R7449 Final technical report Appendices

Appendices 1-2 & 4-11 are attached as electronic copies, 3 is submitted as hard copy

Appendix 1 Parnell, M., Oduor, G., Ong'aro, J., Grzywacz, D., Jones, K, A. and Brown, M., (2002). The strain variation and virulence of granulovirus of diamondback moth (*Plutella xylostella*) isolated in Kenya. *Journal of Invertebrate Pathology* (in Press)

Appendix 2 Grzywacz,-D., Parnell, D, Kibata, G., Oduor G., Ogutu. W. O., Miano D., and Winstanley. (2002) The development of endemic baculoviruses of *Plutella xylostella* (diamondback moth, DBM) for control of DBM in East Africa. In "*The Management of Diamond Back Moth and other Cruciferous Pests Proceedings forth International Workshop on Diamond Back Moth", Melbourne University*, Ridland, P., (Ed) (In Press)

Appendix 3*Oruko, L.O., Asaba, J., F & Kindness, H. M., (2000). Factors effecting uptake and adoption of outputs of crop protection research in peri-urban vegetable systems in Kenya. In "Sustaining Change: proceedings of a workshop on the factors effecting uptake and adoption of Department for International Development Crop Protection Programme research projects" Hainsworth, S. D., and Eden-Green, S. J., (Eds.) Natural Resources International Ltd. Chatham. pp. 27-34.

Appendix 4 Downham, M. C. A., (2001) An appraisal of the pheromone mating-disruption technique for management of diamond-back moth, *Plutella xylostella.* NRI Report. pp 41.

Appendix 5*Downham, M. C. A., (2002) A second appraisal of the pheromone mating-disruption technique for management of diamond-back moth, *Plutella xylostella.* NRI Report. pp 31

Appendix 6* Parnell, M., (2001) Field trials of *Plutella xylostella* granulovirus against diamondback moth on kale, carried out in Kenya during 1998 and 2000. NRI Report pp 17.

Appendix 7 Oruko, L.O., and Ndun'gu, B., (2000) Final Socio-economic report for the vegetable IPM thematic cluster. CAB International Africa Regional Centre and Kenya Agricultural Research Institute Report pp 49.

Appendix 8*Ogutu, W. O., Ogol., C.K.P.O., Oduor G. I., Parnell, M., Miano, D.W. and. Grzywacz D. (2002) Evaluation of a naturally occurring baculovirus for the management of diamondback moth, *Plutella xylostella* L. in Kenya. Paper accepted for International symposium improving biocontrol of Plutella xylostella. 21-24th October 2002, Montpellier, France. pp 8.

Appendix 9 *Grzywacz,-D., Parnell, D, Kibata, G., Oduor G., Ogutu. W. O., Poole , J., and ,Miano D. (2002) The granulovirus of *Plutella xylostella* (diamond back moth DBM) and its potential for control of DBM in Kenya. Paper accepted for International symposium improving biocontrol of Plutella xylostella. 21-24th October 2002, Montpellier, France, pp6.

Appendix 10 Kenyan application form to register a biopesticide

Appendix 11 Data requirements for biopesticide registration dossier

The strain variation and virulence of granulovirus of diamondback moth (*Plutella xylostella*, Lep., Yponomeutidae) isolated in Kenya.

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SUMMARY

The diamondback moth (DBM), *Plutella xylostella*, is a serious pest of brassica crops throughout the world. In Kenya, control of DBM on brassica vegetables is becoming an increasing problem due to escalating resistance to the favoured control option, chemical

insecticides**.** *Plutella xylostella* granulovirus (PxGV) has shown promise for DBM control in other countries and is important as an alternative control method for future development, however the Kenyan authorities do not allow importation of exotic organisms for pest control purposes. Therefore, in order to test the potential of PxGV in Kenya, isolates of this virus indigenous in Kenya had to be found. During a survey of 27 farms in Kenya, 127 diseased or dead DBM larvae were collected from several different locations on the outskirts of Nairobi. Of the 127 samples, 95 were found to be infected with PxGV. Restriction Endonuclease analysis of the viral DNA from infected larvae showed that fourteen of the 95 isolates had between 2 and 6 major band differences in DNA profiles after digestion with *Eco*R1 and *Pst*1 restriction enzymes and varied in molecular weight by up to 6.2 kilobase pairs. Bioassays to compare the efficacy of the Kenyan PxGV strains to each other and to a PxGV strain isolated in Taiwan found that no significant difference in potency existed between any of the isolates. This study forms the basis for future evaluation of PxGV's potential as a control agent of DBM in Kenya, and the genetic variation in Kenyan PxGV isolates provides additional support to the theory that DBM may have originated in southern Africa.

INTRODUCTION

The diamondback moth (DBM) *Plutella xylostella*, feed only on plants from the family Brassicaceae and are a major pest of brassica vegetables (kale, cabbage, rapeseed etc.) throughout Kenya (Michalik, 1994). Presently, conventional chemical insecticides are heavily relied upon to control them (Kibata, 1997). It is well known that DBM has become resistant to chemical insecticides in many countries throughout the world (Roush, 1997) and current programmes underway in Kenya have indicated that chemical resistance in DBM is also occurring there (Kibata, 1997). The chemical insecticides currently recommended for control are expensive, damaging to the environment and in some areas simply not available to the small-scale farmers who account for a high percentage of the brassica vegetable production of Kenya (pers. comm., Kibata). For these reasons a collaborative project between the Natural Resources Institute (NRI), the Kenya Agricultural Research Institute (KARI) and CAB International, Africa Regional Centre (CABI-ARC) was set up to investigate alternative methods of DBM control. One component of the project concentrated on the possibility of using baculoviruses.

In the past, baculoviruses (BV) have been found to infect DBM populations in India (Rabindra, 1997), South East Asia (Kadir *et. al* 1999a) and the Far East (Asayama and Osaki, 1970; Yen and Kao 1972;). Although nuclearpolyhedrovirus (NPV) of *Galleria mellonella* and *Autographa californica* have shown pathogenicity to DBM (Kadir, 1992) the only DBM specific BVs found have been granuloviruses (GV), most of which have been isolated from DBM populations in South East Asia and the Far East (Asayama and Osaki, 1970; Yen and Kao 1972; Kadir *et. al* 1999a). Rules laid down by the Kenyan authorities on the importation and use of insect pathogens in Kenya stipulate that only

indigenous material may be used for any pest control or experimental purposes. Therefore, under Project ZA0078 funded by the Department for International Development (DFID) Crop Protection Program a screening programme for local isolates of BV in Kenyan DBM populations was undertaken. The program concentrated on screening for GV although the possibility of NPV infection was not ignored.

MATERIALS AND METHODS

Individual DBM larvae showing symptoms of GV infection were collected from field DBM populations and after confirmation of GV presence by microscopy, restriction endonuclease analysis (REN) of the viral DNA was performed. Laboratory bioassays of isolates with different DNA profiles were also performed. From here on, GV samples extracted from infected individuals will be referred to as isolates. In order to compare the Kenyan isolates of *Plutella xylostella* GV (PxGV) to a standard, we used an isolate of Taiwanese PxGV (PxGV-Tw) kindly supplied in 1992 by Horticultural Research International (HRI) UK and previously reported on by Kadir (Kadir *et. al*, 1999a and b).

Prevalence of baculovirus in field collected DBM larvae

To collect GV infected DBM larvae, a survey of brassica farms was conducted which concentrated on the region around Nairobi. In total, 27 farms were surveyed in different agroecological (AEZ) zones in seven districts at sites within a radius of 170 km from Nairobi (Table 1). The AEZs visited were Upper Highland (UH), Lower Highland (LH) and Upper Midland (UM) as characterised by agroclimatic factors and soil types (Jaetzold and Schmidt, 1983). The area to the north of the city was surveyed most intensively

because the cooler, wetter climate created good conditions for farmers to grow brassica crops. DBM larvae infected with GV show very distinct symptoms, exhibiting puffy, elongated integument and a change of colour from dull green to pale yellow (Asayama and Osaki, 1970). Such symptoms allowed easy detection of GV infected larvae and each larva suspected of being infected was collected individually in a 1.5ml plastic, capped tube with no additives. All samples were kept refrigerated away from direct light until microscopic examination was possible. Standard, unstained wet mounts of GV infected larvae crushed in 0.5ml of sterile distilled water were examined in the laboratory using a microscope and dark-field contrast at X400 magnification to detect the presence of GV or NPV. All larval samples that were confirmed as having GV infection were selected for further study and given a GV isolate number.

Propagation of GV isolates

To provide enough material for REN analysis and bioassays, each of the virus isolates had to be multiplied. Multiplication of the virus was done in laboratory reared DBM larvae from a colony originating from Kenya. For each isolate 15 second instar DBM larvae were inoculated with GV by painting the virus suspension onto both surfaces of a 10.0cm x 5.0cm Chinese Cabbage leaf at a concentration of $4.0x10^7$ GV occlusion bodies (OB)/ml. To allow even coverage of the waxy leaf surfaces virus suspensions of GV were in 0.01% (v/v) Triton X100 wetting agent in distilled water. Larvae were allowed to feed on the dosed leaves for 24 hours before being transferred to fresh undosed leaves. They were then reared until full GV infection had taken place and were harvested just prior to death. To ensure the virus was propagated unchanged, DNA profiles of progeny

and inoculum viral DNA of several isolates were obtained and checked for differences in banding patterns, none were observed.

Extraction and purification of GV from infected DBM

Larvae infected with individual GV isolates were pooled but each isolate was treated separately to ensure no cross contamination occurred. The progeny virus from each isolate was then extracted and purified by macerating larvae with a small mortar and pestle, filtering the resulting suspension and centrifuging the filtrate on 50 to 70 % sucrose following methods described by Parnell (1999b).

Restriction endonuclease analysis (REN) of GV isolates

REN analysis was performed on each of the GV isolates individually and broadly followed a protocol devised by Smith and Summers (1978). DNA extraction was performed on each virus isolate by addition of 25μl of 0.5 molar (M) EDTA (pH 8) and 3.0μl of proteinase K for 1.5 hours at 37°C, followed by 75μl of 1M sodium carbonate (15 minutes) and 25μl of 10% (w/v) sodium dodecyl sulphate (30 minutes). After treatment with equal volumes of tris-saturated phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1), the extracted DNA was purified by dialysis in tris-acetate buffer (pH 8.3) at 4°C for 36 hours. Restriction enzyme digestions (*Eco*R1 and *Pst*1) were performed on the purified DNA of all virus isolates as specified by the manufacturer (Promega UK Ltd, Delta House, Southampton. S07 7NS). Electrophoresis of the DNA digests were then carried out at 35 volts for 18 hours on 0.6% agarose gels prepared with tris-acetate buffer (pH 8.3) and suspended in tris-acetate

buffer-filled electrophoresis tanks. The PxGV-Tw standard and molecular weight markers (1 kilobase, Life Technologies, and λ mix 19, MBI Fermentas) were run along side the DNA digests on each gel. DNA profiles were stained by submerging the agarose gels in ethidium bromide solution (100μl in 1 litre distilled water) for 30 minutes. DNA profiles of each isolate present in the gels were displayed on an ultra violet transiluminator (Camlab Ltd., Cambridge. CB4 1TH. UK.) and photographs were taken using a Polaroid MP-4 Land Camera with 667 black and white film. Approximate molecular weights of genomes of the GV isolates were estimated from the mobility of DNA fragments relative to fragments of 1Kb ladder and λ Mix19 molecular weight markers (MBI Fermentas, Helena Biosciences, Sunderland, UK).

Comparative pathogenicity bioassays

Test larvae

DBM larvae were used in all bioassays. The larvae used were from a disease free laboratory colony that had been established at NRI from wild *Plutella xylostella* pupae collected from the Ngong region of Kenya in 1996. The colony was maintained on 4-6 week old Chinese Cabbage seedlings at 25°C (± 2°C) under a 12:12 light:dark cycle in clear Perspex cages with cut-out sides covered in muslin for ventilation. To ensure larvae used in the assays were of the same age, fresh seedlings were presented to the adults on a daily basis so that eggs from a single day's lay could be collected. Second instar larvae were chosen for bioassay having first scrutinised the head capsule size to be sure of collecting the desired larval stage.

Bioassay Procedure

The pathogenicity of the different isolates were determined by means of two bioassay methods. Initially comparative bioassays using single discriminate doses aimed at producing between 20 and 70% mortality in test insects were performed on eight GV isolates displaying different DNA profiles in order to ascertain if significant differences in potency existed. Subsequently, in order to obtain LC_{50} values, dose series bioassays were carried out on three of those eight isolates and the PxGV-Tw isolate. For the discriminate dose bioassays a single dose of each of the nine isolates tested was prepared in 0.01% Triton X100 at a concentration of between 2.10×10^6 OB/ml and 5.40×10^6 OB/ml. Although the doses were not prepared to exactly the same concentration for every isolate, it was considered that to cause a significant effect in mortality levels a larger difference in dose than was present would have been required due to the low slope of dose against mortality in bioassays. For the three isolates used in the dose series bioassay, four five-fold dilutions of a top concentration that fell within 2.70×10^7 OB/ml and 3.06×10^8 OB/ml were prepared. Doses were prepared by dilution of purified stock suspension of each isolate tested. The concentration of each was determined by counting the virus using a 0.02mm depth, bacterial spore counting chamber (Weber Scientific International, UK) and a Leica DMRB microscope set to dark phase illumination at x200 magnification. A leaf paint bioassay method was used in both procedures whereby 150µl of virus suspension of each dose was applied to Chinese cabbage leaves of 50mm x 70mm ensuring both surfaces were completely and uniformly covered. Once the virus had dried, leaves were mounted in 10 mls of 0.8% (w/v) molten agar. Dosed leaves were mounted by the stem only, in clear plastic 90mm diameter tubs. Two leaves were prepared for each dose of the isolates tested and 15 second instar DBM larvae were placed on each leaf

(30 larvae/dose) before lids were placed on the tubs. The lids were ventilated with 15 slits produced by a No. 11 scalpel blade and the assays were incubated at 27°C in a 12:12 night:day cycle. After 24 hours of feeding on infected leaf material, all larvae were transferred to freshly mounted, undosed Chinese cabbage leaves and were supplied fresh feeding material as when it was required. The bioassays were run until death or pupation of all larvae and daily monitoring was carried out of larval mortality to monitor speed of kill.

RESULTS

Prevalence of baculovirus in field collected DBM larvae

During the field survey, 127 larvae with disease symptoms were collected from eight of the 27 farms included in the survey. Microscopic examination confirmed that 95 larvae collected from four of the eight farms were suffering from GV infection. The areas in which GV-infected larvae were found covered all three agroecological zones visited and were Nyathuna, South Kinangop and Naivasha. In Nyathuna, 84 GV-infected DBM larvae were collected (isolates Nya-01 to Nya-84), in South Kinangop 9 GV infected larvae were collected (isolates SK-01 to SK-09) and in Naivasha 2 GV-infected larvae were collected (isolates Nva-01 and Nva-02).

Restriction endonuclease analysis of GV infected DBM

The REN analysis of the 95 PxGV isolates showed that 27 had different DNA fragment profiles to any other when cut with either one of the two restriction enzymes. Of those

27, 14 had fragment profiles that could be distinguished from any other with both *Eco*R1 and *Pst*1 cuts (Figure 2). Comparison of these 14 Kenyan PxGV isolates to an isolate of PxGV from Taiwan (PxGV-Tw) revealed that, although the profiles had many similarities, there were major band differences between all isolates. Both the *Pst*1 and *Eco*R1 digests revealed between 2 and 6 major band differences between isolates, even in those collected from the same location (Figure 2).

The level of variation in banding patterns between the PxGV-Tw and any Kenyan isolate was no greater than that seen when comparing Kenyan isolates to each other. The dendrogram in Figure 3 shows the level of homology between the Kenyan and Taiwanese isolates and it can be seen that the PxGV-Tw isolate shares a closer homology to many of the Nyathuna Kenyan isolates than the South Kinangop isolate (SK-01) does. Table 2 presents the estimated molecular weights of all isolates with a different DNA profile. It can be seen that the estimated molecular weights of the Kenyan isolates varied from 92.12 kilobase pairs (kbp) (Nya-52) to 98.32 kbp (Nya-40). The Taiwanese isolate had the lowest molecular weight at 90.71 kbp.

Pathogenicity of different PxGV isolates

Although no lethal time (LT) experiments were conducted the results of bioassays indicated that speed of kill did not vary significantly between any of the Kenyan isolates when compared to the Taiwanese standard or each other. The bioassays showed that time to death ranged from 4 to 8 days post inoculation but in the dose series bioassays speed of kill was generally fastest for high doses. The dose series bioassays showed that for the top concentrations, which fell between 2.70 $x10^7$ OB/ml and 3.06 x 10^8 OB/ml, up to 100% of

the final death toll had occurred by the fourth day post inoculation. However, for the lowest doses, which fell between $1.77x10^5$ OB/ml and $2.45x10^6$ OB/ml, no mortality was observed before day five.

It can be seen from Figure 1 that in the discriminate dose bioassays there was variation in mortality between repetitions of all isolates of PxGV. The error bars of the graph show % mortality for individual isolates differed by up to 6.3 times. A variation in response also occurred in the dose series bioassays where difference in LC_{50} values varied up to nearly seven times within repetitions of the same isolate. If potency ratios of Kenyan isolates to the PxGV-Tw in the dose response bioassays are compared, then an even greater variation of up to nine-fold occurred for sample Nya-01 (Table 4).

In the three repetitions of the discriminate dose bioassay, the control mortality was 20%, 20% and 13%. Therefore, before any data analysis was carried out, Abbot's Correction was applied to the mortality data of each repetition to compensate for the high control deaths. Two-Group Comparisons were carried out in SigmaStat and the PxGV-Tw isolate was compared individually to each of the Kenyan isolates using the "t-test". Results showed every Kenyan isolate to be significantly more potent (Table 3) than the PxGV-Tw over the three repetitions of the assay with average % mortality ranging from 26.2% to 40.3% as compared to 5.2% for the PxGV-Tw (Figure 1). However, when compared to each other, no significant differences in mortality occurred between any of the Kenyan isolates.

No significant differences in LC_{50} values between Kenyan isolates and the PxGV-Tw isolate were observed in the dose response bioassays although small variations did occur

between the four isolates tested. Probit analysis was performed on the mortality data from the dose response bioassays and the results showed that average LC_{50} values for second instar DBM larvae varied from $2.36x10^6$ OBs/ml for Nya-01 PxGV to $3.95x10^7$ OBs/ml for Nya-40 PxGV (Table 4). Average potency ratios of Kenyan isolates compared to the Taiwanese isolate were 4.46:1 (Nya-01:PxGV-Tw), 0.68:1 (Nya-40:PxGVTw) and 3.12:1 (SK-01:PxGV-Tw).

DISCUSSION

The results presented here have shown Kenyan isolates of PxGV to be highly pathogenic to DBM larvae providing encouragement for their future development into biological pesticides. Both subgroups of BV (NPV and GV) are known to infect and kill the diamondback moth but in past laboratory studies GV has proven to be the most pathogenic (Kadir, 1992). No specific NPVs of DBM have ever been found so it was of no surprise that only larvae infected with GV were found. The highest incidence of GV infected larvae was in the Nyathuna Location, Kiambu District. The region was situated on high ground (1800-2100m above sea level) where the cooler, wetter and more overcast conditions lent themselves to the widespread cultivation of brassica crops, which harboured dense populations of DBM. The DBM population levels and climatic conditions could have favoured occurrence of epizootics of the virus so it was not surprising that more GV infected larvae were collected in the north compared to areas south of Nairobi where conditions were hot, dry and sunny and fewer brassica farms existed.

Although there were sixteen different DNA profiles identified from the 95 isolates collected on the survey and distinguished by both restriction enzymes, in most cases each profile was present in more than one infected larva. Only one isolate (SK-01) was found to be infecting just a single larva and from the dendrogram in Figure 3, it can be seen that its profile was the least homologous when compared with the others. The isolate was not significantly less potent than any others included in the dose response assays and its DNA profile did not possess any sub-molar bands so it is unlikely to be a mixture of two competing isolates. However, it was unusual to find an isolate infecting only one larva when the others were all found in several.

The similarities observed between the Taiwanese and Kenyan isolates are consistent with those observed in a previous study of the same Taiwanese isolate, in which it was compared to a Chinese isolate of PxGV (Kadir *et. al*, 1999a). Kadir noted that they appeared very closely related although he did observe between 1 and 3 major band differences between the two isolates after digestion with *Eco*R1, *Hin*dIII and *Bam*H1. The level of variation between isolates in Kadir's study was less than that seen between some isolates collected from the same sites in Kenya even though the Kadir isolates were from two different countries. The high level of variation in the same Kenyan site could indicate a long association between GV and DBM in the region. No such variation has been reported previously and in a field survey carried out in Japan, only one isolate of PxGV was discovered (Yamada and Yamaguchi, 1985). The theory that DBM is an exotic pest in the Far East allows the hypothesis that its diseases travelled with it, and that the PxGV found in Taiwan and China could have originated elsewhere.

The origin of DBM is generally considered to be somewhere in Mediterranean Europe having evolved on cultivated brassicas also believed to have European origin (Hardy, 1938). Recently however, the origin of DBM has been brought into question by Kfir (1998) who noted that 175 wild plant species belonging to the family Brassicaceae have been recorded in South Africa. He also conducted a survey of wild DBM populations in South Africa in which he isolated 22 species of parasitoids and hyperparasitoids. Some of those were found to be specific to DBM and restricted to South Africa. In particular the sexual form of the parasitoid *Diadromus collaris*, which only appears in an asexual form in Europe. Considering that all asexual organisms derive from sexual forms (Mayr, 1965), the author speculated that the diverse fauna of DBM parasitoids and hyperparasitoids, large number of indigenous host plants and existence of the sexual form of *Diadromus collaris* provided compelling evidence that the origin of DBM was southern Africa. The wide variation in genomes of PxGV isolates discovered in Kenya during the present study and apparent lack of diversity in isolates from other regions of the world provides additional support to the theory that the origin of DBM lies in Sub-Saharan Africa.

The information gathered on speed of kill did not show any significant differences between isolates but as other studies have shown a certain degree of dose dependency existed (van Beek *et. al*, 1988; Kadir *et. al*, 1999b). Kadir's study showed a dose dependency existed in bioassays of first instars although the assays performed on second instars did not show a similar trend. Kadir performed 18 repetitions of assays on first instars but only 2 on second instars and commented that dose dependency for time to death was generally only observed where an extensive number of assays had been performed. It is possibly the case that a large number of repetitions are required to show

up trends in dose dependency on time to death. However, only three replicates of the dose response assay in the current study were performed so it may be the case that a dose dependency on time to death does exist in PxGV assays.

The level of response varied considerably in all of the bioassays carried out with up to a seven-fold difference on some occasions. Although no precision tests of the bioassay method were carried out, the level of variation in response was comparable to that shown in other studies involving bioassay of DBM pathogens in which extensive precision tests were done and found to be within acceptable levels (Kadir *et. al*, 1999b). Kadir found that mean LD_{50} value varied up to almost nine-fold from 1.0 to 8.9 OB per neonate larva in a series of bioassays consisting of 18 repetitions. Therefore, the present study showed a similar level of variation to kadir's.

Although the initial discriminate dose bioassays indicated that the Kenyan isolates were all more potent than the Taiwanese isolate the more rigorous dose response method did not support this. There have been no comparative studies of the pathogenicity of different PxGV isolates to DBM larvae in the past although similar results were obtained in studies of GV isolates of other insect species (Crook, 1986; Crook *et. al* 1985). In Crook's studies, the infectivity of five isolates *Artogeia rapae* GV (ArGV) and three different isolates of *Cydia pomonella* GV (CpGV) from different geographical locations were investigated and showed no significant difference in potency between any of the isolates tested. There were no significant differences in potency between any Kenyan isolate indicating that the high level of variation between isolates had no discernible effect on potency. Such a variability in GV could be highly beneficial in the development of future

PxGV-based DBM control strategies in that variation of isolate may be used in resistance management practices.

Many countries have strict rules on importation of exotic organisms for use as pest control measures and in some, the precise legislation is patchy or confused with a blanket ban on exotic isolates creating difficulties in registration of existing products or testing of novel ones. In many cases the restriction on importation of exotics is essential, however, the authors would like to bring the following points to attention. The REN analysis showed that although variation existed between Kenyan and the Taiwanese isolates, many shared a high proportion of similar restriction sites. In fact, there was a greater level of variation between some Kenyan isolates than between Kenyan and the Taiwanese isolate. In addition to that, the bioassays showed no significant difference in activity between any isolate be it from Kenya or Taiwan. Such results indicate that a high level of affinity between isolates from different geographical locations may exist. In such circumstances there would appear to be room for relaxation of certain aspects of legislation on importation of exotic organisms, so long as those organisms were in an original and unaltered state and could be shown to share a high affinity with indigenous isolates.

The average LC_{50} value of the Taiwanese isolate was 7.8 times greater in the present study than was found in a previous study of the same isolate (Kadir *et. al*, 1999b). In comparison, the LC_{50} values of the Kenyan isolates were between 3.6 and 12.5 times greater than Kadir's figures. In his study, Kadir noted that the LC_{50} value of the Taiwanese strain of PxGV placed it amongst GV isolates that are highly infectious to their hosts (Payne, 1986; Payne *et. al*, 1981). Therefore, considering the lack of significant difference found between Kenyan and Taiwanese isolates, the Kenyan isolates

should also be considered in the same group. It is generally considered that highly infectious GV isolates are suitable for use as control products of their hosts, thus justify the further development of Kenyan PxGV as a DBM control measure.

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TABLE 1

Regions, and their location relevant to Nairobi, that were included in the farm survey of baculovirus-infected DBM larvae.

District	Site	Agroecological zone	No. of DBM larvae collected	Position from Nairobi
Kiambu	Nyathuna	Lower Highland	86	
	Karura	Lower Highland	0	
	Kibiko	Lower Highland	0	
Machakos	Athi River	Upper Midland	0	
	Katitu	Upper Midland	0	
Nyandaru	S Kingangop	Upper Highland	26	
	N Kinangop	Upper Highland	0	
	Mukungi	Upper Highland	4	
Muranga	Kangari	Lower Highland	0	
Nakuru	Naivasha	Upper Midland	11	
Kajiado	Ngong	Upper Midland	0	
Kirinyaga	Mwea	Upper Midland	0	

TABLE 2

Estimated molecular weight, in kilobase pairs, of Kenyan and Taiwanese PxGV DNA.

