Source	df	ss	ms	Fvalue	Probability
Replication	3	2.3	0.8	1.8	0.155
Factor A	8	38.2	4.8	10.9	0
Factor B	5	7.3	1.5	3.3	0.007
AB	40	57.5	1.4	3.3	0
Error	159	69.8	0.4		
Total	215	175.1			

Coefficient of variation: **49.97%**
Factor A Treatment
Factor B Collection date

Appendix 1b:

Analysis of variance table for pupae collected

Source	df	ss	Ms	Fvalue	Probability
Replication	3	0.3	0.1	1.5	0.2
Factor A	8	5.5	0.7	9.9	0
Factor B	5	2.5	0.5	7.1	0
AB	40	10.8	0.3	3.9	0
Error	159	11.1	0.1		
Total	215	30.2			

Coefficient of variation: **18.89%**
Factor A Treatment
Factor B Collection date

Appendix 1c:

Analysis of variance table for adults sampled with yellow sticky traps

Source	df	ss	ms	Fvalue
Replication	3	1.3	0.4	1.2
Factor A	8	3.2	0.4	1.2
Factor B	4	56.4	0.14.1	41.4
AB	32	12.7	0.4	1.2
Error	132	44.9	0.3	

Coefficient of variation: **40.34%**
Factor A Treatment
Factor B Collection date

Appendix 2 Analysis of variance tables for experiment 2

Appendix 2a:

Analysis of variance table for larvae collected

Source	Df	ss	Ms	Fvalue	Probability
Replication	3	5.2	1.7	6	0.0011
Factor A	5	41.6	8.3	29	0
Factor B	3	42.1	14	49	0
AB	15	32.3	2.2	7.5	0
Error	69	19.7	0.3		
Total	95	140.8			

Coefficient of variation: **28.02%**
Factor A Collection date
Factor B Stage of spray

Appendix 2b:

Analysis of variance table for pupae collected

Source	df	ss	ms	Fvalue	Probability
Replication	3	0.8	0.264	2.7	0.05
Factor A	5	8	1.6	16.5	0
Factor B	3	2.5	0.8	8.7	0
AB	15	4.7	0.3	3.3	0
Error	69	6.7	0.1		
Total	95	22.7			

Coefficient of variation: **32.82%**
Factor A Collection date
Factor B Stage of spray

Appendix 2c:

Analysis of variance table for yield

Source	df	ss	ms	Fvalue	Probability
Replication	3	679945.8	226648.6	2.6	0.06
Factor A	3	6402690.9	2134230.3	24.5	0
Factor B	4	908683.3	227170.9	2.6	0.04
AB	12	1258506.1	104875.5	1.2	0.3
Error	57	4965457.4	87113.3		

Coefficient of variation: **16.87%**
Factor A Stage of spray
Factor B Plant number

Appendix 2d:

Analysis of variance table for outer score

Source	df	ss	ms	Fvalue	Probability
Replication	3	0.2	0.1	1.5	0.22
Factor A	3	53.6	17.9	338.3	0
Factor B	4	0.7	0.2	3.3	0.12
AB	12	1.8	0.2	2.8	0
Error	57	3	0.1		
Total	79				

Coefficient of variation: **14.48**
Factor A Stage of spray
Factor B Plant number

Appendix 2e:

Analysis of variance table for inner score

Source	df	Ss	ms	Fvalue	Probability
Replication	3	1	0.4	1	
Factor A	3	133.7	44.6	117	0
Factor B	4	3.3	0.8	2.2	0.08
AB	12	3.7	0.3	0.8	
Error	57	21.7	0.4		
Total	79	163.5			

Coefficient of variation: **33.14%**
Factor A Stage of spray
Factor B Plant number