Sample	Molecular weight (Kb pairs)
Nya-01 PxGV[c]	96.679
Nya-02 PxGV	96.420
Nya-03 PxGV	95.720
Nya-06 PxGV	95.420
Nya-07 PxGV	96.720
Nya-14 PxGV	97.950
Nya-15 PxGV	97.770
Nya-25 PxGV	96.970
Nya-27 PxGV	96.120
Nya-29 PxGV	92.720
Nya-40 PxGV	98.320
Nya-42 PxGV	92.450
Nya-52 PxGV	92.120
SK-01 PxGV[b]	95.570
PxGV-Tw[a]	90.710

[a] Refers to samples collected from Taiwan

[b] Samples with prefix SK refer to samples collected from the South Kinangop region of Kenya

[c] Samples with prefix Nya refer to samples collected from the Nyathuna region of Kenya

TABLE 3

"t" test results of discriminate dose bioassay mortality from Kenyan PxGV isolates compared to the Taiwanese isolate.

Isolate of PxGV	SEM	P
PxGV-Tw [a]	1.43	
Nya-01 [b]	14.63	0.07
Nya-02	13.41	0.06
Nya-03	5.20	0.01
Nya-29	6.21	0.03
Nya-37	3.20	0.001
Nya-40	5.82	0.01
Nya-42	8.70	0.04
Nya-83	12.80	0.09
SK-01 [c]	8.70	0.04

SEM = Standard error of means

[a] Refers to samples collected from Taiwan

[b] Samples with prefix Nya refer to samples collected from the Nyathuna region of Kenya

[c] Samples with prefix SK refer to samples collected from the South Kinangop region of Kenya

TABLE 4

Lethal concentration 50 (LC_{50}) values at 27 °C for the three repetitions of the dose response bioassays of Kenyan (Nya & SK) and Taiwanese (PxGV-Tw) isolates of PxGV in second instar DBM larvae.

Isolate of PxGV	Assay Rep.	No. larvae	LC_{50} value (OB/ml)	Lower limit	Upper limit	χ^2	SE[a]	df[b]	Potency ratio to PxGV-Tw
PxGV-Tw	1	30	$1.25x10^7$	$3.89x10^6$	$3.40x10^7$	19.1	0.14	8	
	2	30	$1.94x10^7$	$9.87x10^6$	$3.60x10^7$	12.7	0.16	10	
	3	30	$1.46x10^7$	$4.67x10^6$	$4.09x10^7$	14.5	0.09	11	
Nya-01	1	30	$2.94x10^6$	$8.93x10^5$	$8.42x10^6$	19.1	0.14	8	4.25
	2	30	$2.36x10^6$	$1.05x10^6$	$4.59x10^6$	12.7	0.16	10	8.22
	3	30	$1.62x10^7$	$5.26x10^6$	$6.11x10^7$	14.5	0.09	11	0.90
Nya-40	1	30	$2.01x10^7$	$5.32x10^6$	$5.80x10^7$	19.1	0.14	8	0.62
	2	30	$3.95x10^7$	$1.99x10^7$	$7.55x10^7$	12.7	0.16	10	0.49
	3	30	$1.56x10^7$	$4.94x10^6$	$4.36x10^7$	14.5	0.09	11	0.94
SK-01	1	30	$5.89x10^6$	$1.87x10^6$	$1.79x10^7$	19.1	0.14	8	2.12
	2	30	$1.37x10^7$	$6.62x10^6$	$2.95x10^7$	12.7	0.16	10	1.42
	3	30	$2.51x10^6$	$8.37x10^5$	$7.24x10^6$	14.5	0.09	11	5.82

[a] SE = standard error

[b] df = degrees of freedom

FIG. 1. Bar chart of average mortality of second instar DBM larvae expressed in discriminate dose bioassays of Kenyan and Taiwanese PxGV. Error bars are standard deviations.

FIG.2. Comparison of PxGV isolates. DNA of each isolate was digested with *Pst*1 restriction endonuclease, fragments were separated on 0.6% agarose gel. Track 1, 1kb molecular size standard; tracks 2-16, Kenyan PxGV isolates from Nyathuna (Nya-01, Nya-02, Nya-03, Nya-06, Nya-07, Nya-14, Nya-15, Nya-25, Nya-27, Ny-29, Nya-35, Nya-37, Nya-40, Nya-42, Nya-52 respectively); track 17, PxGV isolate from South Kinangop (SK-01); track 18, Taiwanese PxGV; Track 19, λ 19-Mix molecular size standard.

Note: Track 12 (Nya-35) is a mixed isolate with profiles of Nya-29 and Nya-42 (Tracks 11 and 15). Track 13 (Nya-37) has the same profile as Nya-52 (Track 16).

FIG. 3. Dendrogram showing homology between Kenyan and Taiwanese PxGV isolates. "Tw" is the Taiwanese PxGV-Tw isolate, "Nya" prefix represents samples collected in the Nyathuna region of Kenya, "SK" prefix represents samples collected from the South Kinangop region of Kenya.

The development of endemic baculoviruses of *Plutella xylostella* (diamond back moth DBM) for control of DBM in East Africa.

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Abstract

A project to develop non-chemical methods of DBM control on brassica crops in Kenya has been exploring the use of endemic pathogens as potential control agents. Initial surveys for endemic pathogens identified *P.xylostella* granulovirus (*Plxy*GV) on farms in Kenya. Subsequently 14 genetically distinguishable isolates were identified from field collected material. These were purified and ranging bioassays showed these isolates were pathogenic to Kenyan strains of DBM with $LC_{50's}$ varying from $2.36x10^6$ to $3.95x10^7$ occlusion bodies (OB) per ml for second instar DBM. One isolate (Nya-01) was selected and subsequently used for field trials in Kenya. The trials showed that unformulated *Plxy*GV applied at weekly intervals at a rate of 3.0 $x10^{13}$ OB/ha could control DBM on Kale more effectively than available chemical insecticides. After application, infection rates in DBM can reach 90%. Further field trials are currently underway to determine the lowest effective dose rate for this virus when applied as a formulation. Initial virus production studies using *in vivo*

propagation in 2nd instar DBM reared on cabbage showed an initial productivity of 4.0 $\pm$ 0.44 x10^{10} OB per larva.

Keywords Plutella xylostella, baculovirus, brassicae, granulovirus, biocontrol, Kenya,

Running title Development of endemic baculoviruses of DBM in Kenya

Introduction

The diamondback moth (DBM) *Plutella xylostella*, feed only on plants from the family Brassicaceae and are a major pest of brassica vegetables (kale, cabbage, rapeseed etc.) throughout Kenya (Michalik, 1994). Presently, conventional chemical insecticides are heavily relied upon to control them (Kibata, 1997). It is well known that DBM has become resistant to chemical insecticides in many countries throughout the world (Roush, 1997) and current programmes underway in Kenya have indicated that chemical resistance in DBM is also occurring there (Kibata, 1997). The chemical insecticides currently recommended for control are expensive, damaging to the environment and in some areas simply not available to the small-scale farmers who account for a high percentage of the brassica vegetable production of Kenya (Kibata 1996).

To address this issue, a collaborative project between the Natural Resources Institute (NRI), the Kenya Agricultural Research Institute (KARI) and CAB International, Africa Regional Centre (CABI-ARC) was set up to investigate alternative methods of DBM control. One component of the project investigated the possible use of endemic baculoviruses.

Before this study GVs of *P.xylostella* had been reported from Japan (Asayama and Osaki 1970) Taiwan (Wang & Rose 1978, Kadir 1986), China (Kadir et al 1999) and India (Rabindra 1997) but there were no previous published records from Africa. A number of

other NPVs, some uncharacterised (Padamvathamma and Veeresh 1989), have been reported as infecting DBM but a review of the potential of DBM pathogens concluded that only the GV showed promising levels of pathogenicity (Wilding 1986). More recently an NPV has been identified from *P.xylostella* in China. This was characterised as being genetically similar to, though genetically distinct from, *Autographa californica* MNPV and *A.falcifera* MNPV (Kariuki and McIntosh 1999).

Materials and methods

Pathogen survey and identification

To collect baculoviruses a survey of brassica farms was conducted around Nairobi. In total, 27 farms were surveyed within a radius of 170 km from Nairobi. In field sampling suspect larvae showing signs of baculovirus infection, puffy appearance and the pale-yellow to white coloration (Asayama and Osaki, 1970) were collected and individually stored for later examination. Standard, unstained wet mounts of infected larvae examined using a microscope and dark-field contrast at X400 magnification to detect the presence of baculoviruses. Each candidate GV isolate was propagated *in vivo* in 15 2nd instar DBM following methods described by Parnell (1999).

Restriction endonuclease analysis (REN) of the baculovirus isolates was performed on each of the GV isolates individually following the protocol of Smith and Summers (1978) as modified by Rabindra (1997).

Bioassay of pathogen strains

The pathogenicity of the different isolates were determined by means of two bioassay methods. Comparative bioassays using single discriminate doses were performed on nine

GV isolates displaying different DNA profiles. Subsequently, in order to obtain LC_{50} values, dose series bioassays were carried out on three of those eight isolates and the *Plxy*GV-Tw isolate. The concentration of GV was determined by counting using a 0.02mm depth bacterial spore-counting chamber viewed under dark phase illumination at x200 magnification.

For the discriminate dose bioassays and dose response bioassays were carried as per Parnell (1999).. Bioassay data was corrected using Abbot's correction for control mortality and dose series data analysed using a probit analysis with the SPSS data analysis package.

Field trials

To evaluate the potential of the Kenyan *Plxy*GV to control crop loss caused by DBM, isolate Nya-01 was selected for mass production and use in small-plot field trials. This isolate was selected because it had been indicated as the most pathogenic strain in the lab bioassays. The virus was applied as a simple unformulated suspension using standard farmer equipment. Volume application rate for all treatments was 800 litres/ha. The first field trial was carried out on the research farm at Jomo Kenyatta University of Agricultural Technology (JKUAT) 25 Km outside Nairobi lasting 12 weeks in late1998. This was a randomised-block design trial carried out on small plots of 5m x 5m with a 1m gap between plots and a plant/row spacing of 60cm. Test crop was Kale (var. Thousand headed). This trial compared two virus treatments, a weekly application of high application rate of 3.0×10^{14} (occlusion bodies {OB}) and a medium rate of 3.0×10^{13} OB ha^{-1}. There was a no treatment control and a standard farmer insecticide treatment schedule based upon weekly application of the local standard pyrethroid insecticide (Karate- lamda-cyhalothrin).

A second field trial was carried out at the National Agricultural Research Laboratory (NARL) farm on the outskirts of Nairobi in 2000. In this trial there were five treatments arranged in randomised replicated plot design. The treatments were three virus application rates (3 x

10^{14}, 3 x 10^{13} and 3 x 10^{12} OB ha^{-1}) a no treatment control and a standard insecticide treatment with Karate as before. The plots were 5x5m with a 1m gap between plots and a plant spacing of 60 x 60 cm.

In both trials 10 random plants in the central area of the plot were sampled weekly for numbers of DBM larvae present, numbers showing symptoms of GV infection and damage caused by DBM. In addition, in the second trial yield data was also collected. To assess more precisely the disease incidence in the plots after three weeks of the trial 45 larvae of each instar were collected from each treatment and reared individually in the laboratory and the disease occurrence recorded. The yield data was analysed using 2 way ANOVA on the SigmaStat statistical package (SPSS Inc., USA).

PlxyGV Productivity

In order to estimate the productivity of the *Plxy*GV when produced *in vivo*, two hundred 2nd and 3rd instar larvae were inoculated with a range of concentrations of the strain Nya-01 and reared under standard conditions until death. Progeny virus was collected, counted and its identity confirmed using REN.

Results

During the field survey, 127 larvae with disease symptoms were collected from eight of the 27 farms included in the survey. Microscopic examination confirmed that 95 larvae collected from four of the eight farms were suffering from GV infection. The areas in which GV-infected larvae were found were Nyathuna (84 larvae-two farms), South Kinangop (9 larvae) and Naivasha (2 larvae).

The REN analysis of the 95 *Plxy*GV isolates showed that 14 had fragment profiles that could be distinguished from any other with both *Eco*R1 and *Pst*1 cuts (Figure 1). Comparison of these 14 Kenyan *Plxy*GV isolates to an isolate of *Plxy*GV from Taiwan (*Plxy*GV-Tw) revealed that, although the profiles had many similarities, there were major band differences between all isolates. Both the *Pst*1 and *Eco*R1 digests revealed between 2 and 6 major band differences between isolates, even in those collected from the same location (Figure 1).

Results from the discriminate dose assay showed every Kenyan isolate to be significantly more potent than the PlxyGV-Tw with average % mortality ranging from 26.2% to 40.3% as compared to 5.2% for the *Plxy*GV-Tw (Figure 2). However in the dose response bioassays no significant differences in LC_{50} values between Kenyan isolates and the *Plxy*GV-Tw isolate were observed. Average LC_{50} values for second instar DBM larvae varied from $2.36x10^6$ OBs/ml for Nya-01 *Plxy*GV to $3.95x10^7$ OBs/ml for Nya-40 PlxyGV. In comparison the LC50 for the *Plxy*GV-Tw was $1.55x10^7$ OBs/ml.

The field trials carried out at JKUAT showed that the *Plxy*GV when sprayed using standard farmer application equipment was highly infectious to DBM, spreading rapidly in trial plots and infecting 80-90% of larvae within two to three weeks of application (Figure 3). Very little occurrence of infected insects was recorded from the control or insecticide treated plots. Both the high dose rate of 3.0×10^{14} OB $ha^{-1}$and the lower dose of 3.0×10^{13} OB ha^{-1} reduced DBM damage to crops to below that seen in either unsprayed controls or insecticide treated plots (Figure 4).

In the second trial at NARL the yield data (Figure 5) showed that the highest application rate dose gave significantly higher yield than the no treatment control (37% higher, P=<0.001 df = 4 and 28, F = 6.25) or the insecticide treatment (17% higher, P=<0.001 df = 4 and 28, F = 6.25). The average DBM numbers in each treatment showed an application-rate effect with the lowest numbers occuring in the highest virus rate treatment (Figure 6). In the second

trial average observed DBM infection rates in virus treated plots also showed a clear application-rate trend with the highest dose producing an average of 40% (Figure 7). In this trial there was some infection observed in the control and insecticide plots. From insects sampled from the *Plxy*GV application-rate plots, the true infection rate was much higher than that observed in the field and Table 1 shows the percent virus mortality recorded from insects taken from the plot treated at 3 $x10^{13}$ OB ha^{-1}.

The maximum productivity of the *Plxy*GV was found to be 4.0 $\pm$ 0.44 $x10^{10}$ OB per larva obtained from 2nd instars inoculated with 2.0 $x10^{8}$ OB ml^{-1}.

Discussion

The Pathogen survey revealed that the GV of DBM occurred on 50% of the farms surveyed though in all cases with a relatively low incidence. On no farm were widespread epizootics observed or reported by local farmers questioned. The discovery of so many different genetic isolates (14) in the small number of infected larvae collected is therefore striking. Previously reported work (Kadir et al 1999) has characterised only two genetically distinct isolates one from China and one from Taiwan. Other studies of DBM pathogens have also only reported finding a single genetically distinct isolate from India (Rabindra 1997) and Japan (Yamada & Yamaguchi 1985).

The GV isolates from Kenya are genetically similar to, though genetically distinct from the previously reported Taiwanese isolate. This isolate we now know is itself similar to and closely related to the Chinese isolate (Kadir and Payne 1999). The two isolates studied differed by 1-3 major bands in the *Eco*R1, *Bam*H1 and *Hind*III profiles from each other. The differences in the Kenyan isolates studied here were greater at 2-6 bands with only two profiles *Eco*R1 and *Pst*1, even amongst isolates collected from the same farm. This genetic diversity amongst isolates of *Plxy*GV from Kenya could be extremely useful as a diverse

genetic resource that could be exploited in the development of a GV for DBM control. The high level of variation in the *Plxy*GV isolates could indicate a long association between *Plxy*GV and DBM in the region and could have a bearing on the debate concerning the origin of DBM. This was generally considered to be somewhere in Mediterranean Europe having evolved on cultivated brassicas also believed to have European origin (Hardy, 1938). Recently however, the Mediterranean origin of DBM has been brought into question by Kfir (1998) who hypothesised a southern African origin for DBM on the basis of the diversity of wild hosts and endemic parasitoids found in South Africa. The genetic variation in PlxyGV isolates discovered in Kenya during the present study and apparent lack of diversity in isolates from other regions of the world might be interpreted as providing additional support to the theory that the origin of DBM lies in Sub-Saharan Africa.

The initial discriminant single dose bioassay results showed all the Kenyan isolates to be significantly more pathogenic than the Taiwanese isolate. However the LC_{50} data from the subsequent dose response assays showed no significant differences, even though the mean LC_{50} for Taiwanese isolate was 6.5 times higher that of the most active Kenyan isolate (Nya-01). This result reflects the high variability in response seen with the some Kenyan isolates including Nya-01. These were originally *in vivo* propagated but not cloned, which might have reduced this variability. These isolates have since been cloned and the assays are currently being repeated on these cloned isolates.

The productivity of the Kenyan isolates is high at $4.0 \pm 0.44 \times 10^{10}$ OB per larva, equivalent to 8.0×10^{9} OB per mg. This may be compared with between 1.9×10^{10} and 4.5×10^{9} per larva reported with other GVs produced in Lepidoptera (Evans 1986). High productivity is a valuable asset in a potential biopesticide as it reduces the number of insects needed to produce the desired application rate. At this rate of production the highest application rate used in these trials, 3.0×10^{14} OB ha^{-1} would be equivalent to 7,500 infected larvae per ha.

In comparison most existing commercial baculovirus products are applied at rates of between 50-500 larval equivalents per ha (Moscardi 1999).

The first field trial showed that application of *Plxy*GV at $3x10^{13}$ OB ha^{-1} could reduce DBM damage much better than either the use of the standard chemical insecticide or the no treatment control. The very limited effectiveness of the standard insecticide lamda-cyhalothrin was a finding suggesting significant resistance in DBM. This has since been confirmed by other work in Kenya (J Cooper 2001) and is now no longer recommended for DBM control.

The speed with which weekly sprays of *Plxy*GV initiated infection rates of 90% could indicate that one or two applications of *Plxy*GV at the start of the season might be sufficient to start an epizootic infection in resident DBM populations. However whether augmentative approach alone would be sufficient to produce control of DBM numbers and damage though would need testing under field conditions. While collection of a high percentage of infected insects in virus treated plots suggests that recycling of PlxyGV is very important its precise contribution to control remains to be quantified.

In the second trial the yield results showed that again the *Plxy*GV performed significantly better than the chemical insecticide at the highest application rate used $3x10^{14}$ OB ha^{-1}. A similar result in terms of controlling DBM numbers has been reported previously by Su (1989) using a Taiwanese isolate applied as here at seven day intervals. However direct comparisons are difficult, as in that trial the *Plxy*GV was quantified in terms of larval equivalents per litre and no direct enumeration of the GV was carried out.

Glasshouse trials again have showed that application of the Taiwanese isolate can reduce DBM numbers and that there is a dose response over the range 9 $x10^{11}$ to $9x10^{13}$ and at the highest dose the *Plxy*GV reduced damage as effectively as application of Bt (Kadir 1992).

In addition it was shown that the addition of molasses to a formulation could increase the viruses efficacy by a factor of ten and allow for a consequent reduction in the application rate of *Plxy*GV. This finding closely mirrors that of Ballard et al (2000) who found that addition of 10% molasses produced a similar 10 fold increase in efficacy with the codling moth (*Cydia Pomonella*) granulovirus (*Cp*GV) on apples.

The two granuloviruses that have been comercialised to date *Cp*GV and Adoxophyes orana granulovirus are both sold for application at rates of $1x10^{13}$ OB ha^{-1}. In comparison the rate of *Plxy*GV used here which produced significant increase in yield is $3x10^{14}$ OB ha^{-1}. Even given that the Kenyan PlxyGV seems to be more productive than other GVs this suggests a need to reduce the application rate by a factor of ten if its use is to be commercially attractive.

The trials reported here did not include formulation ingredients and field trials of such a formulation are underway now in Kenya to evaluate the efficacy of reduced rate formulated *Plxy*GV. Formulation might also address the short persistence time on field crops seen with GVs. Kadir (1986) reported that with PlxyGV-Tw exposure of unformulated virus to 7 hours sunlight in Malaysia was sufficient to reduce virus efficacy by 50%. Although the persistence of the Kenya PlxyGv has yet to be quantified it is unlikely to be longer.

In conclusion while the results of these trials of PlxyGV are promising it has yet to be determined that PlxyGV can be effective or reliable enough for consistent control.

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AN APPRAISAL OF THE PHEROMONE MATING-DISRUPTION TECHNIQUE FOR MANAGEMENT OF DIAMOND-BACK MOTH, *PLUTELLA XYLOSTELLA*

Kenya Agricultural Research Institute

CAB INTERNATIONAL
Africa Regional Centre

AN APPRAISAL OF THE PHEROMONE MATING-DISRUPTION TECHNIQUE FOR MANAGEMENT OF DIAMOND-BACK MOTH, *PLUTELLA XYLOSTELLA*

A Technical Report for Project R7449:
Development of Biorational Brassica IPM in Kenya

DFID Crop Protection Programme

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Contents

Executive Summary

A trial of pheromone mating disruption of diamondback moth (DBM), *Plutella xylostella*, L. was carried out at three sites near Nairobi, Kenya from March to August 2000. The synthetic pheromone formulation used was a PVC-based one known as 'Selibate'™ and individual dispensers were set out at a density of approximately 625 ha^{-1}. At each site single, 0.1 ha plots of kale containing 60 and 120 g ha^{-1} of pheromone active ingredient were compared with a control plot. One or two aphicide sprays were made early in the season in all plots, but controls were otherwise untreated. As determined by weekly sampling, populations of pests, including DBM, were low throughout the trial and no clear between-treatment differences in the numbers of DBM larvae and pupae, or in yield, were observed. Pheromone trap monitoring indicated incomplete suppression of catches in the pheromone-treated plots, even in the early stages of the trial, when disruption of pheromone-mediated behaviour should have been greatest. From the results it is concluded that the pheromone treatments did not disrupt mating of DBM. It is concluded that previous, apparently successful, results in Kenya may have been due to insecticide 'resurgence' effects in control plots providing a favourable, but misleading, comparison with the pheromone plots. Through a comparison of trial results from elsewhere, it is further concluded that the only chance of successful mating disruption in the Kenyan, small-holder farmer context lies in increasing pheromone dispenser density and effective plot size (through the use of 'buffer zones'). Accordingly, recommendations are made for a trial incorporating these and other changes.

Acknowledgements

I am greatly indebted to the unstinting efforts of Dr G. Oduor and Mr W. Okello Ogutu from CABI-ARC and Dr G.N. Kibata and Mr D. Miano of KARI who set-up and managed the trial at the three field sites. The respective field teams from both organisations worked tirelessly throughout the long trial period. I am grateful for the assistance of Mr Wanyonyi (Irrigation Manager) and Mr Mbuvi (Farm Manager) at the University of Nairobi farm, Kabete. Similarly, Mr Kaibui (Assistant Farm Manager) at Jomo Kenyatta University of Agriculture and Technology was immensely helpful in managing the trial plots there, as were numerous colleagues at Ongata Rongai farm. I thank the following NRI staff members: Mark Parnell for assistance in finding the trial sites, Dr David Jeffries for his statistical advice, Mrs Margaret Brown for procurement assistance, and Dudley Farman for laboratory analysis of mating disruption dispensers.

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Introduction

Project R7449 aims to develop, evaluate and promote two new biologically based IPM control methods for the diamond-back moth (DBM), *Plutella xylostella,* L. on small-holder vegetable farms in Kenya. These are the use of pheromone-based mating-disruption and an endemic viral bio-pesticide. This report concerns trials to develop the first of these. It has two main objectives: to report detailed results from trials carried out in the 2000 and to discuss these in the context of earlier trials in Kenya during the previous project phase (R6615) (Critchley *et al*., 1998, 1999a, 1999b) and, elsewhere, by others.

The technique of mating-disruption involves the release of pheromone within a crop, such that mate location is disrupted or impaired in some way, and subsequent infestations reduced or eliminated. As in the present case - with brassica crops in peri-urban Kenya - it has generally been developed in crops in which insecticide usage is problematic due the development of resistance, or is undesirable because the product is destined for human consumption.

Several commercial formulations of pheromone dispenser have been developed for mating-disruption. All aim to provide a controlled release of pheromone into the crop over a long period, and protection against chemical degradation as well as ease of application. The formulation used in the current work is based on a poly-vinyl chloride matrix and was originally developed at NRI (Cork *et al*., 1989).

The identity of the sex pheromone of DBM is well established. Many years ago it was shown to comprise a mixture of three components, principally (*Z*)-11-hexadecenal (Z11-16:Ald) and (*Z*)-11-hexadecenyl acetate (Z11-16:Ac) (Tamaki *et al*., 1977; Koshihara *et al*. 1978), with a small quantity of (*Z*)-11-hexadecenol (Z11-16:OH) (Ando *et al*., 1979; Koshihara & Yamada, 1980). The geographic origin of DBM may affect the optimum ratio of blend components. In Canada a 70:30:1 mixture (of Z11-16:Ald: Z11-16:Ac: Z11-16:OH) appears best (Chisholm *et al*., 1979; Chisholm *et al*., 1983) but in Japan 50:50:1 is more effective (references above; Kawasaki, 1984). It was assumed at the outset of the previous project R6615 that the race of DBM in Kenya would more closely resemble that in Asia than that in America (Critchley *et al*., 1998). Therefore the 50:50:1 blend was adopted as standard.

The studies by Critchley and colleagues in Kenya took place against a background of several previous successful trials of mating-disruption of DBM. In Japan, Ohbayashi *et al*. (1992), and to a lesser degree Nemoto *et al*. (1992), found large reductions in catches of males in monitoring traps in plots, lower rates of mating and reduced larval numbers in pheromone treated brassica and vegetable plots, compared with untreated or insecticide treated fields. Similarly, on commercial cabbage farms in Florida, USA McLaughlin *et al*. (1994) and Mitchell *et al*. (1997) obtained good control of the pest in pheromone plots compared to fields treated with insecticides. With the exception of that by Nemoto *et al.* (1992) all these studies had used relatively high ($\geq$ 250 g ha^{-1}) rates of application of the pheromone active ingredient that could not be considered economically viable (Talekar & Shelton, 1993). All were carried out in fields of several hectares or more. Therefore these trials could not be regarded as realistic in terms of the constraints faced by small-holder farmers in Africa. Ohno *et al*. (1992), in Japan, and Schroeder *et al*. (2000) in the USA have carried out trials in much smaller, 0.1 and 0.2 ha plots, respectively (though also with application rates of at least 250 g ha^{-1}), but these yielded conflicting results.

In this context, for work in Kenya it was important to investigate the possibility of mating-disruption of DBM in small plots, typical of those cultivated by Kenyan small-holder farmers, using lower rates of application that were more likely to be economically viable. Work on the previous project phase was carried out in the major rainy seasons of 1997 (May - July) and 1998 (May - September), and the major dry season of 1999 (February - April).

During the first year much of the work consisted of identifying the most practical traps and lures to be used in subsequent work (Critchley *et al*., 1998). Results obtained then dictated the trap and lure types used since then (see Materials and Methods). Two further sets of preliminary observations provided encouraging indications of the feasibility of mating-disruption under Kenyan conditions. Mating-disruption dispensers placed in 12 × 12 m plots depressed catches by traps in the centre of the plots by 73 - 98%, compared to untreated plots, 10 weeks after application, indicating that males' ability to locate individual pheromone sources within small treated areas was greatly reduced over this period. Furthermore, measurements of the persistence of the pheromone in the mating-disruption dispensers showed that most (60 - 70%) remained after two months exposure under field conditions.

In 1998 and 1999 full-scale trials were undertaken in kale crops. In 1998, dispensers were set out in 0.1 ha plots and in 4 - 8 m wide buffer zones around each plot. The density of sources was varied between each of three sites, but by also varying the amount of pheromone within individual dispensers the overall application rate was maintained at approximately 60 g ha^{-1}. Pheromone plots were compared to controls that were intended to be representative of typical farmer practice. Consequently, the latter received several insecticide sprays against DBM and non-DBM pests. The pheromone treated plots received some limited sprays against non-DBM pests. In the following year a similar approach was taken. Identical treatment and control plots were set out at two sites but plots were only 0.05 ha in size, the pheromone plots lacked buffer zones and the application rate was slightly lower (53 g ha^{-1}). Mating-disruption dispensers were placed at the highest and most effective density used in 1998.

In the 1998 trials (Critchley *et al*. 1999a), the effectiveness of the pheromone treatment appeared to be positively associated with the density of mating-disruption dispensers. In the highest source density (625 ha^{-1}) plot, trap catches of males were completely suppressed for more than 10 weeks after application, larval and pupal numbers and plant damage scores were consistently much lower, relative to the control, and total marketable yield was double that of the control plot. There was no evidence of any controlling effect at the lowest density of pheromone dispensers (156 ha^{-1}), while results for the medium density (312 ha^{-1}) plot were intermediate in most respects. In 1999, the effectiveness of the pheromone treatment was much less marked than for comparable results in 1998. At one site complete trap catch suppression was never achieved, and larval numbers and plant damage scores were usually only slightly lower than in control plots, if at all. Nevertheless yield in the pheromone plot was substantially greater at one site; no valid yield data were available at the second site (Critchley *et al*. 1999b).

Materials and Methods

Location of trial sites

The trial was conducted at three sites: the University of Nairobi farm at Kabete, about 6 km north-west of Nairobi; the Jomo Kenyatta University of Agriculture and Technology farm site (JKUAT), situated about 24 km north-east of Nairobi; a small commercial farm at Ongata Rongai, approximately 20 km south-west of Nairobi.

Crops and agronomic practices

The target crop was kale (Collard *var*. Southern Georgia), *Brassica oleracea*. All plants were transplanted into the plots as seedlings with 3 – 6 true leaves at a spacing of 60 × 60 cm. Seedlings used at Kabete and JKUAT came from nursery beds at KARI/NARL (from screen-house and field beds respectively). Those used at Ongata Rongai came from a local commercial nursery. Transplanting at Kabete, JKUAT and Ongata Rongai took place on 10, 14 and 21 March 2000, respectively. Due to dry weather all plots were irrigated, manually or using overhead irrigation equipment, for at least two weeks following transplanting, and thereafter were rain-fed. Despite this, quite large numbers of seedlings died, necessitating gap-filling. This was most severe at Kabete where up to half of the seedlings needed to be replanted 1 – 2 weeks after initial transplanting.

Fertiliser applications and weeding were carried out as indicated in Table 1 below. DAP was applied at planting while CAN was a top dressing at a rate 5 g and 10 g per plant respectively.

Table 1. Dates of fertiliser applications and weeding at the three trial sites.

Operation	Kabete	JKUAT	Ongata Rongai
Fertiliser applications	10 March 5 April	14 March 5 April and 12 June	21 March 11 April
Weeding	28 March 27 April 14 June 26 July	4 April 18 April 10 May 14 June 10 July	10 April 27 April 24 May 21 June 20 July

Early season insecticide applications were made against aphids (mainly *Brevicoryne brassicae*) using aphid-specific products. All plots at Ongata Rongai and Kabete received two sprays, and those at JKUAT one spray. Sprays at Ongata Rongai and Kabete on 4 April 2000 used Pymetrozine 50 WG (200 g in 500 l water ha^{-1}), a novel product from Novartis. At JKUAT on 5 April and at Ongata Rongai and Kabete on 2 – 3 May, sprays were made with Pirimor 50 WP (Pirimicarb, 250 g in 500 l water ha^{-1}).

Plot sizes and treatments

Individual plots were 0.09 - 0.10 ha in area; dimensions varied from 30 × 30 m to 20 × 50 m. Plots were situated at least 50 m apart to minimise the effect of pheromone dispensers in one plot on others. At Kabete and Ongata Rongai plots were at least 100 m from other brassica crops which might act as sources of infestation, but at JKUAT one end of the plots was only 10 m from another field of kale.

Three plots were planted at each site: an untreated control, a pheromone treatment of approximately 60 g a.i. ha^{-1} and one of 120 g a.i. ha^{-1}. For both pheromone treatments the formulation used was a black, PVC 'shoelace' or Selibate™ formulation (Cork *et al*., 1989) produced by Agrisense-BCS, Pontypridd, UK in December 1999. This contained a blend of (*Z*)-11-hexadecenal, (*Z*)-11-hexadeceny acetate and (*Z*)-11-hexadecenol in the ratio 30:30:1, with a total active ingredient content of 7.9 mg g^{-1} (determined by analysis at NRI). The departure of the blend from the nominal 50:50:1 ratio was not considered to be biologically significant. The differences in application rate of the pheromone treatments were achieved by applying different amounts of the formulation; lengths weighing 1 or 2 g were used. These treatments are hereafter referred to as the 1g and 2g pheromone treatments, respectively.

Individual lengths of the formulation were fixed into the split top end of bamboo canes, at a height of 1 m, and applied 4 m apart in a grid pattern (approximating 625 ha^{-1}), the day after transplanting (Figs. 1 and 2). Due to differing plot dimensions the number of dispensers per plot

varied from 64 to 78[1]. Dispensers that were damaged or lost during the course of the trial were replaced with fresh dispensers within a few days.

Monitoring and evaluation of treatments

Daily catches of adult male DBM in three white, sticky-delta pheromone traps placed in each plot were recorded. The traps were baited with polyethylene vial lures (23 mm × 9 mm diameter) containing 0.1 mg (Z)-11-16:Ald, (Z)-11-16:Ac and (Z)-11-16:OH in the respective ratio of 50:50:1, plus 0.2 mg BHT. Sticky card trap inserts were replaced weekly; pheromone lures were replaced every two weeks. Traps and lures were supplied by International Pheromone Systems, Wirral, U.K.

DBM and other invertebrates and diseases were sampled on a weekly basis in each plot. A plant was randomly selected within each plot, this plant and the eight surrounding ones were then sampled. If a plant was missing, the next plant out from the centre of the group was sampled instead. This procedure was repeated a further two times so that 27 plants were sampled per plot. The two outer rows of plants were not sampled. All invertebrates on a selected plant were counted individually, except for aphids. Aphid species and plant diseases were scored as indicated in the Table 2.

Table 2. Scoring system used for sampling of aphids and plant diseases.

Score	Aphid species (on top-most, fully expanded leaf only)	Diseases (whole plant)
0	no aphids present	no symptoms
1	~5% of leaf area covered	~10% of plant affected
2	~10% " "	~25% " "
3	~25% " "	≥50% " "
4	≥50% " "	-

Harvesting commenced 36 – 55 days after transplanting at the different sites. It then continued at 14-day intervals such that 7 – 9 harvests were carried out at each site. On each occasion the total number of marketable and damaged leaves, and their weight, was recorded separately. A distinction was made between all harvests and those up to 20 June only (approximately 12 WAT). This was because the residue data for the pheromone formulation obtained by Critchley *et al*. (1998), and trap-catch data from Critchley *et al*. (1999a,b), indicated that 12 weeks is the

[1] As a result of this the actual dose rates were, for the 1 g treatment, 56.9 - 62.4 g a.i. ha^{-1}; for the 2 g treatment

approximate half-life of the formulation, and is probably the maximum period over which trap-catches in treated plots are suppressed. Together these suggested that any impact on yield should be confined to the first 12 weeks after transplanting. In each plot the initial (after gap-filling) and final (after last harvest) plant stand was also determined.

Quantitative analysis of the mating-disruption formulation was also carried out. Five lengths were retrieved from each treated plot at each site on 8 August 2000 (140 – 151 days after transplanting), wrapped in aluminium foil and refrigerated before being sent back to NRI for analysis. The amount of each pheromone component contained in three sub-sample lengths of the formulation from each plot was then determined and compared to un-exposed samples taken from the packet supplied on 23 March.

Duration of the trial

The original intention was for the trial to last 12 – 15 weeks. However generally low populations of DBM resulted in the decision to leave the crop in the ground, and monitoring to continue, until about 19 weeks after planting, in the hope of encountering sufficiently large populations that between-treatment differences might become apparent.

Data analysis

In the present design there is no suitable method of statistical comparison of monitoring data or sample harvests, as the geographically separate sites cannot be regarded as replicate blocks (D. Jeffries, pers. comm.). This is because the size of any treatment × block interactions cannot be estimated (there was only one replicate of each treatment per site). However, large or temporally consistent differences between data from different plots, may be considered as indicative of real differences.

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they were 113.8 - 124.8 g a.i. ha^{-1}.

Figure 1. Selibate™ DBM mating-disruption dispenser fixed into bamboo cane.

Figure 2. Array of mating-disruption dispensers, immediately after transplanting, in one of the plots at Kabete.

Results

Pheromone trap catches

Catches of male DBM adults were nearly always highest in control plots, especially at the start of the season (Figs. 3 - 5). The difference between catches in the control and pheromone plots tended to decrease through the trial period, so that they were similar in the last few weeks. The relative magnitude of catches in the control and pheromone plots are illustrated in Table 3. General catch levels fluctuated through the season, but trends differed between sites. Peak catches of 20 - 22 male DBM adults per week were reached in control plots at Kabete 18 - 20 WAT and at JKUAT from 3 - 9 WAT (Figs. 3 and 4). Those at Ongata Rongai occurred from 9 - 15 WAT, and were less than half as great (Fig. 5).

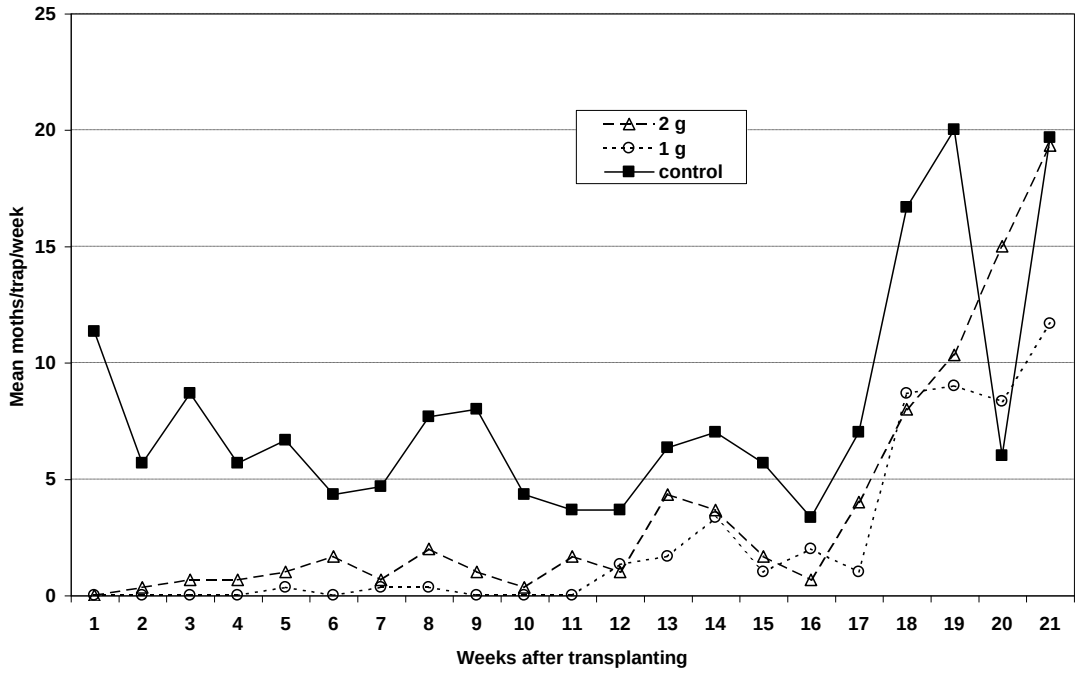

Figure 3. Mean weekly trap-catch results for Kabete (means of 3 traps for each plot).

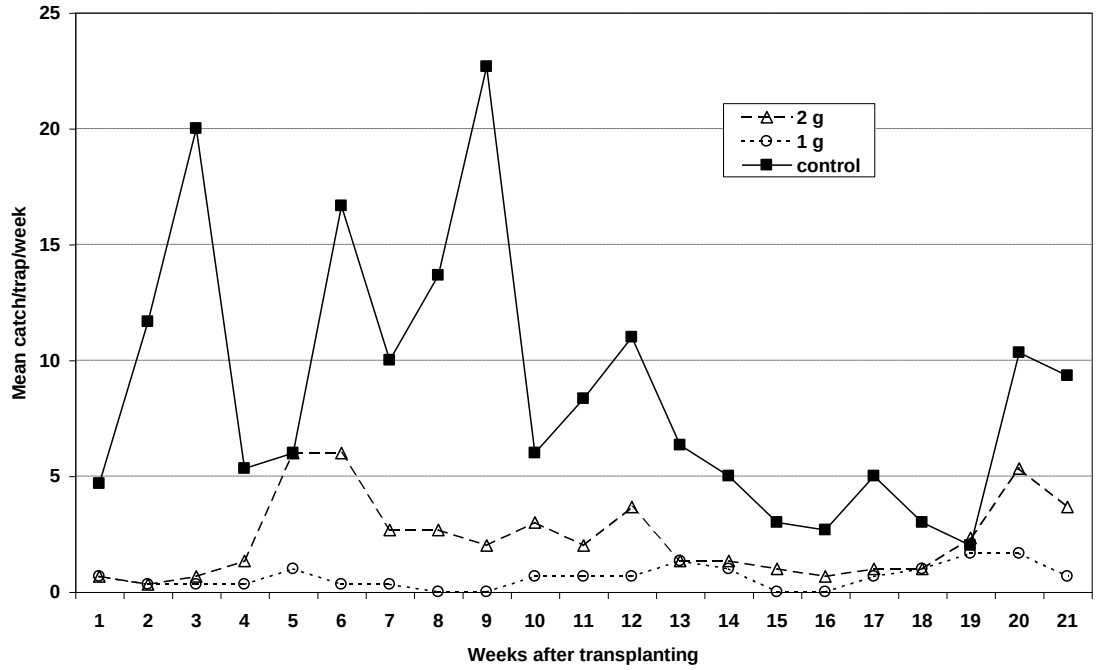

Figure 4. Mean weekly trap-catch results for JKUAT (means of 3 traps for each plot).

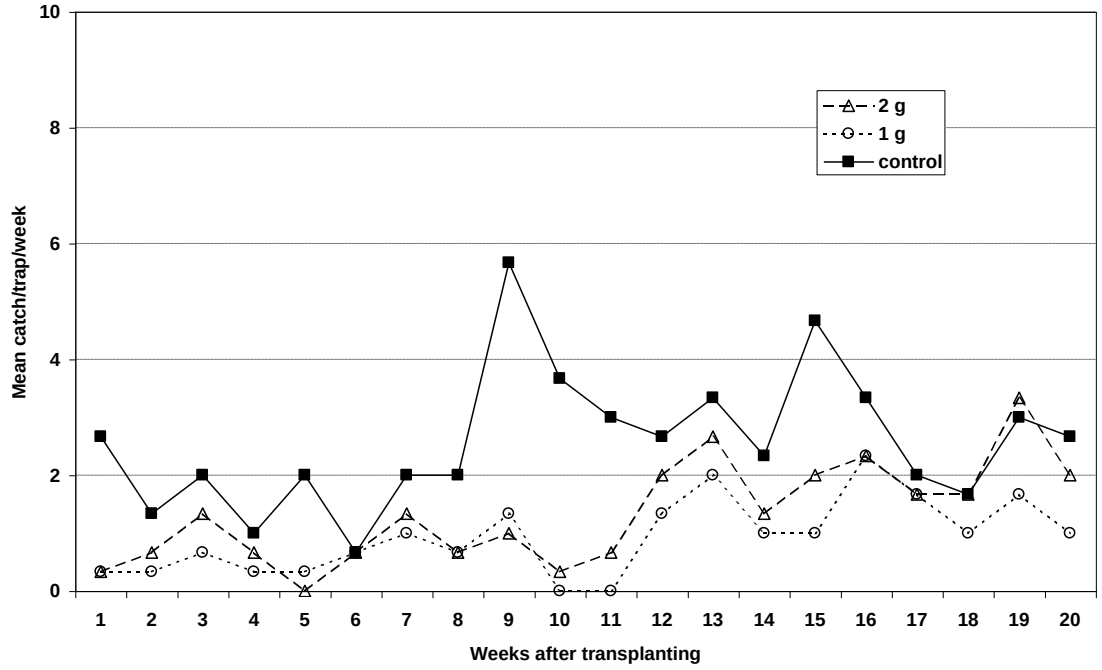

Figure 5. Mean weekly trap catches at Ongata Rongai (means of 3 traps for each plot).

Table 3. *Weekly trap-catches in the pheromone plots at each site, expressed as percentages of the respective control.*

Weeks after planting	Kabete		JKUAT		Ongata Rongai	
	2 g	1 g	2 g	1 g	2 g	1 g
1	0	0	14	14	13	13
2	6	0	3	3	50	25
3	8	0	3	2	67	33
4	12	0	25	6	67	33
5	15	5	100	17	0	17
6	38	0	36	2	100	100
7	14	7	27	3	67	50
8	26	4	20	0	33	33
9	13	0	9	0	18	24
10	8	0	50	11	9	0
11	45	0	24	8	22	0
12	27	36	33	6	75	50
13	68	26	21	21	80	60
14	52	48	27	20	57	43
15	29	18	33	0	43	21
16	20	60	25	0	70	70
17	57	14	20	13	83	83
18	48	52	33	33	100	60
19	52	45	117	83	111	56
20	250	139	52	16	75	38
21	98	59	39	7	-	-

Weekly DBM counts

The majority of DBM found on kale plants during the trial were larvae. Combined larval plus pupal counts are illustrated in Figs. 6 - 8. These show that there were almost universally very low numbers (<1 individual per plant) in all plots throughout the trial, and little evidence of any consistent treatment differences. The only exception to this was at Kabete where after 16 weeks mean counts rose to 2 – 6 DBM per plant, with counts tending to be lowest in the control plot.

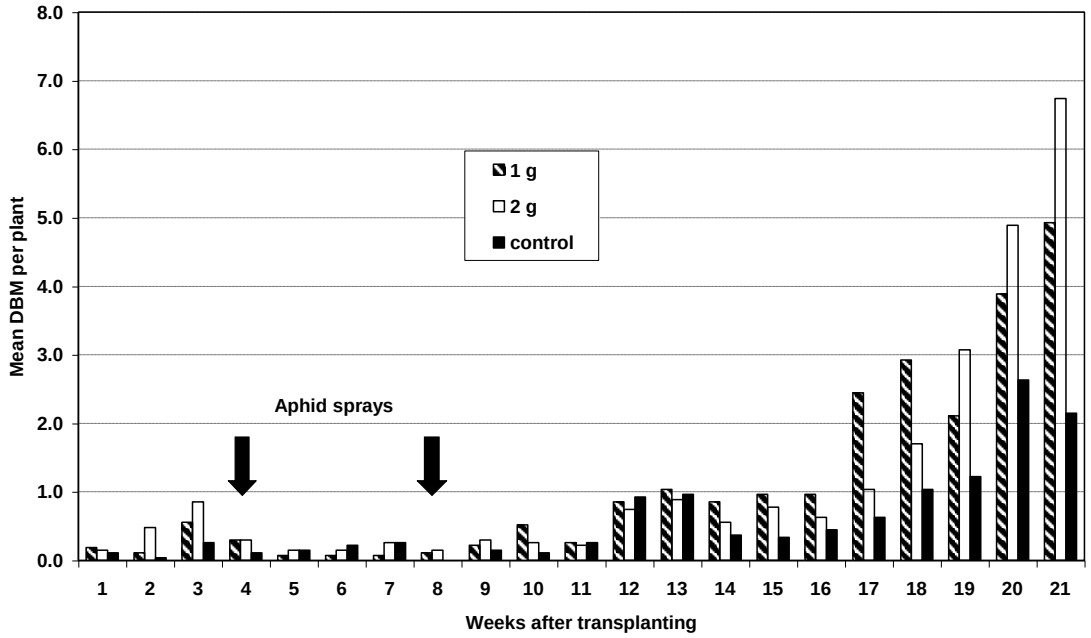

Figure 6. DBM counts (larvae and pupae) at Kabete.

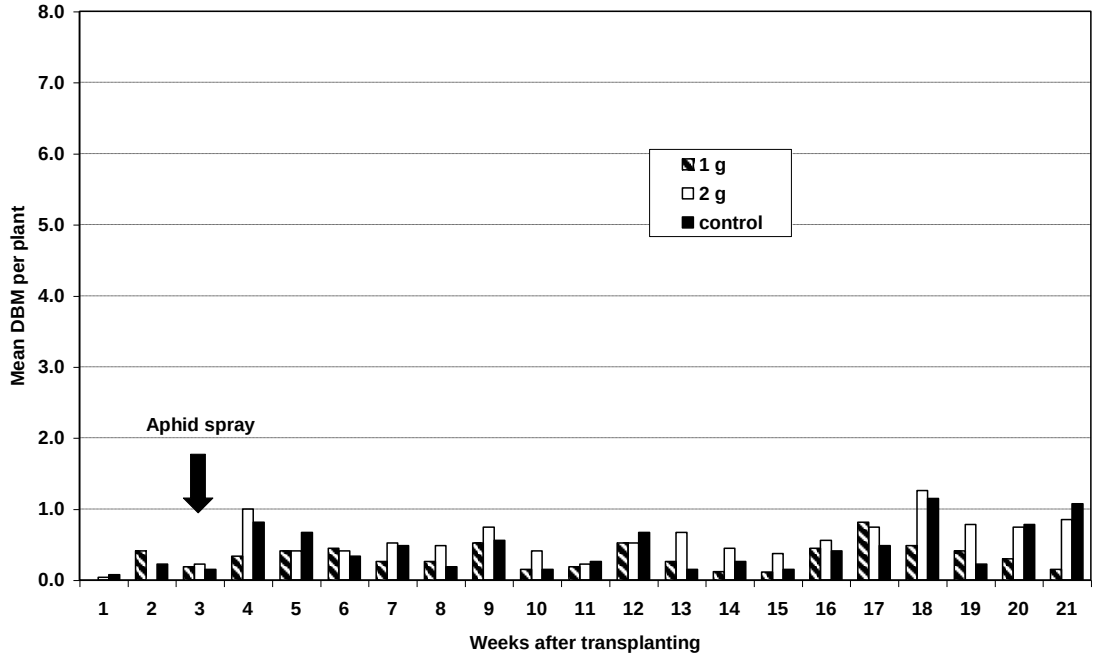

Figure 7. DBM counts (larvae + pupae) at JKUAT.

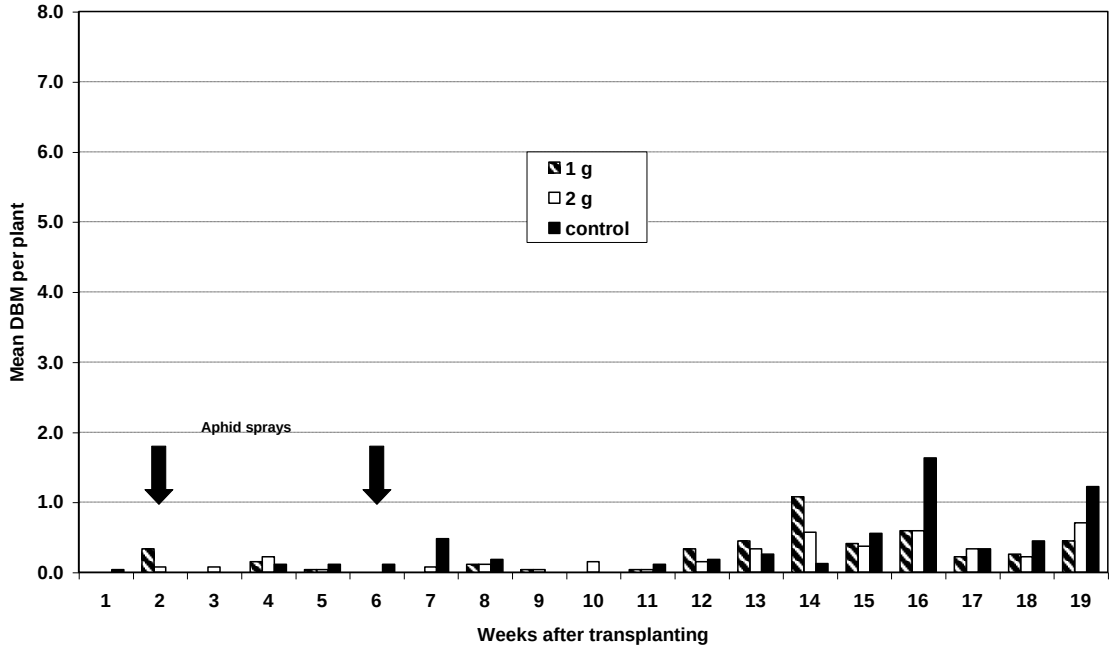

Figure 8. DBM counts (larvae + pupae) at Ongata Rongai.

Non-DBM counts

Comprehensive tables of invertebrate and disease incidence are given in Appendices 1a - c, 2a - c and 3a - c.

There were almost no significant non-DBM pest attacks at any site throughout the trial. Aphids, predominantly *Brevicoryne brassicae*, were the most abundant pests other than DBM. There was some tendency for *B. brassicae* scores to increase through the trial, but the highest were rarely greater than 0.6 (see illustrative data for JKUAT, given as Fig. 9). The low populations of aphids seemed unaffected by the early-season aphicide applications. The occurrence of other pests such as leafminer species, bollworms, *Plusia orichalcea* and thrips was often nil and only rarely exceeded 0.5 individuals per plant.

The occurrence of fungal diseases was negligible, although occasional scores of 1.0 were recorded in respect of powdery mildew at Kabete. However, unspecified plant viruses may have had some general impact on yield. At all sites mean virus disease scores increased steadily through the trial, exceeding 1.0 on most sample dates in the latter half. They were highest at JKUAT (see Fig. 10), where mean scores reached 1.5 – 2.0. There was no

indication that disease scores were linked to the presence or absence of pheromone treatments.

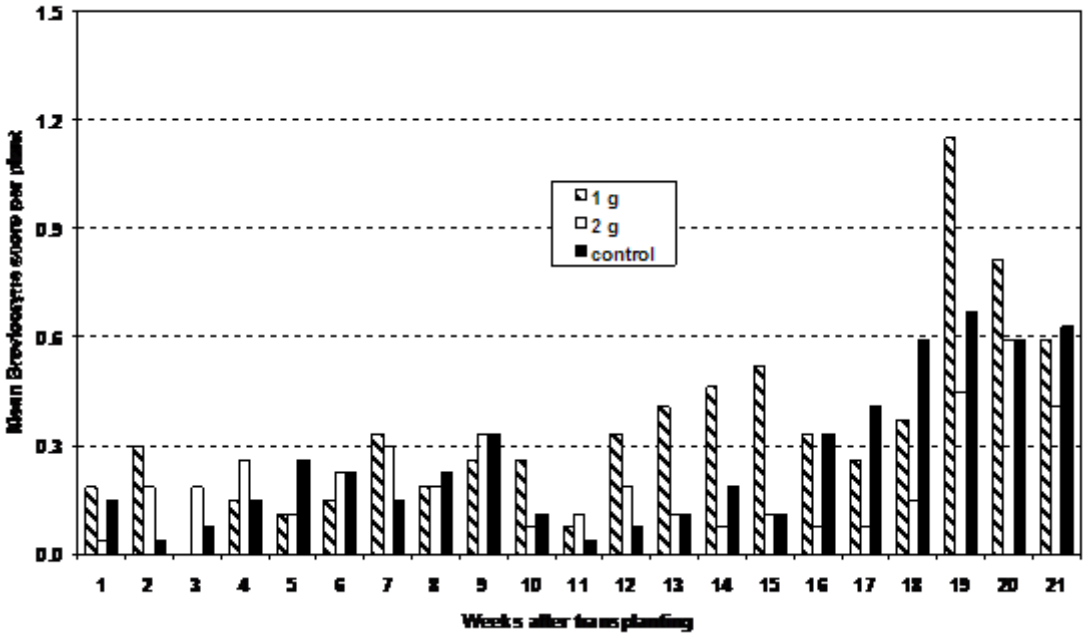

Figure 9. Mean weekly *Brevicoryne* spp. aphid scores at JKUAT.

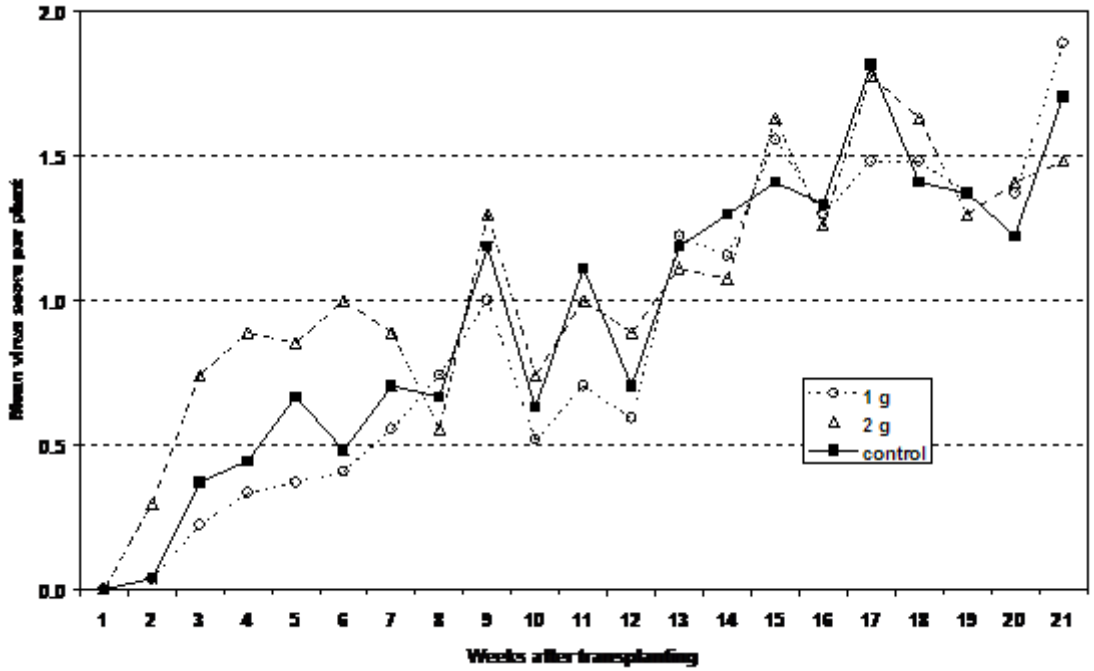

Figure 10. Mean weekly plant virus scores at JKUAT.

Natural enemy counts were low in all plots at all sites, throughout the trial. The incidence of predators such as coccinelids, syrphids, spiders, *Orius* spp. and ants was usually nil and only occasionally rose to more than 0.2 individuals per plant for any species group (Appendices 1a to 3c).

Harvests

Yield data were expressed as accumulated totals for harvests up to 20 June, and for all harvests to the end of the trial (Table 4). Total yields were greatest in the 2 g pheromone plot at JKUAT and in the control plots at Ongata Rongai and Kabete. The lower yields in the pheromone plots at Kabete are partly explained by the lower number of plants in those plots. The percentage by weight of yield considered too damaged to be marketable varied from 9 – 16% for harvests up to 20 June, and from 10 – 18% for all harvests. Differences between treatments in all cases were quite small and showed no consistent trend across sites.

Table 4. Summary harvest data.

	Total harvested (Kg)*		% Harvest damaged by weight		Final plant
	All harvests	To 20 June	All harvests	To 20 June	Stand as % of Initial
JKUAT					
1 g	916	553	14.8	14.5	93.1
2 g	1313	808	15.9	14.4	97.4
Control	983	644	17.2	12.3	88.3
Ongata Rongai					
1 g	1736	1091	12.0	9.3	93.2
2 g	1886	1228	10.4	9.1	90.3
Control	2379	1645	10.9	11.1	90.5
Kabete					
1 g	1847	1328	17.9	15.4	69.1
2 g	1940	1329	12.8	11.8	76.9
Control	2503	1671	13.9	15.6	86.0

* The total number of harvests was 7 at JKUAT, 8 at Ongata Rongai and 9 at Kabete; the number of harvests carried out up to 20 June was 4, 5 and 5 respectively.

Quantitative analysis of Selibate™ dispensers

Analysis of the unexposed sample dispensers, both 1 and 2 mg, showed that the original concentration of active ingredient within the Selibate™ was very close to the intended figure of 8% (Table 5). The relative proportion of the Z11-16:OH minor component was higher than intended – being in the ratio 1:25 to 1:30 with the other components, instead of 1:50. However reported successes with DBM mating-disruption formulations containing a range of varying blends suggests this small variation from the intended ratio should not have had a significant impact on the effectiveness of mating-disruption.

Results for all the exposed samples indicated that total pheromone content was reduced to 15 – 25% of the original value after 19 weeks in the field. Of the remaining pheromone, most consisted of the Z11-16:Ac component, and the ratios of the three components were skewed markedly from the original values. This suggests that the formulation could not have been at all effective in influencing DBM moth behaviour by the end of the trial.

Table 5. Summary results of quantitative analysis of DBM Selibate formulation; figures are all means of three samples.

Sample	Amount of pheromone components (mg/100 mg formulation)			Total (mg/100 mg)	Component ratios		
	Z11-16:Ald	Z11-16:Ac	Z11-16:OH		Ald:Ac	Ald:OH	Ac:OH
Unexposed 1g	3.83	3.95	0.13	7.91	0.97	29.60	30.49
Unexposed 2g	3.79	3.88	0.16	7.84	0.98	24.09	24.58
JKUAT 1g	0.53	1.44	0.01	1.98	0.36	64.96	178.28
JKUAT 2g	0.05	1.40	0.01	1.46	0.04	7.83	205.75
Kabete 1g	0.23	1.07	0.01	1.31	0.21	21.19	97.44
Kabete 2g	0.12	1.04	0.01	1.17	0.14	10.93	89.35
Ongata Rongai 1g	0.08	2.14	0.01	2.23	0.04	7.82	220.97
Ongata Rongai 2g	0.08	1.83	0.01	1.92	0.04	8.16	205.12

Formulation produced by Agrisense-BCS 22 December 1999; unexposed samples taken from bags 23 March 2000; exposed samples all removed from fields 8 August 2000; analysis carried out 17 November 2000.

Discussion

The present results

It is clear from the present results that both levels of pheromone treatment had little, if any, effect in reducing DBM pest incidence or damage. The lower, 1g, pheromone treatment was equivalent in source density and unit-area dose-rate to the 'best' (*i.e.* 625 sources ha^{-1}) treatment identified by Critchley *et al*. (1999a,b). Thus there is a striking contrast between the results for 2000 and the findings in 1998 and 1999.

There are three possible explanations for this:

1. low pest incidence in 2000 which prevented any between-treatment differences being manifested;
2. greater use of insecticides, particularly in control plots, by Critchley *et al*. leading to 'resurgence' of DBM in controls which in turn produced apparent, beneficial effects of the pheromone treatments in 1998 and 1999;
3. use by Critchley *et al*. of 'buffer zones' of extra dispensers around pheromone plots, effectively increasing plot size.

Pest Incidence

Although DBM incidence was low in 2000, and damage by the pest was never serious, inspection of the data for 1998 and 1999 (Table 6) indicates that pest numbers were not much higher than in 2000. Yet there were marked differences between treatment and control plots in the Critchley *et al*. results. Trap catches in pheromone plots (all sites, both years) were more strongly suppressed than in 2000; the pheromone treatment equivalent to the present 1g treatment apparently resulted in strong suppression of larval and pupal numbers, and greater yield, in 1998 and at one of the two sites in 1999. Considering all these facts, if the pheromone treatments deployed in 2000 were at all effective, at least some impact on pest incidence could have been expected, even if there were no effect on yield. Thus the first explanation above does not seem adequate.

Table 6. Summary comparison of results between data of Critchley *et al*. (1999a,b) and 2000 trials in Kenya.

	1998 & 1999	2000
Mean weekly trap catches (control plots)	Mostly 2 - 10, peaks 25 - 45	Mostly 5 - 10, peaks 20 - 25
Larval + pupal incidence per plant	Mostly 0.5 - 2.0, end-of-season peaks 4.5 - 35.0 (control plots)	Always < 1.0, except end-of-season peak at Kabete of 6.0 (treatments and controls)
Yield	Yield in 'best' density pheromone plot $\times 2$ that in control; no effect at lower pheromone densities	No consistent differences in % yield loss between control and pheromone plots

Resurgence Effects

There is good evidence that 'resurgence' effects could explain the results obtained by Critchley et al. (1999a,b). In those trials the control plots were intended to reflect farmer practice and as such, consistently more insecticides were applied than in the respective pheromone treated plots (Table 7).

In three separate comparative pesticide trials carried out in Kenya under the Vegetable IPM project (A0848), DBM larval numbers were consistently higher in plots treated with Pirimicarb and the pyrethroid λ-Cyhalothrin, than in unsprayed plots. Populations of syrphid fly larvae (predators of DBM) were also reduced in the sprayed plots. The same effects were noted in respect of λ-Cyhalothrin in three 'hand-picking' trials, and in these cases there was evidence that spider populations were also reduced. It is possible that similar effects may have occured with other pesticides used in the 1998 and 1999 pheromone trials, particularly the pyrethroid α-Cypermethrin. It can be further noted that populations of syrphid fly larvae were lower in control plots at two of the three sites in the 1998 trials (no data on beneficial arthropods were recorded for 1999), and that pyrethroids were only sprayed in control plots. Thus, there is a very strong argument that the lower populations of DBM observed in the pheromone plots of 1998 and 1999 were due to a lack of resurgence effects there, compared to the more heavily insecticide-sprayed control plots. Such could not have been the case in 2000.

Table 7. Summary comparison of insecticide applications in 1998, 1999 and 2000 trials in Kenya.

	1998	1999	2000
Sprays in pheromone plots	1 - 3	0 - 2	1 - 2
Sprays in controls	2 - 5	4 - 9	1 - 2
Insecticides used	Fenitrothion, Pirimicarb, λ-Cyhalothrin*	Including: Fenitrothion, Pirimicarb, Dimethoate, α-Cypermethrin* Chlorpyrifos	Pymetrozine, Pirimicarb

* Not used in the respective pheromone plots

Use of 'Buffer Zones'

In their 1998 trials Critchley and his co-workers employed 4 - 8 m wide 'buffer zones' around the edges of the pheromone plots. It is conceivable that these contributed to the apparent beneficial effects of pheromone treatment observed, as they would have nearly doubled the effective plot size. In support of this it may be noted that results in 1998 were somewhat better than in 1999, when buffer zones were not used.

Plot size is usually considered an important factor governing the effectiveness of mating-disruption treatments - and most successful control has been in plots of at least 1 ha. Having a large, contiguous area of treated crop is advantageous in two ways. Firstly, the diluting effect of wind upon aerial concentrations of the pheromone within the plot is minimised, at least away from the field edges. Secondly, if immigration of previously mated female insects occurs, it is less likely that all of the plot will be affected if it is sufficiently large.

Other mating-disruption studies with DBM

As noted in the Introduction, trials conducted by Ohbayashi *et al*. (1992), Nemoto *et al*. (1992), Ohno *et al*. (1992), McLaughlin *et al*. (1994) and Mitchell *et al*. (1997) have all produced good control of DBM in pheromone plots compared to their respective controls. All of these results were obtained using commercial ‘rope’ formulations consisting of a tube filled with the pheromone and stiffened with wire, and nearly all utilised only the two major pheromone components, Z11-16:Ald and Z11-16:Ac.

All of the above-mentioned trials used at least 250 g ha^{-1} of pheromone, with the exception of that by Nemoto *et al*. (1992), which employed only 50 g ha^{-1}. Interestingly, it was in this case that the results were least convincing. Specifically, trap captures of male DBM in the pheromone plots were less suppressed, relative to untreated controls, than in the other trials and larval incidence was lower than the controls only on the last sampling date. This seems to underline the importance of high application rates.

Ohbayashi *et al*. (1992) reported a series of nine trials, using unsprayed controls, carried out in open fields over a range of locations, and plot sizes – mostly between 3 and 14 ha. Application rates were 250 g ha^{-1} a.i. Trap catches in pheromone plots were typically reduced by 90% compared to controls, levels of mating suppression ranged from 50 – 100%, while larval reduction varied from 10 – 99%. Control was generally poorer in smaller fields, and in fields exposed to winds. In one field of 0.8 ha control was good in the centre, but poor at the edge. In a separate trial, mating suppression of almost 100% was achieved in 0.1 ha plots that were enclosed under a greenhouse, so as to be airtight. In the same plots mating suppression was poor when the plot was ventilated. Ohbayashi *et al*. (1992) concluded that successful mating disruption was not possible in small plots and at sites exposed to wind. These conclusions were somewhat contradicted by the findings of Ohno *et al*. (1992). They found strong (~90%) trap-catch and mating suppression, and lower incidence of larvae in open field plots of only 0.1 ha (one in an exposed location), also treated at a rate of 250 g ha^{-1}. Here again, controls were untreated.

McLaughlin *et al*. (1994) and Mitchell *et al*. (1997), working on commercial cabbage farms in Florida, used pheromone plot sizes of 8.1 and 24.6 ha, and application rates of 250 and 406 g ha^{-1}, respectively. In these cases the control plots received many more applications of insecticide than the pheromone plots. Thus the results - strong trap-catch suppression and reduction of larval numbers below an economic threshold in pheromone plots, relative to controls - might be ascribed to resurgence effects in the controls, as with the Critchley *et al*. results. However, the strong suppression of mating of sentinel females in the pheromone plots indicated a large mating disrupting effect, and argued against that conclusion. In the pheromone plots, greater crop damage was noted at the edges than in the centre. This was consistent with mating disruption working over most of the plots, but breaking down at the edges.

With regard to plot size the findings by Schroeder *et al*. (2000), also in small (0.2 ha) plots of cabbage, were interesting. A series of three trials compared pheromone applied at 275 g ha^{-1} with untreated controls and, unlike most other trials, treatments were replicated. Several field cages were deployed within plots; at the start of the trials these were inoculated with different densities of DBM pupae. Pheromone traps and 'sentinel' females were deployed within the field cages to assess the ability of males to locate females. Irrespective of the initial DBM density, no effect of the pheromone treatment was found on the proportion of mated sentinel females, or on the numbers of F_1 larvae and pupae found on cabbage plants. Significant trap-catch suppression only occurred in two of the three trials. Although the use of the field cages allowed the effect of the pheromone to be tested under several simulated population densities, and allowed possible effects of immigration or emigration to be discounted, they may have masked mating disruption effects. This could be because within the cages, males were never far from sentinel females, and did not need to follow their pheromone plumes far upwind, against a background of competing plumes from synthetic sources, as would otherwise be the case in a mating disruption plot. Instead, matings could have occurred following chance encounters. A similar effect could have occurred with respect to trap catches.

Limitations of the methods used in the 2000 trials

The design of the trial - with single replicates of each treatment and the control at separate sites, meant that there was no useful statistical analysis that could be applied to the data. In the present case this may not greatly matter, since visual inspection of the data is sufficient to indicate that no treatment differences occurred (except perhaps in the case of the pheromone trap catches). However, clearly it would be preferable to be able to analyse the data with some form of parametric test. The problem can only be avoided if there are at least two replicates of each treatment at each site.

The larval sampling method examined three groups of nine neighbouring plants per plot. Given the large size of the plots, a better, more representative sampling method might look at a larger number of groups, consisting of fewer plants.

Pheromone traps were monitored in order to provide a measure of the extent to which mating of DBM was disrupted in the respective plots. This assumed that the ability of males to orient towards both traps and females, is well correlated. In general this may not always be

justified[2], particularly if the pheromone blend used in the trap lures differs from the natural blend either quantitatively or qualitatively. Data on trap-catches and mating frequency has justified the assumption for some species (Critchley *et al*., 1991; Nakache *et al*., 1992; Chamberlain *et al*., 1993) but not others (Kehat *et al*., 1985; Palaniswamy *et al*., 1986; Downham *et al*., 1995).

With DBM, Chisholm *et al*. (1984) demonstrated equivalent reductions in trap catches in pheromone treated plots, whether they were baited with synthetic lures or virgin females. This suggested that trap catch reductions should form a good index of mating disruption. This was borne out by Ohbayashi *et al*. (1992), Nemoto *et al*. (1992), Ohno *et al*. (1992), McLaughlin *et al*. (1994) and Mitchell *et al*. (1997). However Schroeder *et al*. (2000) found trap catch suppression without any corresponding effect on mating. Given the doubt on this point, and the desirability of showing at least some definite evidence of mating disruption, a future trial should include some mating assessment, if feasible. At present it is probably best to assume that trap-catch suppression is not a reliable indicator of mating suppression.

Results of the quantitative analysis of the Selibate™ dispensers indicated that the pheromone content had fallen to ineffective levels by 19 weeks after application. However, it cannot be determined from the data how long the dispensers did remain effective. Results from the various Critchley trials suggested that 12 weeks was the maximum effective life-span of the formulation; trapping data from the present work agree with this to the extent that trap-catch suppression no longer occurred consistently after this period. Clearly, it will be advisable in future trials to retrieve mating disruption dispensers at regular intervals so that the change in pheromone content through time is monitored more closely.

[2] To understand why requires some understanding of the principles of mating disruption. Three main possible mechanisms are usually given (Minks & Cardé, 1988; Cardé & Minks, 1995; see also Sanders, 1997):

- 'confusion' or 'trail-masking' in which the pheromone plume of the female is rendered indistinguishable from the 'cloud' of synthetic pheromone produced by the sources;
- 'false-trail' following in which synthetic sources effectively compete with females, and males are diverted from them (but the respective plumes are distinguishable);
- adaptation of antennal receptors or habituation of the insect's central nervous system, this raises the male response threshold so that orientation to female-emitted plumes is reduced or absent.

Pheromone blend will strongly influence the mechanism and efficacy of mating disruption. In general, a complete rather than partial or unnatural blend will be most effective (Minks & Cardé, 1988). This is because insects are generally very good at distinguishing the neighbouring, even intermingled, pheromone plumes from sources releasing full and incomplete pheromone blends (*e.g.* Liu & Haynes, 1992).

Conclusions

It is concluded that:

1. The mating disruption trials for DBM conducted in Kenya in 2000 showed no evidence of successful mating disruption in that no beneficial effects were noted in respect of larval numbers or yield loss.

2. Positive results from previous trials in Kenya were mostly due to 'resurgence' effects in control plots, possibly aided by the use of buffer zones in pheromone plots.

3. Future trials are only likely to succeed if the plot sizes or unit-area dose rates are increased. Dose-rates greater than the higher of the two used in 2000, 120 g ha^{-1}, will probably never be economically viable. The use of commercial farm plots of several hectares probably offers the best technical chance of successful mating disruption of DBM, but as the overall project aim is to aid small-holder farmers for whom such large plots are not realistic, there is only very limited scope for increasing plot size.

4. There were limitations in the 2000 trials in terms of overall trial design, and to a lesser extent in the larval sampling methodology, the absence of a method for assessing mating frequency of female DBM in the trial plots and the need to determine pheromone loss from the dispensers more frequently.

Specific Recommendations for a Further Trial

1. Future trials should use 0.1 ha plots as before, but should compare untreated controls with mating-disruption plots where the array of dispensers covers the kale plot itself ***and*** a substantial 'buffer zone' outside so that a total area of 0.2 ha is covered.

2. A single dose-rate of 120 g ha^{-1} should be tested and the density of dispensers within the treatment plots should be doubled to 1250 ha^{-1}.

3. The trial design should consist of 3 controls and 3 treated plots, if possible at a single site (so that analysis of variance can be applied to any results). As before, trial plots (including buffer zones) should be separated by a minimum of 50 m.

4. The larval sampling method should examine seven groups of four neighbouring plants per plot.

5. Some assessment of mating by female DBM should be attempted on at least two occasions during the trial.

6. Samples of Selibate™ dispensers should be taken from the fields at 2 week intervals for subsequent quantitative analysis.

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Appendix 1a. Mean weekly pest, natural enemy and disease incidence in the 1 g pheromone mating-disruption plot at JKUAT.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.00	0.19	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00		
2	0.00	0.41	0.30	0.00	0.15	0.00	0.04	0.00	0.04	0.00	0.00	0.04	0.00	0.00	0.04	0.00		
3	0.00	0.19	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.00	0.04	0.07	0.00	0.00	0.22	0.00		
4	0.00	0.33	0.15	0.07	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.33	0.00		
5	0.00	0.41	0.11	0.00	0.11	0.00	0.00	0.00	0.04	0.00	0.07	0.11	0.00	0.00	0.37	0.00		
6	0.00	0.44	0.15	0.15	0.15	0.00	0.00	0.04	0.11	0.00	0.00	0.19	0.00	0.00	0.41	0.00		
7	0.00	0.26	0.33	0.00	0.07	0.00	0.00	0.00	0.04	0.04	0.07	0.07	0.11	0.00	0.56	0.00		
8	0.00	0.26	0.19	0.07	0.07	0.00	0.00	0.00	0.07	0.00	0.00	0.11	0.00	0.00	0.74	0.00		
9	0.00	0.52	0.26	0.15	0.04	0.04	0.00	0.07	0.11	0.00	0.00	0.15	0.00	0.00	1.00	0.00		
10	0.04	0.11	0.26	0.15	0.04	0.04	0.00	0.26	0.00	0.00	0.00	0.04	0.00	0.00	0.52	0.00		
11	0.04	0.15	0.07	0.19	0.07	0.04	0.00	0.07	0.00	0.00	0.00	0.11	0.00	0.00	0.70	0.00		
12	0.00	0.52	0.33	0.22	0.15	0.00	0.00	0.11	0.00	0.00	0.04	0.19	0.00	0.00	0.59	0.04		
13	0.15	0.11	0.41	0.19	0.19	0.07	0.00	0.22	0.00	0.00	0.07	0.11	0.00	0.00	1.22	0.00		
14	0.00	0.12	0.46	0.08	0.00	0.00	0.00	0.00	0.00	0.04	0.19	0.08	0.00	0.00	1.15	0.00		
15	0.04	0.07	0.52	0.22	0.04	0.00	0.00	0.26	0.00	0.00	0.04	0.15	0.00	0.00	1.56	0.00		
16	0.00	0.44	0.33	0.15	0.07	0.00	0.00	0.44	0.00	0.07	0.04	0.07	0.00	0.00	1.30	0.00	0.00	
17	0.04	0.78	0.26	0.37	0.19	0.00	0.00	0.33	0.00	0.00	0.19	0.11	0.00	0.00	1.48	0.00	0.00	
18	0.00	0.48	0.37	0.52	0.26	0.00	0.00	0.11	0.04	0.00	0.07	0.04	0.00	0.00	1.48	0.00	0.00	
19	0.00	0.41	1.15	0.74	0.00	0.00	0.00	0.07	0.00	0.22	0.19	0.22	0.00	0.00	1.37	0.00	0.00	0.15
20	0.04	0.26	0.81	0.37	0.04	0.00	0.00	4.89	0.00	0.07	0.11	0.00	0.00	0.00	1.37	0.00	0.00	0.04
21	0.00	0.15	0.59	0.48	0.00	0.00	0.00	4.96	0.00	0.00	0.26	0.00	0.00	0.00	1.89	0.04	0.07	0.00

Appendix 1b. Mean weekly pest, natural enemy and disease incidence in the 2 g pheromone mating-disruption plot at JKUAT.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.04	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
2	0.00	0.00	0.19	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.30	0.00		
3	0.04	0.19	0.19	0.04	0.07	0.00	0.00	0.00	0.07	0.00	0.04	0.04	0.00	0.00	0.74	0.00		
4	0.00	1.00	0.26	0.07	0.00	0.04	0.00	0.00	0.04	0.04	0.19	0.15	0.00	0.00	0.89	0.00		
5	0.00	0.41	0.11	0.04	0.19	0.07	0.00	0.00	0.15	0.00	0.30	0.15	0.00	0.00	0.85	0.00		
6	0.00	0.41	0.22	0.04	0.04	0.00	0.00	0.00	0.07	0.04	0.00	0.07	0.00	0.00	1.00	0.00		
7	0.04	0.48	0.30	0.07	0.04	0.00	0.00	0.00	0.11	0.00	0.00	0.07	0.00	0.00	0.89	0.00		
8	0.04	0.44	0.19	0.00	0.07	0.07	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.56	0.00		
9	0.00	0.74	0.33	0.07	0.11	0.00	0.00	0.11	0.00	0.00	0.00	0.26	0.00	0.00	1.30	0.00		
10	0.00	0.41	0.07	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.26	0.00	0.00	0.74	0.00		
11	0.00	0.22	0.11	0.11	0.07	0.00	0.00	0.07	0.00	0.04	0.00	0.19	0.00	0.00	1.00	0.00		
12	0.11	0.41	0.19	0.00	0.15	0.00	0.00	0.07	0.04	0.00	0.00	0.11	0.00	0.00	0.89	0.00		
13	0.04	0.63	0.11	0.15	0.44	0.00	0.00	0.48	0.00	0.19	0.11	0.00	0.00	0.00	1.11	0.00		
14	0.07	0.37	0.07	0.15	0.15	0.00	0.00	0.00	0.04	0.00	0.11	0.07	0.00	0.00	1.07	0.00		
15	0.04	0.33	0.11	0.19	0.07	0.00	0.00	0.67	0.00	0.11	0.07	0.15	0.00	0.00	1.63	0.00		
16	0.00	0.56	0.07	0.07	0.04	0.00	0.00	1.00	0.04	0.04	0.07	0.07	0.00	0.00	1.26	0.00	0.04	
17	0.07	0.67	0.07	0.22	0.11	0.00	0.00	0.26	0.00	0.07	0.04	0.07	0.00	0.00	1.78	0.00	0.11	
18	0.00	1.26	0.15	0.33	0.19	0.00	0.00	1.30	0.00	0.07	0.07	0.11	0.00	0.00	1.63	0.00	0.00	
19	0.04	0.74	0.44	0.41	0.11	0.00	0.00	0.67	0.00	0.00	0.11	0.11	0.00	0.00	1.30	0.00	0.11	0.22
20	0.07	0.67	0.59	0.33	0.11	0.00	0.00	6.44	0.00	0.19	0.04	0.11	0.00	0.00	1.41	0.00	0.04	0.26
21	0.04	0.81	0.41	0.22	0.07	0.00	0.00	8.19	0.00	0.15	0.22	0.07	0.00	0.00	1.48	0.04	0.11	0.00

Appendix 1c. Mean weekly pest, natural enemy and disease incidence in the control plot at JKUAT.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.07	0.00	0.15	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00		
2	0.00	0.22	0.04	0.07	0.04	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.00	0.04	0.00		
3	0.00	0.15	0.07	0.00	0.04	0.04	0.00	0.00	0.00	0.04	0.07	0.07	0.00	0.00	0.37	0.00		
4	0.00	0.81	0.15	0.07	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.44	0.00		
5	0.00	0.67	0.26	0.07	0.26	0.04	0.00	0.00	0.04	0.00	0.19	0.15	0.00	0.00	0.67	0.00		
6	0.00	0.33	0.22	0.00	0.04	0.00	0.00	0.00	0.11	0.04	0.00	0.30	0.00	0.00	0.48	0.00		
7	0.00	0.48	0.15	0.07	0.04	0.04	0.00	0.00	0.07	0.00	0.00	0.15	0.00	0.00	0.70	0.00		
8	0.00	0.19	0.22	0.15	0.11	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.67	0.00		
9	0.00	0.56	0.33	0.00	0.07	0.04	0.00	0.04	0.00	0.00	0.00	0.19	0.00	0.00	1.19	0.00		
10	0.00	0.15	0.11	0.00	0.04	0.00	0.00	0.19	0.07	0.00	0.00	0.22	0.00	0.00	0.63	0.00		
11	0.00	0.26	0.04	0.07	0.22	0.00	0.00	0.11	0.00	0.04	0.00	0.11	0.00	0.00	1.11	0.00		
12	0.04	0.63	0.07	0.04	0.11	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.00		
13	0.04	0.11	0.11	0.07	0.30	0.07	0.00	0.56	0.00	0.00	0.00	0.11	0.00	0.00	1.19	0.00		
14	0.00	0.26	0.19	0.04	0.11	0.00	0.00	0.15	0.07	0.07	0.00	0.00	0.00	0.00	1.30	0.00		
15	0.04	0.11	0.11	0.22	0.07	0.04	0.00	0.19	0.00	0.00	0.04	0.15	0.00	0.00	1.41	0.00		
16	0.00	0.41	0.33	0.44	0.00	0.00	0.00	0.07	0.00	0.11	0.00	0.04	0.00	0.04	1.33	0.00	0.00	
17	0.04	0.44	0.41	0.33	0.15	0.00	0.00	0.22	0.00	0.04	0.07	0.07	0.00	0.00	1.81	0.00	0.00	
18	0.11	1.04	0.59	0.48	0.15	0.00	0.00	1.33	0.00	0.00	0.04	0.00	0.00	0.00	1.41	0.00	0.00	
19	0.11	0.11	0.67	0.56	0.15	0.04	0.00	1.00	0.00	0.00	0.07	0.19	0.00	0.00	1.37	0.00	0.00	0.22
20	0.15	0.63	0.59	0.33	0.11	0.00	0.00	6.89	0.00	0.07	0.15	0.07	0.00	0.00	1.22	0.00	0.00	0.07
21	0.19	0.89	0.63	0.37	0.07	0.00	0.00	6.81	0.00	0.22	0.07	0.15	0.00	0.00	1.70	0.00	0.04	0.04

Appendix 2a. Mean weekly pest, natural enemy and disease incidence in the 1 g pheromone mating-disruption plot at Ongata Rongai.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.00	0.07	0.15	0.19	0.00	0.07	0.04	0.00	0.00	0.00	0.07	0.00	0.00	0.11	0.00		
2	0.00	0.33	0.15	0.04	0.15	0.00	0.07	0.04	0.00	0.04	0.00	0.26	0.00	0.04	0.22	0.00		
3	0.00	0.00	0.15	0.04	0.07	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.11	0.07		
4	0.07	0.07	0.33	0.30	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00		
5	0.00	0.04	0.26	0.19	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.70	0.04		
6	0.00	0.00	0.33	0.04	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.11		
7	0.00	0.00	0.26	0.07	0.52	0.00	0.00	0.04	0.00	0.00	0.07	0.22	0.00	0.00	0.78	0.22		
8	0.00	0.11	0.22	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.81	0.04		
9	0.00	0.04	0.07	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	1.04	0.04		
10	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.22	0.00		
11	0.00	0.04	0.07	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.15	0.00	0.00	0.93	0.22		
12	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.04		
13	0.04	0.41	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.07	0.00	0.00	1.59	0.07		
14	0.04	1.04	0.78	0.00	0.07	0.00	0.00	0.41	0.00	0.00	0.22	0.15	0.00	0.00	1.19	0.11		
15	0.04	0.37	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	1.00	0.07	0.00	0.07
16	0.04	0.56	0.19	0.07	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.11	0.00	0.00	1.04	0.30	0.04	0.37
17	0.00	0.22	0.15	0.00	0.00	0.00	0.00	1.19	0.00	0.00	0.04	0.00	0.00	0.00	1.07	0.00	0.00	0.00
18	0.00	0.26	0.67	0.19	0.00	0.00	0.00	0.52	0.00	0.04	0.00	0.15	0.00	0.00	1.00	0.11	0.04	0.19
19	0.00	0.44	0.44	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	1.41	0.11	0.00	0.00

Appendix 2b. Mean weekly pest, natural enemy and disease incidence in the 2 g pheromone mating-disruption plot at Ongata Rongai.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.00	0.04	0.11	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00		
2	0.00	0.07	0.07	0.07	0.22	0.00	0.12	0.07	0.04	0.00	0.00	0.07	0.00	0.00	0.11	0.00		
3	0.00	0.07	0.07	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.07	0.04		
4	0.00	0.22	0.15	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.19	0.00		
5	0.00	0.04	0.11	0.11	0.22	0.00	0.00	0.00	0.04	0.00	0.00	0.26	0.00	0.00	0.48	0.04		
6	0.00	0.00	0.19	0.04	0.15	0.00	0.00	0.00	0.00	0.00	0.15	0.04	0.00	0.00	0.48	0.04		
7	0.00	0.07	0.63	0.04	0.33	0.00	0.00	0.00	0.04	0.00	0.11	0.15	0.00	0.00	0.89	0.00		
8	0.00	0.11	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.04	0.07	0.00	0.00	0.63	0.00		
9	0.00	0.04	0.11	0.04	0.07	0.00	0.00	0.00	0.04	0.00	0.04	0.07	0.00	0.00	0.59	0.00		
10	0.04	0.11	0.11	0.00	0.11	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.00		
11	0.00	0.04	0.15	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.07	0.15	0.00	0.00	1.07	0.00		
12	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	1.04	0.00		
13	0.00	0.33	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	1.52	0.00		
14	0.01	0.56	0.27	0.05	0.04	0.00	0.02	0.30	0.01	0.00	0.01	0.11	0.00	0.00	0.48	0.03		
15	0.00	0.37	0.11	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.11	0.07	0.07
16	0.07	0.52	0.15	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.19	0.04	0.07
17	0.04	0.30	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.96	0.04	0.04	0.00
18	0.04	0.19	0.74	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.15	0.00	0.00	1.33	0.33	0.11	0.11
19	0.11	0.59	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.04	0.00	0.00	1.37	0.22	0.00	0.00

Appendix 2c. Mean weekly pest, natural enemy and disease incidence in the control plot at Ongata Rongai.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.04	0.22	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
2	0.00	0.00	0.11	0.00	0.04	0.00	0.00	0.00	0.07	0.00	0.00	0.19	0.00	0.00	0.22	0.00		
3	0.00	0.00	0.04	0.19	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.07	0.04		
4	0.04	0.07	0.11	0.11	0.00	0.07	0.04	0.00	0.11	0.00	0.00	0.11	0.00	0.00	0.15	0.00		
5	0.07	0.04	0.07	0.15	0.04	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.44	0.04		
6	0.00	0.11	0.04	0.04	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.44	0.07		
7	0.07	0.41	0.00	0.00	0.26	0.00	0.00	0.00	0.04	0.00	0.11	0.15	0.00	0.00	0.70	0.04		
8	0.04	0.15	0.67	0.15	0.11	0.04	0.00	0.00	0.00	0.00	0.07	0.04	0.00	0.00	0.00	0.04		
9	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	1.07	0.00		
10	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	1.30	0.00		
11	0.04	0.07	0.44	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	1.52	0.00		
12	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.96	0.04		
13	0.07	0.19	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.00	0.00	1.33	0.04		
14	0.01	0.11	0.19	0.04	0.09	0.00	0.00	0.01	0.01	0.00	0.03	0.08	0.00	0.00	0.91	0.04		
15	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.93	0.11	0.04	0.04
16	0.04	1.59	0.07	0.07	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.33	0.19	0.07
17	0.04	0.30	0.44	0.00	0.00	0.00	0.00	0.56	0.00	0.04	0.00	0.04	0.00	0.00	0.85	0.15	0.00	0.00
18	0.04	0.41	0.59	0.04	0.04	0.00	0.04	0.74	0.00	0.07	0.04	0.04	0.00	0.00	1.22	0.67	0.04	0.11
19	0.04	1.19	0.70	0.00	0.07	0.00	0.00	0.59	0.00	0.00	0.19	0.19	0.00	0.00	1.04	0.07	0.00	0.00

Appendix 3a. Mean weekly pest, natural enemy and disease incidence in the 1 g pheromone mating-disruption plot at Kabete.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.19	0.11	0.22	0.11	0.04	0.07	0.37	0.00	0.00	0.00	0.04	0.00	0.00	0.11	0.00	0.00	
2	0.00	0.11	0.04	0.15	0.04	0.00	0.11	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	
3	0.07	0.48	0.26	0.15	0.04	0.00	0.07	0.00	0.04	0.00	0.00	0.04	0.00	0.00	0.22	0.00	0.00	
4	0.04	0.26	0.15	0.04	0.15	0.00	0.00	0.04	0.00	0.00	0.04	0.07	0.00	0.00	0.11	0.00	0.00	
5	0.00	0.07	0.04	0.11	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00	
6	0.04	0.04	0.30	0.07	0.00	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.74	0.00	0.00	
7	0.00	0.07	0.30	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.63	0.74	0.00	
8	0.04	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.89	0.00	0.00	
9	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.85	0.48	0.00	
10	0.07	0.44	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.52	0.00	0.00	
11	0.04	0.22	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.67	0.00	0.00	
12	0.11	0.74	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.74	0.00	0.00	
13	0.11	0.93	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.56	1.22	0.00	
14	0.15	0.70	0.33	0.04	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.89	0.04	0.00	
15	0.00	0.96	0.00	0.00	0.00	0.00	0.00	0.22	0.04	0.00	0.07	0.00	0.00	0.00	1.33	0.07	0.00	
16	0.11	0.85	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	1.30	0.00	0.00	
17	0.30	2.15	0.04	0.00	0.04	0.00	0.00	0.41	0.00	0.00	0.04	0.00	0.00	0.00	1.30	0.44	0.26	
18	0.11	2.81	0.26	0.00	0.04	0.00	0.00	0.00	0.04	0.00	0.00	0.07	0.00	0.00	0.89	0.15	0.11	
19	0.11	2.00	0.19	0.04	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.11	0.41	
20	0.44	3.44	0.15	0.11	0.00	0.00	0.00	0.96	0.00	0.00	0.04	0.00	0.00	0.00	0.81	0.00	0.00	0.00
21	0.00	4.93	0.04	0.04	0.00	0.00	0.00	0.96	0.00	0.00	0.11	0.07	0.00	0.00	0.96	0.22	0.48	0.00

Appendix 3b. Mean weekly pest, natural enemy and disease incidence in the 2 g pheromone mating-disruption plot at Kabete.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.15	0.07	0.11	0.00	0.00	0.04	0.19	0.00	0.00	0.00	0.07	0.00	0.00	0.04	0.00	0.00	
2	0.00	0.48	0.07	0.30	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
3	0.00	0.85	0.11	0.26	0.11	0.00	0.00	0.07	0.00	0.00	0.11	0.04	0.00	0.00	0.19	0.00	0.00	
4	0.00	0.30	0.04	0.00	0.04	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.00	0.00	
5	0.00	0.15	0.26	0.00	0.00	0.04	0.00	0.15	0.04	0.00	0.00	0.15	0.00	0.00	0.37	0.15	0.00	
6	0.00	0.15	0.11	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.07	0.00	0.00	0.63	0.00	0.00	
7	0.00	0.26	0.37	0.04	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.37	0.00	
8	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.00	
9	0.00	0.30	0.07	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.19	0.00	
10	0.04	0.22	0.11	0.00	0.07	0.00	0.00	0.00	0.11	0.00	0.00	0.04	0.00	0.00	0.74	0.07	0.00	
11	0.11	0.11	0.11	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.52	0.00	0.00	
12	0.11	0.63	0.30	0.00	0.00	0.00	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.70	0.00	0.00	
13	0.00	0.89	0.26	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.11	0.00	0.00	0.85	0.11	0.00	
14	0.04	0.52	0.67	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.04	0.00	0.00	1.04	0.00	0.00	
15	0.00	0.78	0.11	0.04	0.00	0.00	0.00	0.56	0.00	0.04	0.15	0.04	0.00	0.00	1.30	0.11	0.00	
16	0.00	0.63	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.30	0.04	0.00	
17	0.04	1.00	0.19	0.11	0.00	0.04	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00	1.26	0.26	0.00	
18	0.26	1.44	0.30	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.15	0.00	
19	0.00	3.07	0.04	0.04	0.00	0.00	0.00	0.19	0.00	0.00	0.04	0.00	0.00	0.00	0.63	0.00	0.00	
20	0.15	4.74	0.19	0.15	0.00	0.00	0.00	1.70	0.00	0.00	0.00	0.00	0.00	0.04	0.93	0.04	0.00	0.00
21	0.00	6.74	0.04	0.15	0.00	0.00	0.00	1.74	0.00	0.00	0.04	0.07	0.00	0.00	0.85	0.04	0.11	0.00

Appendix 3c. Mean weekly pest, natural enemy and disease incidence in the control plot at Kabete.

Week	DBM (actual counts)		Aphid Scores (0-4)			Pests (actual counts)				Natural Enemies (actual counts)					Diseases scores (0-3)			
	Pupae	Larvae	*Brevicoryne*	*Myzus*	*Lipaphis*	*Plusia*	Leafminer	Thrips	Bollworm	Coccinelids	Syrphids	Spiders	Orius	Ants	Virus	Powdery mildew	Black rot	Downy mildew
1	0.00	0.11	0.33	0.00	0.07	0.04	0.11	0.22	0.00	0.00	0.00	0.07	0.00	0.00	0.04	0.00	0.00	
2	0.00	0.04	0.33	0.07	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	
3	0.04	0.22	0.15	0.11	0.04	0.00	0.15	0.00	0.00	0.00	0.07	0.04	0.00	0.00	0.26	0.00	0.00	
4	0.04	0.07	0.11	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.04	0.00	0.00	0.11	0.00	0.00	
5	0.00	0.15	0.26	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.07	0.00	0.00	0.22	0.00	0.00	
6	0.15	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00	0.00	
7	0.11	0.15	0.07	0.00	0.04	0.00	0.00	0.04	0.07	0.00	0.00	0.00	0.00	0.00	0.56	0.04	0.00	
8	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.85	0.04	0.00	
9	0.07	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	1.00	1.07	0.00	
10	0.00	0.11	0.00	0.00	0.07	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.44	0.15	0.00	
11	0.00	0.26	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.52	0.26	0.00	
12	0.22	0.70	0.07	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.22	0.04	0.00	
13	0.07	0.89	0.22	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.07	0.00	
14	0.07	0.30	0.30	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.04	0.00	0.00	0.00	0.70	0.00	0.00	
15	0.04	0.30	0.11	0.00	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00	1.19	0.19	0.00	
16	0.04	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.78	0.00	0.00	
17	0.07	0.56	0.11	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.04	
18	0.04	1.00	0.19	0.00	0.00	0.00	0.00	0.11	0.11	0.00	0.00	0.07	0.00	0.00	0.56	0.00	0.00	
19	0.07	1.15	0.07	0.04	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.04	0.00	0.00	0.59	0.07	0.30	
20	0.26	2.37	0.22	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.04	0.00
21	0.00	2.15	0.11	0.00	0.00	0.00	0.00	1.48	0.00	0.00	0.04	0.00	0.00	0.00	0.81	0.00	0.30	0.00

A SECOND APPRAISAL OF THE PHEROMONE MATING-DISRUPTION TECHNIQUE FOR DIAMOND-BACK MOTH, *PLUTELLA XYLOSTELLA*

A Technical Report for Project R7449:
Development of Biorational Brassica IPM in Kenya

DFID Crop Protection Programme

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Executive Summary

A trial of pheromone mating disruption of diamondback moth (DBM), *Plutella xylostella*, L. was carried out at the Jomo Kenyatta University of Agriculture and Technology farm near Nairobi, Kenya from October 2001 to January 2002. Following an earlier trial in 2000, this trial incorporated a number of changes to design and methodology that were intended to improve treatment effectiveness as well as evaluation of results. The synthetic pheromone formulation used was a PVC-based one known as 'Selibate'™ and individual dispensers were set out at a density of approximately 1425 ha^{-1} in treated plots. The trial consisted of six 0.1 ha plots of kale, three treatment and three untreated controls, in a randomised-block design. In the treated plots, mating-disruption dispensers were distributed over an area of a 0.2 ha centred on the kale plot, *i.e.* there was an unplanted, but treated 'buffer zone' of 6.6 m around each treated plot. The rate of application of the active ingredient was 110 g ha^{-1}. No pesticide applications were made in any of the plots. As determined by weekly sampling, populations of DBM larvae were generally higher than in the previous trial. There were slightly fewer larvae in treatment plots and leaf damage scores were consistently slightly lower; total marketable yield from 6 harvest dates was 50% higher. However these differences were not statistically significant. Four separate sets of observations to determine rates of mating of female DBM indicated that the pheromone treatment did not suppress mating as envisaged. Pheromone trap monitoring showing incomplete suppression of catches in the pheromone-treated plots, even in the early stages of the trial, strengthened this conclusion. Quantitative analysis of pheromone dispensers exposed under field conditions indicated that the half-life of the pheromone within the dispensers was about eight weeks. Even if mating-disruption of DBM was effective early in the trial, it would have been weak or absent after this period. The fact that between-treatment differences in DBM larval numbers, damage scores and marketable yield continued until the end of the trial lends further weight to the conclusion that these apparent beneficial effects were due to non-treatment related effects. These results reinforce earlier conclusions that pheromone mating-disruption of DBM in the context of small-holder farmers in Kenya is not feasible, and future trials could only possibly succeed if it were practical to increase plot sizes, unit-area dose rates and physical isolation of plots.

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Introduction

Project R7449 aims to develop, evaluate and promote two new biologically based IPM control methods for the diamond-back moth (DBM), *Plutella xylostella*, L. on small-holder vegetable farms in Kenya. These are the use of pheromone-based mating-disruption and an endemic viral bio-pesticide. This report details results from the second of two trials of pheromone mating-disruption for management of DBM. Results of the first trial in 2000, together with a discussion of earlier trials in Kenya (Critchley *et al*., 1998, 1999a, 1999b) and elsewhere, were reported previously (Downham, 2001).

The first trial was carried out at three sites near Nairobi, Kenya from March to August 2000. The synthetic pheromone formulation used was a PVC-based one known as 'Selibate'™ and individual dispensers were set out at a density of approximately 625 ha^{-1}. At each site single, 0.1 ha plots of kale containing 60 and 120 g ha^{-1} of pheromone active ingredient were compared with a control plot. One or two aphicide sprays were made early in the season in all plots, but controls were otherwise untreated. As determined by weekly sampling, populations of pests, including DBM, were low throughout the trial and no clear between-treatment differences in the numbers of DBM larvae and pupae, or in yield, were observed. Pheromone trap monitoring indicated incomplete suppression of catches in the pheromone-treated plots, even in the early stages of the trial, when disruption of pheromone-mediated behaviour should have been greatest.

From the results of the first trial it was concluded that the pheromone treatments had not disrupted mating of DBM. Earlier, apparently successful, results in Kenya may have been due to insecticide 'resurgence' effects in control plots providing a favourable, but misleading, comparison with the pheromone plots. Through a comparison of trial results from Japan and the US, which involved much higher application rates and/or greater plot sizes, it was further concluded that the only chance of successful mating disruption in the Kenyan, small-holder farmer context lay in increasing pheromone dispenser density and effective plot size. Based on this and other conclusions, several recommendations were made. These, and some other changes, were incorporated in the second trial although much of the basic methodology remained the same.

Three important changes in the second trial were:

- a doubling of pheromone dispenser density (while using the higher of the two application rates from the first trial);
- deployment of pheromone dispensers, not only within the planted area, but in 'buffer-zone' around it, effectively doubling the area covered;
- using a lower dispenser height (~ 50 cm, compared to 1 m).

The aim of these changes was to create a denser and more homogenous cloud of pheromone within the crop and thus increase its effectiveness.

Another significant alteration was that the trial should consist of replicated control and treatment plots, *at a single site*, thus allowing analysis of variance to be employed in the statistical evaluation of results. Equally importantly, direct methods of assessing mating of female DBM within plots were used for the first time in Kenya. This was to determine directly whether the envisaged mode of action of the treatments – suppression of mating – was actually occurring. Finally, a more thorough quantitative analysis of pheromone in dispensers was carried out to determine the amounts of pheromone remaining in the dispensers through time – and hence their effective longevity.

Materials and Methods

Location and duration of trial

The trial was conducted in three fields at the Jomo Kenyatta University of Agriculture and Technology farm site (JKUAT), situated about 24 km north-east of Nairobi. It began on 8 October 2001 and ran until 28 January 2002.

Crops and agronomic practices

The target crop was kale (Collard *var*. Southern Georgia), *Brassica oleracea*. All plants were transplanted into the plots as seedlings with 3 – 6 true leaves at a spacing of 60 × 60 cm. Seedlings came from field nursery beds sown at JKUAT on 4 September 2001 for the trial. Transplanting took place on 8 October. Due to dry weather all plots were irrigated using overhead irrigation equipment, for at least two weeks following transplanting, and thereafter were rain-fed. Despite this, some seedlings died, necessitating gap-filling which was carried out on a piecemeal basis for about 2 weeks after initial transplanting.

Fertiliser applications and weeding were carried out as indicated in Table 1 below. No insecticide applications were made in any of the plots.

Table 1. Dates of fertiliser applications and weeding during the trial.

Operation	Dates	Details
Fertiliser applications	8 October (transplanting)	DAP[1], 5 g per plant hill
	31 October	CAN[2], 10 g per plant hill
	3 December	CAN, 10 g per plant hill
Weeding	30 October	
	14 November	
	27 November	
	14 December	
	29 December	
	12 January	

[1]Diammonium Phosphate. [2]Calcium Ammonium Nitrate.

Trial design and treatments

The trial consisted of three pheromone treated plots of kale and three untreated controls. A 'blocked' trial design was achieved by positioning one treated and one control plot in each of three separate, rectangular-shaped fields, varying in area from 0.7 to 1.6 ha. In order to avoid pheromone-mediated interactions between plots, plots within each field were positioned at least 50 m apart at opposite ends of the respective fields. Maize was subsequently planted in 20 – 30 m wide strips in the middle portion of each field to act as a further barrier to pest and pheromone movement between plots.

Individual plots planted to kale were 0.10 ha in area; dimensions of those in blocks 1 and 3 were 31.8 × 31.8 m; in block 2 they were 20 × 50 m. However, with the treated plots the areas covered by pheromone dispensers extended beyond the plot boundaries by 6.6 m in each dimension to cover an area of slightly more than 0.2 ha in each case.

The pheromone formulation used was the same as that for the previous trial, in 2000, *i.e.* a black, PVC 'shoelace' or Selibate™ formulation (Cork *et al*., 1989) produced by Agrisense-BCS, Pontypridd, UK in August 2001. This contained a blend of (*Z*)-11-hexadecenal, (*Z*)-11-hexadeceny acetate and (*Z*)-11-hexadecenol in the ratio 55:45:1, with a total active ingredient content of 7.6 mg g^{-1} (determined by analysis at NRI). The departure of the blend from the nominal 50:50:1 ratio was not considered to be biologically significant.

The pheromone formulation was applied on 11 October. Individual lengths weighing 1 g were fixed into the split top end of bamboo canes, at a height of 0.5 m, and these were positioned 2.78 m apart in a grid pattern. This produced a density of approximately 1425 sources ha^{-1} and a pheromone application rate of 110 g a.i. ha^{-1}. Due to differing plot dimensions the actual numbers of dispensers per treatment plot were 289 (blocks 1 and 3) and 299 (block 2). Dispensers that were damaged or lost during the course of the trial were replaced with fresh dispensers within a few days.

Monitoring of pheromone trap catches

Daily catches of adult male DBM in three white, sticky-delta pheromone traps placed in each plot were recorded. The traps were baited with polyethylene vial lures (23 mm × 9 mm diameter) containing 0.1 mg (Z)-11-16:Ald, (Z)-11-16:Ac and (Z)-11-16:OH in the respective ratio of 50:50:1, plus 0.2 mg BHT. Sticky card trap inserts were replaced weekly; pheromone lures were replaced every two weeks. Traps and lures were supplied by International Pheromone Systems, Wirral, U.K. Traps were set out on 11 October 2001.

Assessments of mating rates

Mating rates of adult female DBM were assessed by four different methods. Firstly, the method of Ohno *et al*. (1992) was adapted as follows. One to 2 day-old virgin females from the KARI culture were tethered by one wing using white cotton thread, then placed in plots between 5 and 7 pm. The loose end of the thread was secured between the plastic pot used for transportation (10 cm diameter × 4 cm deep) and its lid; females were positioned on kale seedlings 4 – 5 m apart in a central region of plots. They were retrieved between 7.00 and 8.30 am the following morning. This procedure was carried out three times, when between 24 and 39 females were placed on each occasion (4 – 7 females per plot).

The following methodology was adapted from Schroeder *et al*. (2000). The wings of 1 – 2 day-old virgin females from the KARI culture were clipped. The females were left overnight in plots in plastic containers (21 × 11 cm, by 5 cm deep) whose inner walls are painted with fluon to prevent escape. The containers were open to the air and placed on the ground. This was carried out twice, with 60 females set out (10 per plot) on each occasion. As with the tethered females, they were placed in plots in the early evening and collected the following morning.

Wild females were also collected from plots on three occasions (10 – 12 per plot collected between 6 and 7pm). Once collected from plots the tethered, clipped-wing and wild females were returned to KARI laboratories and maintained individually in plastic containers with access to honey solution and pieces of fresh kale leaves, which were replaced daily. The

leaves were checked regularly for the presence of larvae for up to 2 weeks. Individuals producing viable eggs were considered to have mated.

A fourth, and less direct, assessment method was employed on four occasions. In addition to the standard pheromone traps with synthetic lures, a further three sticky, delta traps were positioned per plot, each containing virgin females from the KARI culture. Females were confined to containers made of 3 – 4 cm lengths of polythene hose suspended within the traps. The open ends of the tubes were covered with gauze, allowing some airflow through the tube. The tubes were positioned such that their long axes were parallel to that of the trap. On the first two occasions, 16 – 17 October and 11 – 12 November, two females were trap were used and thereafter four females per trap. Females were 1 – 2 days old, except on the first occasion when they were 3 – 4 days old. Captures of males in the traps were recorded in the usual way.

Pest sampling and damage assessment

DBM and other invertebrates and diseases were sampled on a weekly basis from 28 plants in each plot beginning on 22 October (11 days after transplanting) and ending on 28 January. Seven groups of four plants each were sampled in each plot, as follows. A single plant was randomly selected from within each plot (excluding the outer two rows). Sampling was carried out on this plant and three adjacent ones to the front and right of the randomly selected plant, as viewed from a consistent direction. If a plant was missing, another adjacent plant was sampled instead. All invertebrates on sampled plants were counted individually, except for aphids. Aphid species, plant diseases and leaf damage were scored by visual inspection as indicated in the Table 2.

Table 2. Scoring system used for sampling of aphids, plant diseases and leaf damage.

Score	Aphid species (top-most, fully expanded leaf only)	Diseases (whole plant)	Leaf Damage (whole plant)
0	no aphids present	no symptoms	no symptoms
1	~5% of leaf area covered	≤10% of plant affected	≤10% of leaf area affected
2	~10% " "	11 - 50% " "	11 - 50% " " "
3	~25% " "	≥50% " "	≥50% " " "
4	≥50% " "	-	-

Harvesting

Harvesting commenced on 12 November (4 weeks after transplanting) in all plots then continued at 2-week intervals until 21 January, making 6 harvests in all. On each occasion the total number of marketable and damaged leaves, and their weight, was recorded separately, together with a count of the plant stand in each plot.

Quantitative analysis of the Selibate ™ dispensers

Quantitative analysis of the mating-disruption formulation was carried out in order to determine the amount of pheromone remaining in dispensers through the course of the season. On 11 October (day 0), 60 dispensers were placed on a group of canes (two dispensers per cane) on the JKUAT farm more than 50 m from the nearest experimental plots. On day 0, day 11 then subsequently every seven days until 21 January, three randomly selected dispensers were removed from the sticks. These were wrapped in aluminium foil and placed in a fridge, until sent back to NRI in late January for laboratory analysis. The amount of each pheromone component contained in three sub-sample lengths of the formulation from each date was determined and compared to day 0 samples.

Data analysis

Most data sets considered hereafter were subjected to one-way analysis of variance (with blocking) (ANOVA) using Genstat 5 (Release 4.1) for Windows™. Where significant treatment effects were indicated this was confirmed by calculation of the appropriate least significant difference (5% level).

For trap-catch data (synthetic lures) the mean catch per trap in each plot, for successive weekly periods, was first calculated. From these values, the mean weekly catch per trap, for the first eight weeks and throughout the trial, was calculated for each plot and these values subjected to ANOVA. The period of eight weeks related to the expected effective longevity of the mating-disruption dispensers and this point is considered more fully in the Discussion.

Similarly for pest sampling and damage data, the mean value (of 28 plants) for each plot was first determined for each sample date; the mean value for all sample dates, and for sample dates up to week 8, were then calculated for each plot and subjected to ANOVA. Yield data from each harvest date, for each plot, were adjusted for plant stand differences by multiplying by the factor (initial plant stand count/plant stand count at harvest). Adjusted yield data were then summed across the first three sample harvests and for all harvest dates, and total yield data values used in respective ANOVAs. Mating assessment data from wild, caught females and

from the third sample date using tethered females were arc-sine transformed (Mostella & Youtz, 1961) prior to ANOVA. Female-baited trap data were not analysed due to very low catches, while the numbers mated and unmated in remaining assessment tests were analysed using Fisher's Exact test.

Results

Pheromone trap catches

Figure 1 illustrates trends in trap captures of males in the treated and control plots. Catches were consistently lower in treated plots throughout the trial though never, except for the first week, entirely absent. The average of catches per trap in the first eight, and in all, weeks was significantly lower in the treated plots (Table 3). Total weekly catches per trap in control plots only rarely reached five, whereas at the same site in the previous trial in 2000 catches in the only control plot were in the range 5 – 15 for most of the trial period. Table 4 shows the relative magnitude of catches in treatment and control plots. From this it can be seen that catches in pheromone treated plots mostly ranged between 10 and 40% of those in the controls, which was in rough agreement with corresponding data for the 2000 trial.

Table 3. Overall mean catches per trap per week over first eight weeks and all weeks of trial.

	Mean catch trap^{-1} week^{-1}	
	First eight weeks	All weeks
Treatment plots	0.75 (± 0.40)	0.81 (± 0.30)
Control plots	3.58 (± 0.53)	3.33 (± 0.41)

Differences between the respective treatment and control means in each column were statistically significant ($P < 0.05$, LSD following ANOVA). Figures in parentheses are the standard errors of the respective means.

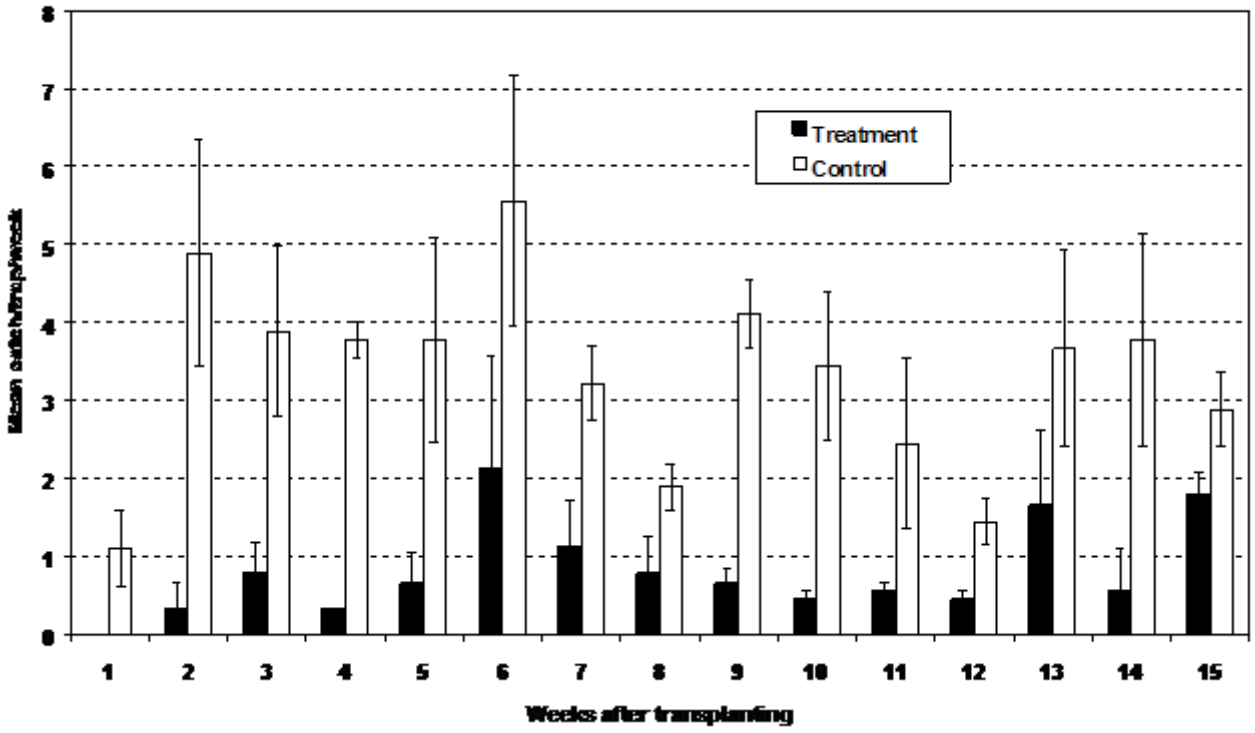

Figure 1. Mean weekly trap-catches in pheromone treated and control plots (error bars indicate standard errors of the respective means).

Table 4. Mean weekly trap-catches in the pheromone plots expressed as a percentage of those in the control plots.

Week	Ending	Treatment as % of Control
1	18-Oct-01	0.0
2	25-Oct-01	6.8
3	1-Nov-01	20.0
4	8-Nov-01	8.8
5	15-Nov-01	17.6
6	22-Nov-01	38.0
7	29-Nov-01	34.5
8	6-Dec-01	41.2
9	13-Dec-01	16.2
10	20-Dec-01	12.9
11	27-Dec-01	22.7
12	3-Jan-02	30.8
13	10-Jan-02	45.5
14	17-Jan-02	14.7
15	24-Jan-02	61.5

Mating Assessments

Tables 5 and 6 summarise the results of the various mating assessments. The strongest mating disruption effects could have been expected during the first 8 weeks of the trial, up to mid-December. Thus it was unfortunate that, for a variety of reasons, data from this period were limited.

Only five males were trapped in treatment plots over the four nights that female baited traps were set out, compared to 14 in control plots. However, as seven of the latter were from a single trap that included a mating pair, the female of which had perhaps been responsible for attracting most of the males, it cannot be said that treatment catches were reduced compared to controls. Low catches (see Table 5) were quite consistent with those from the standard, synthetically baited traps but may have been exacerbated by poor survival of the bait females, at least on the first two occasions. Alternatively, it is possible that the females' pheromone plume did not diffuse out of the holding tube effectively.

Table 5. Summary of results for pheromone traps baited with virgin DBM females.

	Catches in 9 traps (3 per plot)		Female survival	
	Control plots	Treatment plots	Treatment plots	Control plots
16-17 October	0	0	7 of 18	8 of 18
11-12 November	1	1	10 of 18	9 of 18
23-24 November	12*	1	26 of 36	31 of 36
16-17 January	1	3	No data (n = 36)	No data (n = 36)

* Figure includes 7 from a single trap, 2 of whom were a mating pair.

Except for the third tethering night, 23 – 24 January, survival of virgin females set out in plots, either with tethered or clipped wings, was poor (Table 6). On one occasion this could be attributed to heavy overnight rain, but otherwise it must be assumed the majority of females escaped or were taken by predators. On 23 - 24 January mating of tethered females was significantly reduced – from 83% to 56% – and on the earlier tethering nights no females had been mated in treated plots, compared to a few in controls. However, on 26 - 27 November both of the surviving females, from treated plots, had mated. Thus, although the data is weak up to the end of December it appears some mating of females within treated plots did occur.

Mating rates among wild females, which could have mated outside the plots, varied from 83 - 100% in treated plots and from 67 - 100% in control plots. No significant differences were observed in this respect on any of the sample days.

Table 6. Summary of results for mating assessments involving tethered, clipped-wing and wild, collected DBM females.

Date	Number of females		Number mated among surviving females		Statistical significance
	Set out	Survived overnight	Treatments	Controls	
Tethered					
16-17 October	24	11	0 of 5	2 of 6	NS[1]
16-17 January	24	6	0 of 2	1 of 4	NS[1]
23-24 January	39	36	10 of 18	15 of 18	$P < 0.05$[2]
Clipped-wings					
26-27 November	60	2*	2 of 2	-	No test
15-16 January	60	16	3 of 10	2 of 6	NS[1]
Wild females					
26 November	-	-	26 of 31	28 of 33	NS[2]
7 January	-	-	25 of 29	21 of 31	NS[2]
23 January	-	-	29 of 29	28 of 30	NS[2]

*Heavy overnight rain drowned the majority of females. [1]$P > 0.27$, Fisher's Exact test, 1-tail. [2]LSD, following ANOVA using arc-sin transformed data.

Weekly DBM and leaf damage counts

Numbers of DBM larvae recorded in treatment plots were slightly, but quite consistently, lower than in control plots (Fig. 2). However, this difference was not significant either after eight weeks or throughout the trial (Table 7). The mean numbers per plant varied through the trial, reaching a maximum of about 5 larvae per plant 13 weeks after transplanting. The average figure throughout the trial was 1.5 – 2.0 (Table 7). Thus larval populations were substantially higher than at the same site in the previous trial, when they rarely reached one larva per plant, although they cannot be considered very high in absolute terms. The corresponding figures for DBM pupae were much lower, averaging about 0.1 pupae per plant throughout the trial (Table 7). They showed little variation through time, and did not differ between treatment and control plots.

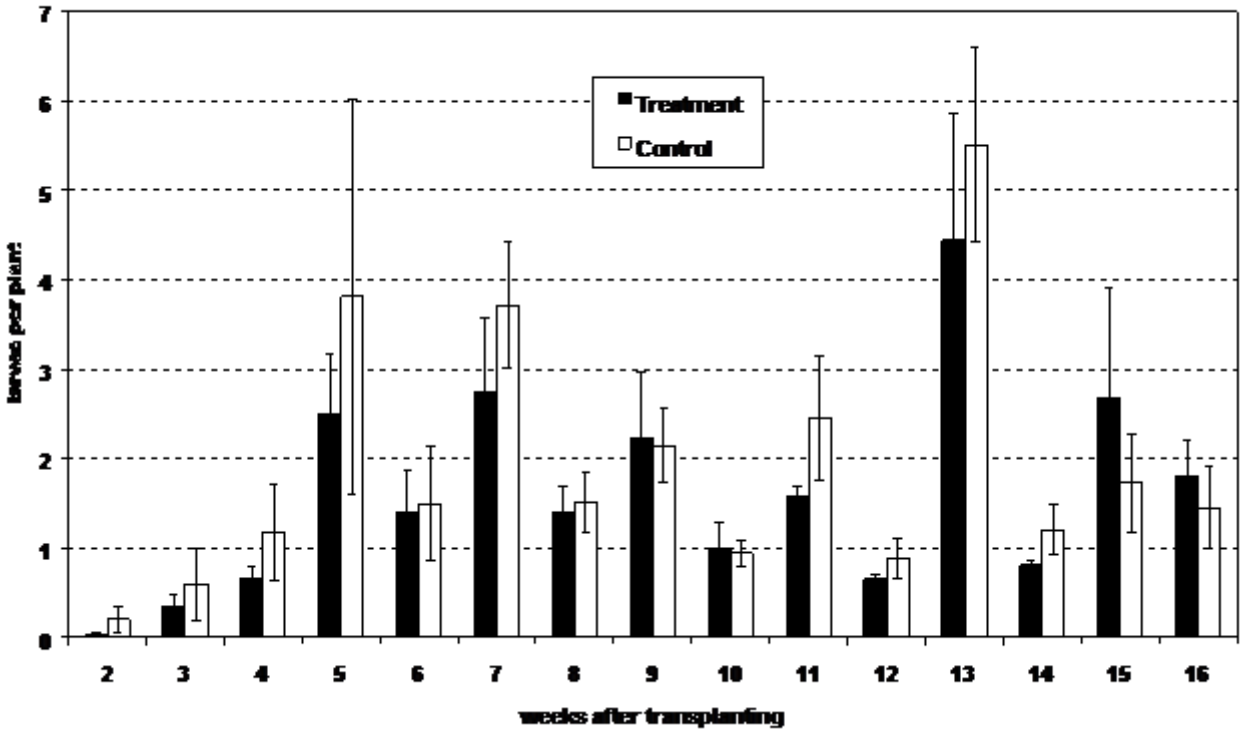

Figure 2. Mean number of DBM larvae per plant in pheromone treated and control plots (error bars indicate standard errors of the respective means).

Table 7. Mean numbers of DBM larvae and pupae per plant sampled over first eight and all weeks of the trial.

	Mean larvae plant^{-1}		Mean pupae plant^{-1}	
	First 8 weeks	All weeks	First 8 weeks	All weeks
Treatment plots	1.41 (± 0.32)	1.62 (± 0.10)	0.11 (± 0.05)	0.12 (± 0.02)
Control plots	1.83 (± 0.51)	1.92 (± 0.19)	0.10 (± 0.02)	0.10 (± 0.02)

Differences between the respective treatment and control means in each column were not statistically significant ($P > 0.25$, F-ratio of ANOVA). Figures in parentheses are the standard errors of the respective means.

Leaf damage scores in the first 2 – 3 weeks of the trial were very low, but rose steadily to a maximum of around 1.0 (on a scale of 0 – 3) at around 8 – 9 weeks after transplanting (Fig. 3). A score of 1.0 indicated a maximum of 10% of leaf area missing or damaged and thus equated to relatively little damage. Damage scores tended to be slightly lower in treatment plots compared to controls on most sample dates. The difference averaged over the first eight weeks of the trial was not statistically significant; however, that averaged over all weeks was significant (Table 8).

Table 8. Mean leaf damage scores over first eight and all weeks of trial.

	Mean damage score	
	First eight weeks	All weeks
Treatment plots	0.54 (± 0.07)	0.72 (± 0.03)
Control plots	0.67 (± 0.13)	0.77 (± 0.03)

The difference between the treatment and control means for the first 8 weeks was not statistically significant (P = 0.18, F-ratio of ANOVA), while that for all weeks was significant (P < 0.05, LSD following ANOVA). Figures in parentheses are the standard errors of the respective means.

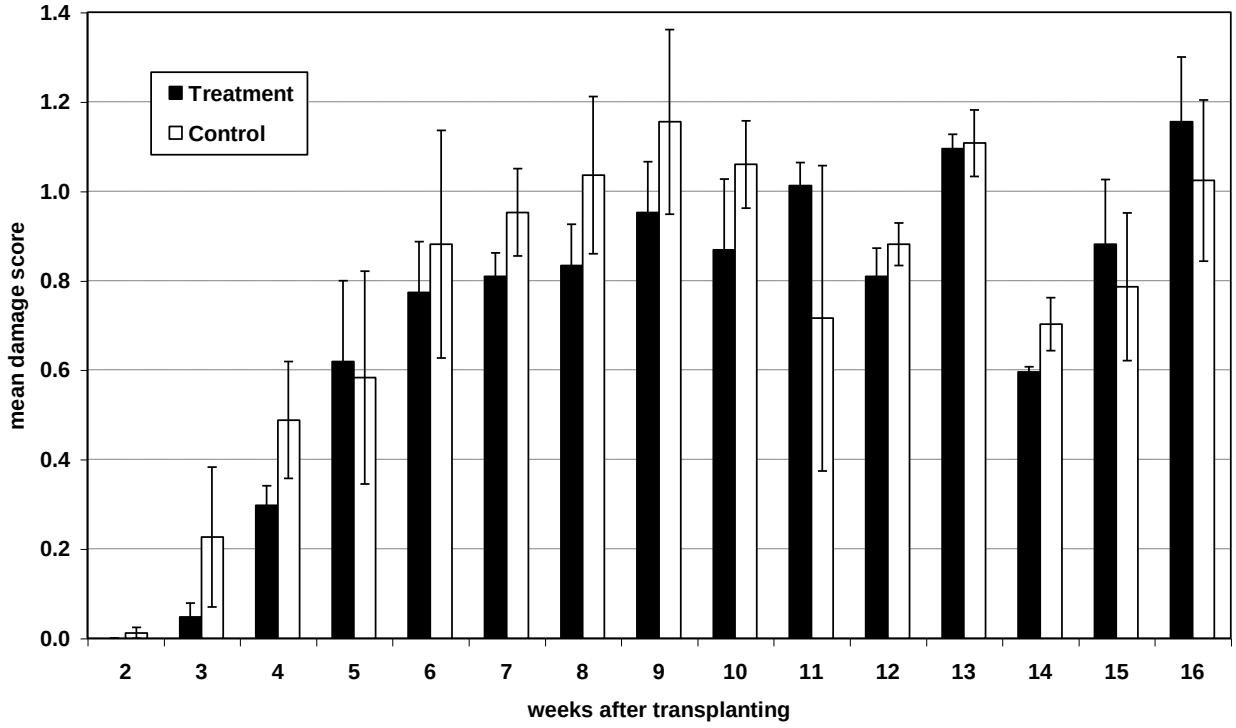

Figure 3. Mean leaf damage score (scale 0 – 3) per plant in pheromone treated and control plots (error bars indicate standard errors of the respective means).

Non-DBM counts

Comprehensive tables of invertebrate and disease incidence are given in Appendices 1a - b, 2a - b and 3a - b.

Aphids and thrips were the only other pests of note, besides DBM, throughout the trial. Among the aphids, *Brevicoryne brassicae* was the dominant species but *Myzus persicae* and *Lipaphis erysimi* were also present. Mean *B. brassicae* scores remained below 0.1 per plant until 8 weeks after transplanting (scale, 0 – 4) then increased sharply to reached 0.5 per plant by the end of the trial. Aphid scores did not differ between treatment and control plots

(Table 9). Counts of thrips averaged well below one individual per plant, and did not differ between treatment and control plots. They were quite variable; occasionally much higher counts were noted in one or two plots that were not sustained on subsequent sample dates (Table 9).

Table 9. Mean scores per plant of three aphid species, of virus diseases and mean number of thrips sampled over all weeks of the trial.

	Mean pest score per plant				Mean thrips per plant
	B. brassicae	*M. persicae*	*L. erysimi*	Virus diseases	
Treatments	0.22 (± 0.06)	0.13 (± 0.02)	0.12 (± 0.04)	1.19 (± 0.11)	0.71 (± 0.59)
Controls	0.20 (± 0.04)	0.14 (± 0.01)	0.12 (± 0.03)	1.24 (± 0.05)	1.25 (± 1.07)

Differences between the respective treatment and control means in each column were not statistically significant ($P > 0.39$, F-ratio of ANOVA). Figures in parentheses are the standard errors of the respective means.

Although aphid populations were low, through the spread of virus diseases, they may have had an indirect effect on yield. Plant virus scores rose steadily through the trial, reaching a mean of about 2.0 per plant by the end. However scores did not differ between pheromone treated and control plots (Table 9).

Harvests

Heavy rain during November resulted in flooding in block 1 of the trial, particularly in the control plot. Thus many plants were lost there and the final plant stand count, as a percentage of the initial value, was only 52%. The corresponding figures for the other plots varied between 66% – 75%. Yield data for each sample date were corrected such that they represented values that would have been obtained with a 100% plant stand. Corrected data for each sample date were then summed over the first three harvests (to 10 December) and over all harvests.

The most notable aspect of the results was a consistently higher mean marketable weight in treatment plots (Fig. 4), which translated to a total figure of 800 Kg plot^{-1} (8.0 t ha^{-1}), compared to 529 Kg plot^{-1} (5.3 t ha^{-1}) in controls (Table 10). Most of this difference was produced by higher yields in blocks 1 and 2 – in block 3 treatment and control yields were similar. Neither this difference, nor the corresponding one for the first three harvests alone, was statistically significant ($P > 0.12$, F-ratio of the ANOVA). Mean damaged yield weight

was similar in treatment and control plots; thus total yield was greater in treatment plots but this effect was also not significant (Table 10).

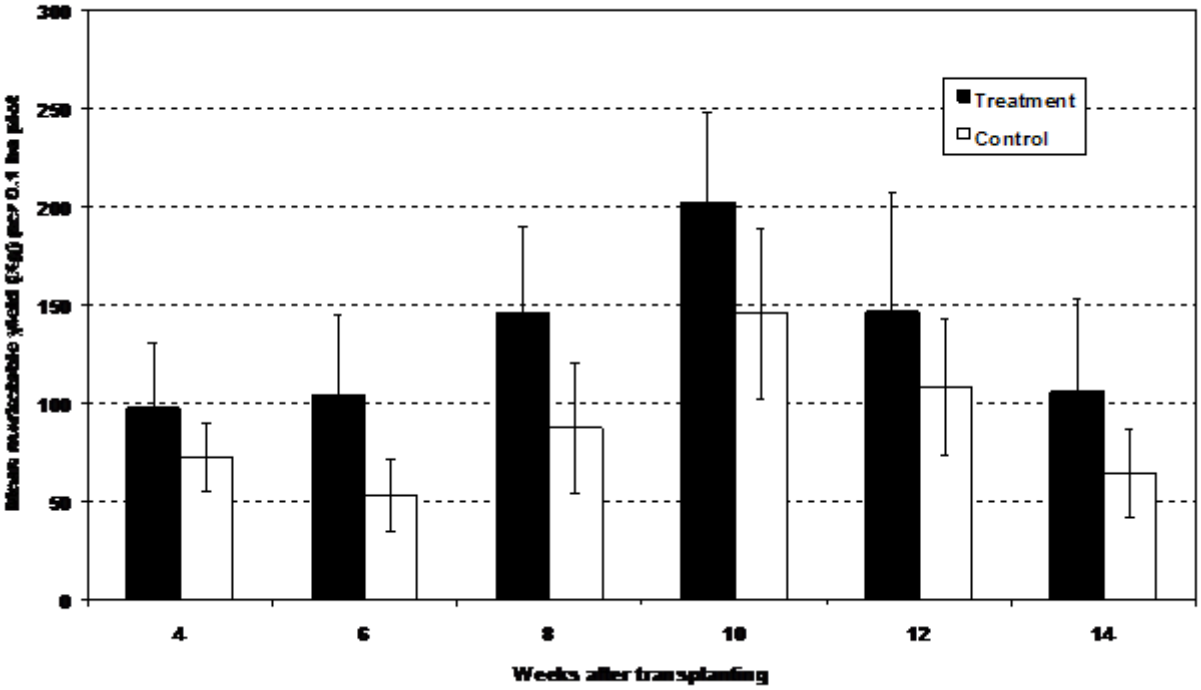

Figure 4. Mean weight of marketable yield per 0.1 ha plot (in Kg) in pheromone treated and control plots on successive harvest dates (error bars indicate standard errors of the respective means).

Table 10. Mean weight of damaged, marketable and total yields per 0.1 ha plot in first three and all harvests combined (all data following correction to 100% plant stand).

	Yield harvested (Kg plot^{-1})		
	Damaged	Marketable	Total
First 3 Harvests			
Treatments	180.9 (± 68.7)	346.1 (± 117.3)	527.0 (± 162.6)
Controls	177.5 (± 55.3)	211.7 (± 66.3)	389.2 (± 96.8)
P for differences*	0.82	0.13	0.17
All Harvests			
Treatments	622.4 (± 131.2)	799.7 (± 258.0)	1422.1 (± 364.7)
Controls	612.3 (± 99.5)	529.1 (± 148.5)	1141.4 (± 242.9)
P for differences*	0.81	0.14	0.16

*P is for the F-ratio of the relevant ANOVA. Figures in parentheses are the standard errors of the respective means.

Paralleling the marketable weight, the mean number of marketable leaves was greater in treatment plots. Although this difference was not significant in respect of the first three

harvests or all harvests, there were significantly more damaged leaves in control plots over both periods (Table 11).

Table 11. Mean number of damaged and marketable leaves and their total per 0.1 ha plot in first three and all harvests combined (all data following correction to 100% plant stand).

	Number of leaves harvested		
	Damaged	Marketable	Total
First 3 Harvests			
Treatments	15546 (± 3585)	19806 (± 4713)	35352 (± 5384)
Controls	18905 (± 3876)	15048 (± 2841)	33953 (± 3966)
P for differences*	0.05	0.14	0.54
All Harvests			
Treatments	39898 (± 4898)	40064 (± 8908)	79961 (± 9554)
Controls	46540 (± 3963)	32528 (± 6124)	79068 (± 8230)
P for differences*	< 0.01	0.13	0.64

*P is for the F-ratio of the relevant ANOVA. Figures in parentheses are the standard errors of the respective means.

Quantitative analysis of Selibate™ dispensers

Analysis of the unexposed (day 0) Selibate™ dispensers showed that the original concentration of pheromone active ingredient within the formulation was 8.6% – slightly higher than the intended figure of 8% (Table 12). Initially the proportion of the Z11-16:Ac pheromone component was about 44% of the total, and that of Z11-16:Ald about 56% – in contrast to the intended 50:50 ratio. However, as expected Z11-16:Ac was more persistent so that the actual ratio of these two major components was close to 50:50 for the first 50 – 60 days of the trial.

Figure 5 shows the total pheromone remaining at each sample date, as a percentage of the amount at day 0. From this it can be seen that on the last sample date (21 January), 102 days after setting out the dispensers, the amount of total pheromone remaining was approximately 30% of the day 0 value. The data show an exponential decline in pheromone remaining, that would have been reflected in the amount released per unit time. The data are well fitted[3] (F-ratio = 492.0, ANOVA of the regression model, model d.f.=2, residual d.f.=13, $P < 0.001$) by the equation:

[3] Note that the fit of the linear relation, $Y = 86.1 - 0.6X$, to the data while also significant, is poorer (*c.f.* F-ratio = 96.6, ANOVA of the regression model) than the exponential.

$Y = \exp(4.52 - 0.011X)$

From the equation, the half-life of the pheromone formulation can be calculated by letting Y = 50%, *i.e.* $t_{½}$ = 55.3 days.

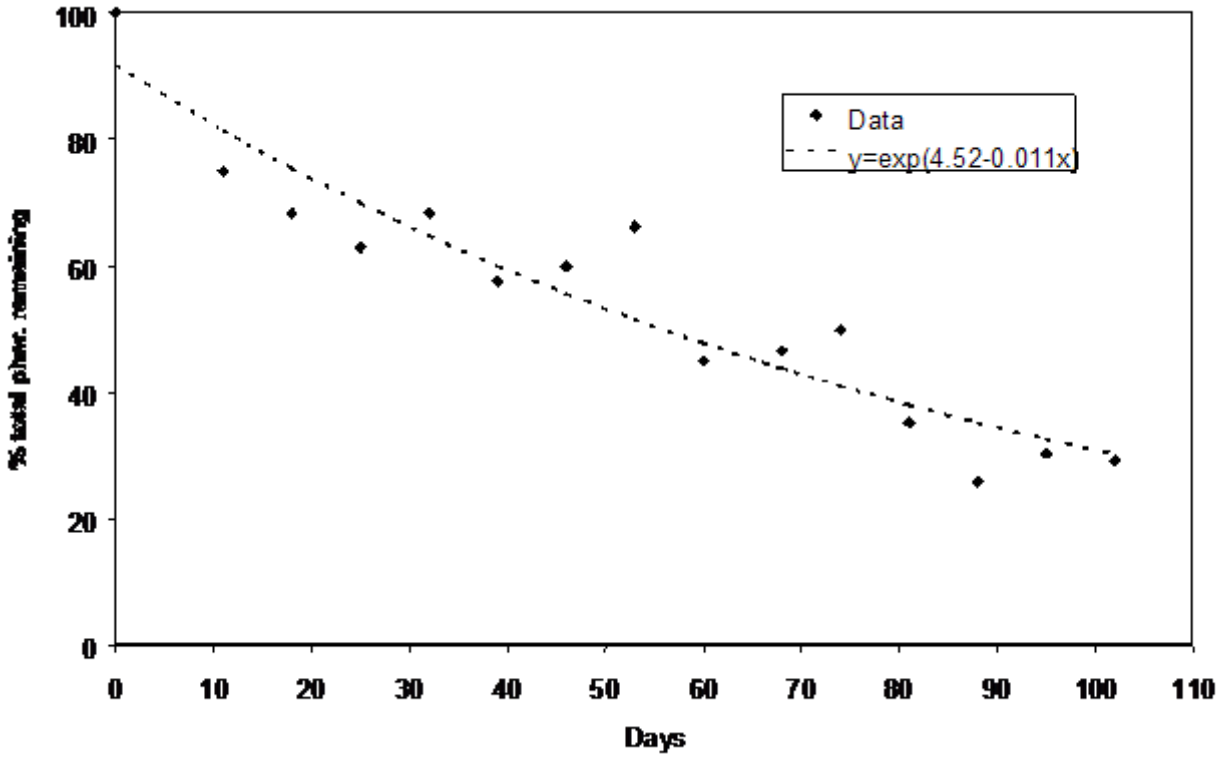

Figure 5. Amount of pheromone active ingredient remaining in Selibate™ dispensers at each sample date, together with the fitted curve.

Table 12. Summary data from quantitative analysis of Selibate™ dispensers of different sample dates (data are means of three sub-samples).

Day	Amount of pheromone components* within Selibate™ formulation (mg/100 mg)			% Z11-16:Ac of total pheromone
	Z11-16:Ald	Z11-16:Ac	Total	
0	4.79	3.83	8.62	44.4
11	3.14	3.32	6.46	51.4
18	2.60	3.29	5.89	55.9
25	2.41	3.01	5.42	55.6
32	2.63	3.26	5.89	55.4
39	2.07	2.89	4.96	58.3
46	2.17	2.99	5.16	58.0
53	2.53	3.18	5.71	55.6
60	1.39	2.49	3.88	64.2
68	1.44	2.57	4.01	64.1
74	1.68	2.62	4.30	60.9
81	0.81	2.22	3.03	73.2
88	0.67	1.56	2.22	70.1
95	0.70	1.91	2.61	73.3
102	0.65	1.86	2.52	74.1

* Amounts of the minor component, Z11-16:OH, were too small for reliable estimation of the amounts remaining, therefore these data are omitted from the table.

Discussion

To put the various measures of effectiveness of the mating-disruption treatment into context, it is useful, first, to consider the results of the quantitative analysis of the mating-disruption dispensers. The data of Fig. 5 and Table 12 show that total pheromone remaining in the dispensers was half of its initial value after about eight weeks. By comparison Critchley *et al.* (1998) found that 61 – 70% of pheromone remained in Selibate™ dispensers 64 days after transplanting. The greater overall longevity in that case may have been related to the experiment having been undertaken at a different time of year (*i.e.* during the long rains).

Since the present data show an exponential decline the amount of pheromone released into the crop per unit time would have fallen in a parallel manner. Moreover, after about eight weeks the balance of the two major blend components was increasingly skewed away from the ideal 50:50 ratio. In reality the practical effectiveness of the pheromone treatments would have declined in a gradual manner, and the fixing of any particular time-frame of effectiveness is somewhat arbitrary. However, given the results for amount of pheromone remaining and blend balance, eight weeks seems the maximum period for which good control of DBM might, theoretically, be possible. It would have been weak or absent thereafter.

From the results there were several small indications of treatment effectiveness, specifically:

- slightly lower DBM larval numbers in treatment plots;
- slightly leaf damage scores in treatment plots;
- higher marketable weights in treatment plots;
- lower numbers of damaged leaves in treatment plots.

As noted in the Results section, most of these effects were not statistically significant, but their consistent occurrence through the trial is nevertheless suggestive of a slight controlling effect of the pheromone treatment. To weigh this possibility, against the alternative one that lower DBM populations/higher yields (if real) were unrelated to treatment effects, it is necessary to consider other aspects of the results.

Clearly, the best determinant must be the mating assessment data. Although these were limited for a variety of practical reasons, particularly for the first eight weeks of the trial, it is possible to draw some conclusions. Combining the tethered- and clipped-wing female data for October – November, when mating suppression should have been strongest, two of seven females were mated from treatment plots, compared to two of six from controls. These were females that must have mated within the plots; the numbers are not indicative of suppression of mating in the treatment plots. A significant reduction of mating was seen using tethered-females in late-January. In the author's view this was probably a chance effect, since by then the mating-disruption dispensers would not have been very effective. In any case more than half of the females in the treated plot were still mated, so the practical usefulness of the result is doubtful.

With the female-baited traps, for the period to the end of November, two males were trapped in treated plots and 13 in controls. However, seven of the latter can be discounted for reasons noted in the Results section. This result suggests that at least some males were able to locate females in the treatment plots.

Considering the data for wild females, which were more numerous, it was very clear that there was no suppression of mating in treatment plots on any of the three sample dates – including the 26 – 27 November. While it can be argued that many of the females could have mated outside the plot, then flown in, such an effect is one that would have to be contended with by any control method. For mating-disruption treatments the only ways it could be minimised is through the use of large plot sizes and extreme isolation of the plot from other sources of DBM.

Overall, the mating data indicate that suppression of mating – the envisaged mode of action – was not occurring in the treatment plots. The catch data for the standard pheromone traps strengthen this conclusion. If the success of males at locating the traps in the treated plots was 10 – 40% of that in control plots, as the results show, then at least that proportion of females (probably more) would have been found and mated by males.

The other way of determining whether the pheromone treatments were having a real controlling effect is to look at the period over which relevant trends persisted. As argued above, control through mating suppression would have been strongest during the first eight

weeks of the trial and largely absent thereafter. Inspection of Figs. 2 – 4 shows that the apparently beneficial between-treatment differences in DBM larval populations, leaf damage and marketable yield did very largely continue through the latter half of the trial. Since the pheromone treatments could not have been effective at that time, this is good evidence that the earlier trends were also unrelated to the pheromone treatment.

Conclusions

The results presented and discussed above reinforce the conclusions of the trials carried out in 2000 that:

1. Pheromone mating-disruption of DBM in the context of small-holder farmers in Kenya is not feasible.

2. Future trials could only succeed if it were practical to increase plot sizes, unit-area dose rates and, ideally, physical isolation of plots.

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Executive Summary

Vegetable production in the peri-urban area surrounding Nairobi has expanded over the years in response to the increasing demand for food from a rapidly growing urban population. However, the resulting opportunities and constraints to expanded vegetable production pose new challenges to crop management. The Department for International Development (DFID), Crop Protection Programme managed by NR International has invested in research aimed at developing novel pest management technologies for use in the peri-urban vegetable system. Focusing mainly on the rational use of chemicals and alternatives to chemicals as a way of controlling vegetable pests and diseases, this research has been guided by a perceived existing demand. A key finding in an earlier demand assessment for alternative pest management technologies revealed that whilst pest and diseases are the most important production constraint, there was widespread misuse of chemical pesticides by farmers. The survey presented here attempts to characterize the peri-urban vegetable production system in order to identify the specific farmer characteristics, and investigates more closely the perceived pest problems that lead to the current production practices. The survey was carried out on 200 farms in peri-urban Nairobi between July and August 2000.

Characteristics of peri-urban vegetable farmers
The survey revealed that peri-urban vegetable farms average 1.3 hectares in size. Vegetable crops cover an average of 0.4 hectares per farm, with tomatoes and brassicas dominating. The majority of peri-urban farmers irrigate their vegetables, improve soil fertility using inorganic fertilizers and use chemical pesticides to control pests. The peri-urban farm operators also have a high literacy level, with 78% having completed at least primary school level of education. Cal J, Collard and Gloria F1 are the preferred varieties of tomato, kale and cabbage, respectively.

The returns to vegetable farming (gross margins) range from £2699 to £264 per hectare per cropping season depending on the crop. Across the districts, tomato farming yields the highest gross margins ranging from £1000 to £6000 per hectare. Also, the high input scenario for all the crops yields a higher gross margin than the average and low input scenarios. Notably also, the cost of irrigation constitutes a large proportion of total variable costs. A 50% reduction in irrigation cost nearly doubles the gross margins.

The cost of crop protection varies from 20 – 65% of the variable costs. However, where the cost of crop protection is high the gross margins are also high. Regressing reported expenditures on crop protection on; value of marketed output per acre, education level, location of the farm, total farm size and experience with vegetable farming yields similar results for tomatoes, kale and cabbage. A positive and significant linear relationship exists between expenditure on crop protection and farms located in Kajiado and Machakos. A similar relationship exists between level of education and expenditure on crop protection while total farm size has a positive linear relationship with expenditure on crop protection in the case of cabbage and tomatoes.

Perception on pests and their control strategies

The survey confirmed the importance of pests and diseases as the main constraint to vegetable production. The African bollworm is perceived to be the most problematic pest for tomato crops whilst Aphids and Diamondback moths are the most problematic for kale and cabbage crops. Farmers perceive blight to be the most important disease of tomatoes. Viral infection and blackrot are the diseases that most concern farmers growing kale and cabbage. Karate (*Lambda – cyhalothrin*) is used to control African bollworm and aphids in tomatoes, DBM, aphids and loopers in kale and cabbage. Dimethoate is used to control African bollworm in tomatoes and DBM in kale and cabbage. Diazinon is used to control aphids in tomatoes and Furadan *(Carbofuran)* for nematodes in tomatoes. Dithane M45 *(Mancozeb)* is used to control blight in tomatoes and blackrot in kale and cabbage. Ridomil *(Metalaxyl)* is also used to control blight. 99% of the peri-urban farmers apply pesticides to control vegetable pest. Out of 200 farms surveyed, a maximum of 5 cited the use of botanicals to control pests although cultural practices were used alongside chemical pesticides.

From this study it is evident a key motivational factor for using chemicals to control pests is the corresponding high returns observed in high input farms. However, with the problems of pest resistance, the sustainability of this strategy is questionable. Opportunities for higher returns and more sustainable practices lie in efficacious alternatives to chemical control.

The large majority of farmers use chemical pesticides. It is therefore important that correct specifications in terms of application levels, protective gear and pre-harvest intervals are observed.

Farmers were able identify and rank virus as important pests of brassicas. They also spray Karate (*Lambda – cyhalothrin)* to control viral infection. Whether farmers are able to link viral infection with specific pests that are vectors of viruses is not clear.

Finally, the Peri-urban vegetable farmers are responsive to market demand. Some of the preferred varieties may be susceptible to the common pests and diseases. The challenge to research therefore is that of developing alternative crop protection technologies for varieties with suitable attributes.

Acknowledgments

This survey was funded through the Peri-Urban Cluster of Projects. Valuable inputs were obtained from Jackie Leslie of VEERU, Department of Agriculture, Reading, U.K. during the design of this survey. In addition, Anthea Cook, Nicola Spence, David Grzywacz, Jerry Cooper and George Oduor advised on the biophysical aspects of the study while Jane Poole assisted in data analysis. We also thank the enumerators; Chacha, Gatambia, Wachira, Opiyo and Kajuju, who with their patience and tenacity completed the survey of 200 farm households in 20 days.

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Introduction

1.1 Kenya's Vegetable Production System

Cultivation of vegetables in Kenya occurs across different agro-climatic conditions, ranging from semi-arid to high altitude (Table 1.1). Derived from an earlier classification scheme in the Farm Management Handbook of Kenya (Jaetzold & Schmidt, 1983), elevation, precipitation and ambient temperatures are the definitive characteristics of a given agro-climatic zone. The traditional vegetable growing areas occur predominantly in the mid to high altitude zones. This is particularly true for brassica production. Table 1.2 indicates the production and consumption trends for brassicas in the traditional production zones of Kenya. The table shows that whilst high and mid altitude zones are net producers of brassicas, all other regions of Kenya are net deficit areas.

Table 1.1 Agro-climatic conditions for vegetable production zones in Kenya

Zone	Elevation (m)	Rainfall (mm)	Temperatures (^{o}C)
High Altitude	2400 – 3000	1000-2000 (Annual)	Min 3^{o}C (June-July)
Mid to High Altitude	1800 – 2400	350-800 (Mar-June) 350-700 (Oct-Dec)	Min 7^{o}C (Mar-Apr) Min 8^{o}C (Oct-Nov)
Mid-Altitude	1150 – 1800	350-700 (Apr-June)	
Semi-Arid	600 – 1150	325-600 (Oct-Dec)	Max 30^{o}C (Annual average)
Coastal Lowlands	0 - 800	400-800 (Apr-June) 350-800 (Oct-Dec)	Max 30^{o}C (Annual average)

Adapted from Kamau & Mills (1998)

Table 1.2 Production and consumption trends for brassica in Kenya

Zone	Estimated land area (hectares)	Estimated Production (tons)	Estimated Consumption (tons)
High Altitude	5,215	75,800	32,410
Mid to High Altitude	16,403	251,500	122,989
Mid-Altitude	15,407	191,400	211,739
Semi-Arid	935	11,900	17,087
Coastal Lowlands	421	2,300	32,064
Rest of Kenya	0	0	116,703

Adapted from Kamau & Mills (1998)

Over the years population pressure in the high to mid-altitude areas, defined as surplus production zones for brassicas, has resulted in migration to more arid areas. These migrants continue to practice arable farming and by employing improved crop management practices, such as irrigation, that modify the production environment,

vegetable production has expanded to the semi-arid zones. Rural to urban migration has also resulted in a rapid growth of urban areas in Kenya, particularly Nairobi. The resulting high population density and an ever-increasing demand for food has caused a change in land-use systems in peri-urban areas of Nairobi. The area to the North and North-West of Nairobi was traditionally a coffee growing zone. This zone currently comprises upper market residential areas, smallholder vegetable farms and medium scale flower farms. Likewise, previously large-scale ranches to the east of Nairobi have now been subdivided into smallholder farms with plots close to Athi River producing vegetables both for the export and local markets through irrigation.

1.2 The peri-urban production system

Peri-urban area by definition would be a region in the environs of an urban centre. Although much debate still goes on about the definition of the peri-urban system, especially the geographic scope, the recent focus is on functional attributes. According to the United Nations Food and Agricultural Organisation "Urban and peri Urban Agriculture is perceived as agriculture practices within and around cities which compete for resources (land, water, energy, labour) that could also serve other purposes to satisfy the requirements of the urban population" (FAO 1999).

Likewise, according to the Department for International Development of the United Kingdom (DFID), Natural Resource Systems Programme, the *peri-urban interface* is created by urban development. "Rural activities pre-exist. As urban activities proliferate and grow, linkages relating to them are built from either the town or the countryside. These cause changes to existing production systems and create new ones that can affect the poor in urban and rural areas. Opportunities arise relating to easy access to markets and services, with ready supplies of labour. Problems arise from shortage of land and risks from pollution and continued urban growth"(http://www.nrinternational.co.uk/).

A study commissioned by DIFD, Crop Protection Programme (CPP), on factors affecting uptake and adoption of outputs of crop protection research in peri-urban vegetable systems in Kenya (Project R7512, ZA0357) identified the peri-urban vegetable production system as an area in the immediate environs of an urban boundary where the land use pattern, particularly vegetable production is influenced by the presence of a given urban centre. In addition to a possible comparative advantage in vegetable production arising from proximity to a market outlet, the peri-urban vegetable producers target the dry season for production of most commodities. During the dry season, the vegetable markets in urban centres are essentially a sellers' market, with demand outstripping supply. The study also identified as peri-urban vegetable producing areas surrounding Nairobi the administrative districts of; Kiambu, Machakos, Thika and Kajiado (Otieno Oruko *et al*, 2000). Agro-climatically, these areas fall in the High to Midland zones (Jaetzold & Schmidt, 1983 a & b).

Noteworthy about the above definitions are the competing opportunities for resources especially land and labour, the primary factors of production in smallholder agriculture in developing countries. In addition, easier access to input services and output markets arising from proximity to urban centres and high opportunity cost of land and labour provide a recipe for the development of a commercially oriented smallholder agricultural production system.

1.3 The CPP Peri-urban cluster of projects

The Department for International Development (DFID) Crop Protection Programme (CPP) *Purpose* of the Peri-urban Production System is to improve the volume, quality and seasonal availability of food and crop products through the reduction of physical and economic losses caused by pests. Previous studies (Oduor *et al*, 1998) identified pests and diseases as the major production constraint. In addition, where chemicals are used to control pests, there is widespread misuse causing environmental damage, promoting the development of pesticide resistance in the pests and causing health problems (Cooper 1999). In order to address this situation (CPP) supports a thematic cluster of research projects in the peri-urban vegetable systems in Kenya. The projects aim to develop improved chemical and non-chemical control methods. In the peri-urban vegetable systems in Kenya, these projects include the following.

- Integrated management of root-knot nematodes on vegetables in Kenya (R7472, 1999-2002)
- Pest management in horticultural crops; an integrated approach to vegetable pest management with the aim of reducing reliance on pesticides in Kenya (ZA0082/3, 1996 – 1999; 1999-02)
- Development of biorational brassica IPM in Kenya (A0869X1, 1999-2002)
- Management of viruses and important vegetable crops in Kenya (ZA0376/R7571, 2000-2003)

A Participatory Rural Appraisal (PRA) undertaken in 1996 indicated that four main crops (kale, cabbage, tomato and spinach) accounted for over 95% of peri-urban vegetable production. Thus, these crops became the major focus of subsequent research activities. Subsequent on-farm surveys to determine the incidence of pests and diseases in different agro-ecological zones led to the prioritisation of the major pest and disease problems and identification of areas of pest management which required improvement. Diamond Back Moth (DBM), aphids and semi looper were found to be important pests of brassicas while on spinach and tomatoes, leaf miner, aphids, semi looper and thrips were more important. Diseases were not found to be particularly important on brassicas, the most damaging being black rot, ring spot, downy mildew and virus. In contrast, diseases were found to be extremely damaging on spinach and tomato including early blight, late blight, virus and leaf spot. Also, the diversity and severity of pests were higher in the lower, warmer locations i.e. Athi River, whereas in higher, cooler locations i.e. Nyathuna, the incidence and severity of diseases was greater.

1.4 Objectives of the present survey

A socio-economic survey was commissioned as part of the Peri-Urban Production System Cluster of Projects, to establish farmer practices and key characteristics of peri-urban farms. The survey was designed with the following objectives:

- To characterise peri-urban vegetable systems
- To determine the main constraints to vegetable production in peri-urban areas
- To determine farmer problems, perceptions and practices in relation to vegetable pests and diseases, with particular reference to the Diamondback moth, root-knot nematodes, aphids, blight and virus disease.
- To establish coping strategies for pests and diseases and implications for the peri-urban cluster of projects.

Sampling Strategy and Data Collection

2.1 Sampling

A sampling frame was constructed to obtain a representative sample of peri-urban vegetable producers around Nairobi. Given the available time and resources it was decided that this survey would involve 200 of these peri-urban farms selected through a stratified multi-stage sampling in order to obtain a representative sample. Initially, consultations with research officers from the Kenya Agricultural Research Institute (KARI) and the Extension Department of the Ministry of Agriculture identified the following as the key attributes of peri-urban Nairobi vegetable farmers.

1) Proximity to Nairobi,
2) Predominance of vegetable production, specifically brassicas and tomatoes,
3) Evidence of smallholdings producing largely for the local market.

Accordingly, the main administrative divisions bordering Nairobi in Thika, Kiambu, Machakos and Kajiado Districts were identified. These divisions were subsequently visited by research officers from CABI, KARI-Thika and Divisional Agricultural Extension Officers. During the visits, a listing of locations and, where possible, sub-locations where cultivation of brassicas and tomatoes are prevalent was compiled. Given that sub-locations are the smallest administrative unit in Kenya, frontline extension staff, Assistant Chiefs and village elders provided a list of specific areas or villages. Village elders then helped compile a listing of all residents in these villages. The geographical locations, relative to Nairobi, of the selected areas are shown on the map (Fig. 2.1). From these lists a random sample of farmers was chosen. The number of farms selected in each district and village was based on a proportion of the total number of vegetable farmers, listed for all villages and districts. 68 farms were sampled in Thika District, 62 in Kiambu, 40 in Machakos and 30 in Kajiado.

Figure 2.1 Map showing Nairobi and the surrounding peri-urban survey sites

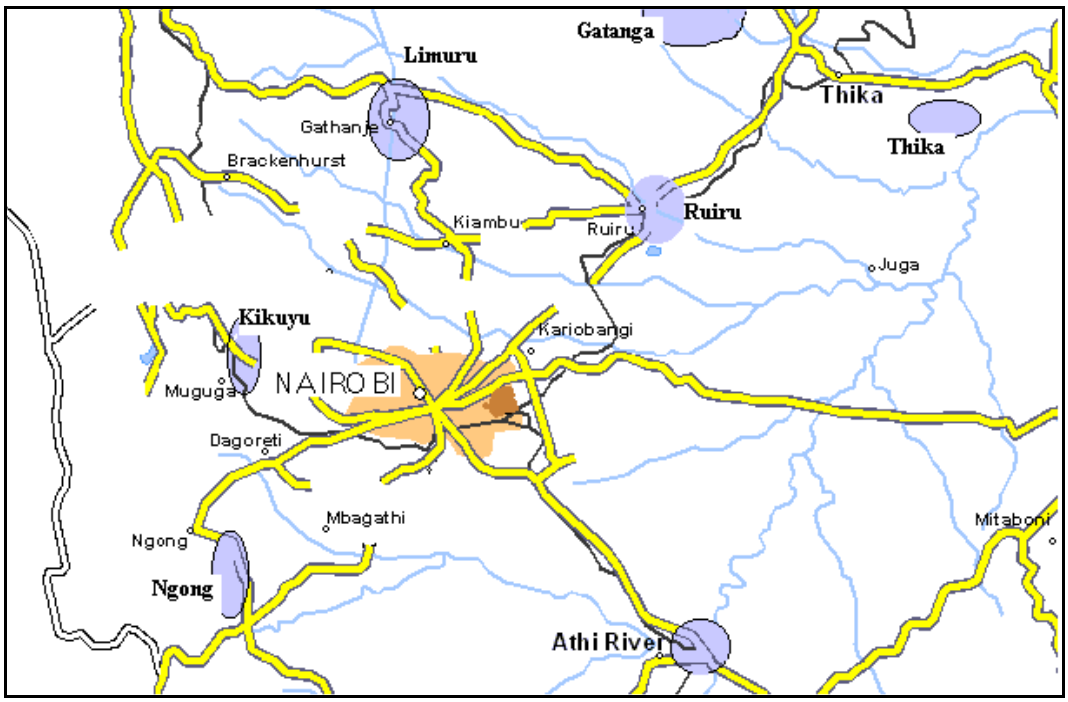


Table 2.1 Peri-urban sampling Areas

Area	**Division**	**Village**	**Agro Ecological Zones**	**Average Annual Rainfall (mm)**
Thika	Thika (Gatuanyaga)	Mbagathi Thika River	Upper Midlands 4 (UM 4)	800
	Ruiru	Kiua Murera	Upper Midlands 4 (UM 4)	800
	Gatanga	Ndunyu Chege Gathece Kiawaira Mkarara Valley Along Chania Nduachi River	Upper Midlands 3 (UM 3)	1200
Kiambu	Kikuyu	Gitiba Valley Karinde Valley Mutua Valley Samiti Kaimba Valley	Lower Highlands 2 (LH 2)	1400
	Limuru	Tharuni	Lower Highlands 2 (LH 2)	1400
Machakos	Athi River	Town Kinanie Area 39	Upper Midlands 6 (UM 6)	500
Kajiado	Ngong	Kiserian Town Kiserian Valley 1 Kiserian Valley 2	Upper Midland 6 (UM 6)	500

AEZ and rainfall figures obtained from (Jaetzold & Schmidt, 1983)

2.2 Data Collection

Data were collected through single visit interviews. The survey questionnaire was developed from an initial checklist compiled by a team of socio-economists and biophysical scientists. The questions were then pre-tested on 4 smallholder farms; 2 in Kandara (Thika) and 2 in Githunguri (Kiambu). Subsequently, the results of the pre-test were discussed with the principal investigators and other scientists based in Nairobi. It was observed that the questionnaire took approximately 3 hours to fill in. In addition, responses to many subjective questions such as estimated yield loss associated with specific pests, given the number pests in each crop and possible confounding from other factors were inconsistent. Assessment of volume type and quantity of chemical pesticides applied and comparing their effectiveness also proved unreliable. Consequently, some questions were removed and the final draft circulated to all principal investigators for comment. Four enumerators were hired to assist in data collection. Each of them had formal university level training in agriculture, specialising in crop protection. They subsequently went on an induction course on interviewing techniques and data coding. The final draft of the questionnaire was pre-tested together with the enumerators as part of their training. The survey team liased with the local extension and administrative

officials in each division to identify the sample farms and inform them about the interviews in advance. All the interviews were conducted on the farms, in the absence of local extension officials who might have influenced the responses. It was established, during the pre-test, that each interview would take between one and one and a half hours. Each enumerator was therefore assigned three farms per day, in order to enhance accuracy and avoid fatigue. The survey was conducted over a period of one month from July 15th to August 15th 2000. Data was analysed using Statistical Package for Social Sciences (SPSS) Version 7.5, GENSTAT 5 and Microsoft Excel 2000.

Characteristics of Peri-urban Vegetable Farmers

3.1 Education, Gender and Farm Location

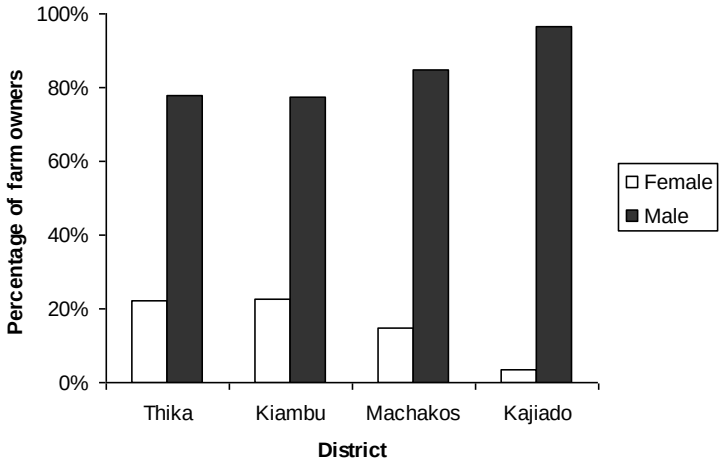
Typical of most smallholder farms in sub-Saharan Africa, the majority of peri-urban vegetables farms were operated (managed on a day-to-day basis) by owners, spouses or offspring of the farm owners. It is worth noting though that 12% of the sample farms had employed farm managers who made decisions on production plans and enterprise mix. Hiring of professional farm managers appears to be a feature of the peri-urban vegetable production system. Furthermore, the majority of farm manager-run farms were found in Athi River (Machakos district). On these farms, in addition to tomatoes and brassicas, Asian vegetables, green beans and other vegetables are grown for export.

Table 3.1 The relationship of farm operators to farm owners

Farm operator	District				Percentage of farms
	Thika	Kiambu	Machakos	Kajiado	
Owner	44	28	8	17	48.5
Spouse	3	11	6	4	12.0
Offspring	19	18	1	4	21.0
Sibling	2	1	2	1	3.0
Employee (Farm manager)	0	4	19	1	12.0
Tenant	0	0	3	3	3.0
Partner	0	0	1	0	0.5

The majority of both farm owners and farm operators were male (82% and 77.5%, respectively). This is typical of Kenya's land tenure system where only male offspring inherit family land, especially farmland.

Figure 3.1 Percentage of male/female farm owners in each district



Almost all of the farm operators had attained some level of formal education with 79% completing at least their primary education (Tables 3.2 and 3.3). This level of literacy is

above the national average of Kenya, which stands at 50% (RoK, 1999). This makes the peri-urban vegetable farmers a suitable target group for conventional extension methods such as print media. Table 3.2 shows the number of farm operators in each education category. A chi-squared test for association between district and education level of farm operator showed a significant result ($p=0.002$). Farm operators in Machakos and Thika are, in general, more highly educated than the operators in Kiambu and Kajiado Districts. This could be related to the fact that a large proportion of farm operators in Machakos are employee farm managers (hired professionals) who have undergone some basic training in crop production.

Not surprisingly, there was a difference in the education levels of the male and female farm operators - the males tend to be more educated. A chi-squared test of association for Table 3.3 shows a significant association ($p<0.001$) between gender of farm operator and education level. 84% of male farm operators had completed at least primary level education with only 62% of female farm operators achieving this level. 11% of female farm operators had received no formal education, whereas only 1% of males were in this situation. Although the current primary school enrolment levels reflect some degree of gender balance (48% girls), completion rate is much lower for girls (RoK 1997). This translates into fewer women in higher institutions of learning.

Table 3.2 Number of farmers at each education level by district

Education level	(number of farmers in each category - 'expected' number, assuming no association, in brackets)				All districts
	Thika	Kiambu	Machakos	Kajiado	
None	**2 (2)**	**5 (2)**	**0 (1)**	**0 (1)**	**7**
Some Primary	**9 (12)**	**16 (11)**	**2 (7)**	**9 (5)**	**36**
Complete Primary	**25 (24)**	**16 (22)**	**15 (14)**	**16 (11)**	**72**
Secondary	**31 (28)**	**25 (25)**	**21 (16)**	**4 (12)**	**81**
Tertiary	**1 (1)**	**0 (1)**	**2 (1)**	**1 (1)**	**4**
All levels	**68**	**62**	**40**	**30**	**200**

Table 3.3 Education level and gender of farm operators

Gender	**Education level of farm operator** (percentages, by gender, in brackets) None	Some Primary	Complete Primary	Secondary	Tertiary	Total
Female	5 (11.1)	12 (26.7)	20 (44.4)	8 (17.8)	0 (0)	45
Male	2 (1.3)	24 (15.5)	52 (33.5)	73 (47.1)	4 (2.6)	155
Total	7 (3.5)	36 (18.0)	72 (36.0)	81 (40.5)	4 (2.0)	200

The average age for farm operators was 38, and for farm owners it was 49 years old. Ages of both farm owners and farm operators ranged from 20 to 90 years old. 27% of farm operators were below the age of 30, but by contrast, only 9% of farm owners were below this age. Table 3.4 shows that the higher educated farm operators and owners were, on average, younger than their less educated neighbours.

Table 3.4 Age of farm owners and farm operators in each education category

Education level	**Average age** (standard deviations in brackets) Farm owner	Farm operator
None	54 (9)	54 (9)
Some Primary	52 (18)	45 (17)
Complete Primary	47 (17)	35 (10)
Secondary	49 (15)	35 (9)
Tertiary	37 (10)	32 (6)
All levels	49 (16)	38 (12)

In order to gain insights into the relative wealth status of the farmers, farmers were asked questions concerning ownership of cattle, motor-vehicle and water tank (Table 3.5). Water tank and cattle ownership is particularly high in Kiambu district while Machakos district has the highest proportion of farmers owning a vehicle.

Table 3.5 Ownership of water tank, cattle and motor vehicle by district

District	(percentage within district in brackets) Water Tank	Cattle	Vehicle
Thika	14 (21)	44 (65)	4 (6)
Kiambu	41 (66)	52 (84)	13 (21)
Machakos	15 (38)	16 (40)	16 (40)
Kajiado	10 (33)	10 (33)	5 (17)
All Districts	80 (40)	122 (61)	38 (19)

On average, farmers in Thika district lived the furthest from an all-weather road. They also had the greatest distance to travel to market, 14.4 km on average.

Table 3.6 Average distances of farms from all weather roads and market

	District				Across districts
	Thika	Kiambu	Machakos	Kajiado	
Distance to all weather road (km)	4.2	2.6	3.0	0.8	3.0
Distance to market (km)	14.4	9.6	3.2	5.3	9.3
Time to get to market (mins)	54	54	36	42	48

3.2 Discussion

Family members managed most peri-urban vegetable farms, comparable to family farm units in the developed world. However, there were cases where professional farm managers who are experienced in vegetable production were hired to manage the farms. The peri-urban vegetable farm operators were also on average more educated than the national average. However, farm operators in Machakos, a large proportion being hired farm managers, had higher levels of formal education. Also, a larger proportion of farm owners in Machakos owned motor vehicles, an indication of their wealth status. It is therefore possible that Machakos farmers were wealthier or more commercially oriented or that the farms were more productive. Chapter 4 analyses other indicators of resource endowment and farm productivity.

Vegetable Production and Marketing Systems

4.1 Cropping System - Land area under vegetables

It was hypothesised in Chapter 1 that smallholder farms typify the peri-urban vegetable production system. Table 4.1 shows that the average farm size was 1.3 hectares, with a cropped area of 0.8 hectares. Farms in Machakos district were, on average, larger than the other survey districts. In all districts except Thika, vegetable area constituted over 50% of the cropped area at the time of the survey. This proportion is highest in Kajiado district where 85% of the cropped land on a farm was given over to vegetable production. The percentage of Kajiado farmers renting land for vegetable production (53%) is also higher than the other districts (23-26%).

Table 4.1 Average farm size, cropped and vegetable areas (in hectares)

(average areas in hectares)	District				All districts
	Thika	Kiambu	Machakos	Kajiado	
Vegetable area of farm	0.3 (0.3)	0.4 (0.4)	0.7 (0.6)	0.4 (0.3)	0.4 (0.4)
Cropped area of farm	0.8 (0.8)	0.7 (0.6)	1.1 (1.1)	0.5 (0.4)	0.8 (0.8)
Total farm size	1.1 (1.0)	1.2 (1.1)	2.3 (2.1)	0.8 (0.6)	1.3 (1.4)
Vegetable area as percentage of cropped farm area	39%	59%	65%	85%	55%
Vegetable area as percentage of total farm	28%	35%	32%	50%	33%
Number of farmers renting land for vegetable crop	17 (25%)	16 (26%)	9 (23%)	16 (53%)	58 (29%)

(standard deviations in brackets for first three rows)

Table 4.3 and Figure 4.1 give a breakdown of vegetable area into the three vegetables, tomatoes, kale and cabbage. The average size of vegetable area is also shown as a proportion of the cropped area within a farm.

Table 4.2 Number of farmers growing vegetables in each district

	District				
	Thika	Kiambu	Machakos	Kajiado	All districts
Tomato	59 (87)	24 (39)	34 (85)	25 (83)	142 (71)
Kale	54 (79)	46 (74)	34 (85)	25 (83)	159 (80)
Cabbage	5 (7)	43 (69)	32 (80)	18 (60)	98 (49)

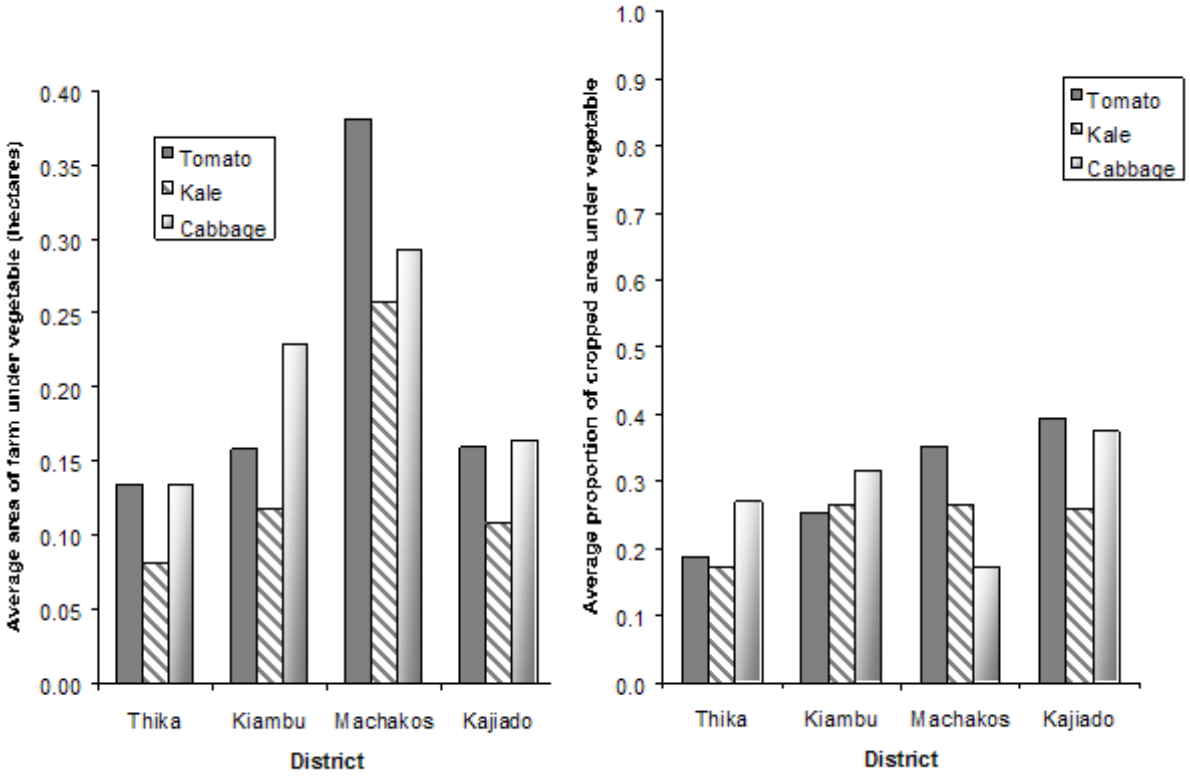
(Percentage of farmers in brackets)

Table 4.3 Average cropped area by district (hectares)

(average areas in hectares)	District				All districts
	Thika	Kiambu	Machakos	Kajiado	
Tomato crop land area	0.13 (0.13)	0.16 (0.10)	0.38 (0.36)	0.16 (0.16)	0.21 (0.24)
Proportion of cropped land under tomatoes	0.19 (0.20)	0.25 (0.20)	0.35 (0.24)	0.39 (0.29)	0.27 (0.24)
Kale crop land area	0.08 (0.06)	0.12 (0.09)	0.26 (0.25)	0.11 (0.09)	0.13 (0.15)
Proportion of cropped land under kale	0.17 (0.22)	0.27 (0.21)	0.26 (0.23)	0.26 (0.18)	0.23 (0.22)
Cabbage crop land area	0.13 (0.15)	0.23 (0.15)	0.29 (0.38)	0.16 (0.11)	0.20 (0.20)
Proportion of cropped land under cabbage	0.27 (0.68)	0.32 (0.24)	0.17 (0.19)	0.37 (0.29)	0.29 (0.45)

(standard deviations in brackets)

Figure 4.1 Average vegetable crop size (hectares) and proportion of cropped area of farm under each vegetable.



4.2. Varietal adoption and seed sources

The predominant tomato variety grown is Cal J. This variety produces hard fruit and has good storage quality (long shelf life) compared with the Moneymaker variety. Cal J is

therefore easier to transport in bulk with minimal losses due to "squashing" and is preferred by small-scale retail outlets since it keeps longer. The majority of farmers (75%) grow the Collard variety of kale. Collard is a leafy variety with a short harvesting season. Conversely, the Thousand headed variety, grown by 24% of farmers has a long harvesting season with late flowering. The most popular cabbage variety planted by 79% of cabbage farmers was Gloria F1. This variety of cabbage produces compact cabbage heads that weigh more than the less popular varieties. Since market price of cabbage is determined by weight, among other attributes, heavier heads fetch a higher price.

The majority of vegetable farmers obtained their seed from retail outlets known as stockists. These retail outlets stock a variety of agricultural inputs including fertilizer and chemical pesticides. All cabbage seed (98 farms) was purchased from a stockist, as was the majority of tomato and kale seed (96% and 70% of farms, respectively). Other sources of seed were; fellow farmer, own seed and seed hawkers. Three farmers (2%) purchased tomato seed from a fellow farmer and 28 farmers (18%) purchased kale seed from a fellow farmer. One farmer used his own tomato seed and only kale seed was purchased from the hawkers (4%).

Table 4.4 Tomato, kale and cabbage varieties

Vegetable	Variety and percentage of farmers		
Tomato	Cal J (86.8)	Moneymaker (7.4)	Caltana II (4.4)
Kale	Collard / Georgia (75.4)	Thousand headed (23.8)	Local variety (9.8)
Cabbage	Gloria F1 (78.9)	Copenhagen Mkt (14.7)	Pintor (6.3)

(N.B. Numbers of farmers identifying their varieties were, 136 for tomatoes, 122 for kale and 95 for cabbage)

4.3 Irrigation

The majority (82%) of the sample farms irrigated their vegetable crops. There was some variation between districts, in Kiambu only 70% irrigated, whereas in Machakos 90% irrigated. Machakos District has low rainfall and additional watering of vegetables is essential. Conversely, Kiambu district receives a higher amount of rainfall and less input is required from irrigation systems. It is also possible that some farmers in Kiambu grow vegetables in the rainy season only.

Methods of irrigation varied across farms and districts. Table 4.5 lists six of the main methods of irrigation, along with the numbers of farmers in each district who used each. Note that some farmers used a combination of irrigation methods.

Table 4.5 Methods of irrigation used by peri-urban farmers in each district

Method of irrigation	District				
	Thika	Kiambu	Machakos	Kajiado	All districts
Overhead sprinkling from river (pumped)	5	20	0	10	35
Basin from river (pumped)	27	16	33	12	88
Channels and gravity	2	1	1	0	4
Own well (by hand)	8	1	0	0	9
Communal well (by hand)	5	1	0	0	6
River (by hand)	17	2	0	1	20
Total number of farmers	68	62	40	30	200

The majority of farmers pumped water from nearby rivers for irrigation. In terms of water application, basin irrigation system was the predominant method used by farmers. This was followed by overhead sprinkling and application by hand. Kiambu had a higher proportion of farmers using sprinklers than the other districts. Ownership of wells and application from the river, by hand was more prevalent in Thika. A plausible explanation for this trend is that whilst farms located close to rivers tend to use pumps, those located inland (e.g. Gatanga in Thika) need a well both for domestic and irrigation water. Also, pumping would be feasible along relatively larger rivers such as Athi, Thika and Nyathuna. Farmers close to streams may prefer to fetch water by hand in compliance with water abstraction requirements.

4.4 Soil fertility management

The majority of the peri-urban farmers surveyed apply inorganic fertilizer to their vegetable crops. Of the sample farms 96% added manure or fertilizer to the soils. Nitrogen based fertilizers Di-ammonium phosphate (DAP), Calcium Ammonium Nitrate (CAN) and Urea were the most frequently used inorganic fertilizers in kale and tomato fields. The use of foliar feed was not widespread, only 23% of farmers applied this, with brand names such as Green Gold and Booster the most widely applied. The use of cattle manure was fairly limited despite the high proportion of households who owned cattle.

Table 4.6 Number of farmers using a given fertilizer by crop

	DAP	CAN	UREA
Tomato	120 (60)	111 (56)	57 (29)
Kale	114 (57)	125 (63)	60 (30)
Cabbage	78 (39)	72 (36)	27 (14)

NB some farmers used more than 1 fertilizer, percentage of farmers in brackets

4.4 Production and Marketing Constraints

An earlier study of factors influencing uptake and adoption of crop protection research output in the peri-urban systems indicated a high degree of awareness about pests among farmers. Furthermore, the existence of pest and disease damage and the production losses arising from the same were considered economically important hence the high incidence

of chemical pesticide use to control pests and diseases. The present survey confirmed these findings. The interview respondents were asked to identify and rank production and marketing constraints for vegetables. Subsequently they were asked to give a combined ranking for marketing and production constraints (Table 4.7).

Table 4.7 Production and marketing constraints to vegetable production ranked highest.

Production constraints	Marketing constraints	Production/Marketing, combined constraints
Pests and Diseases (67%)	Low product prices (68%)	Pests and diseases (78%)
Inadequate capital to purchase inputs (15%)	No market when products are available (8%)	Low product prices (54%)
Inadequate irrigation water (10%)	High cost of transport (5%)	In adequate capital to purchase inputs (52%)

(Percentage of farmers shown in brackets)

Insect pests and diseases of vegetables were cited as the main constraint to vegetable production by 67% of farmers. Inadequate capital to purchase inputs was ranked the highest constraint by 15% of farmers and inadequate irrigation water was ranked highest by 10% of farmers. Other production constraints cited by farmers included, inadequate knowledge on pesticide use, ineffective pesticides, substandard seed and declining soil fertility. Sixty-eight percent of farmers cited low product prices as their main marketing constraint. Lack of market during certain seasons, possibly reflecting competition from the traditional vegetable growing areas was cited as the main marketing constraint by 8% of farmers. The combined ranking of production and marketing constraints emphasises the importance of pest and diseases as a constraint to peri-urban vegetable farmers.

4.5 Discussion

The proportion of cropped land allocated to vegetables demonstrates the importance of vegetables in the peri-urban cropping system. It is also evident that Machakos farmers own larger land holdings, the other hypothesised indicator of wealth. Adoption of improved varieties and crop management was also high among the peri-urban vegetable farmers. Given that there are few if any indigenous cabbage and kale varieties, the observed varietal adoption pattern is not altogether surprising. Likewise, use of inorganic fertilizer for soil fertility management, irrigation and use of certified seed are indications of high input crop management practices. The observed level of crop management in the peri-urban vegetable production system is typical of smallholder cash crop systems such as tea, coffee, cotton and paddy rice that are cultivated largely for the market rather than for home consumption. Also, market determined attributes such as keeping quality in the case of tomatoes or head weight in the case of cabbage in addition to yield in the case of kale are the main factors influencing varietal choice. This confirms the hypothesis that peri-urban vegetable production is guided by market demand. The importance of crop pests as a production constraint is confirmed and the next chapter analyses farmers' perceptions of and coping strategies for different pests and diseases.

Vegetable Pests and Diseases

5.1 Introduction

The ranking of pests and diseases in chapter 4 confirmed the findings of earlier studies namely; a PRA conducted in 1996 to determine the pest management practices in the peri-urban vegetable system and an analysis of factors influencing the uptake of crop protection research outputs in the peri-urban vegetable systems in 2000. In both studies, farmers cited the high risks associated with pest and disease infestation on yield and quality as the key criteria for ranking pests and diseases highly. In the present study, one of the objectives was to assess farmers' knowledge about pests and diseases. Accordingly, farmers were asked several questions about what pests and diseases they saw in their crops and photographs of the cited pests and diseases shown to the survey respondents for identification. They were also asked to give the 'top 3' rankings for the pests and diseases. In addition, the respondents were asked to describe the nature and existence of damage to crops. Where there was a crop in the field, crop damage due to pests and disease was assessed together with the enumerators.

5.2 Tomatoes

Of the farmers surveyed, 142 grow tomatoes and these were asked to identify the pests and diseases that they observed in their tomato crop. They were then asked to name and rank 3 most important pest and disease problems.

5.2.1 Tomato pests

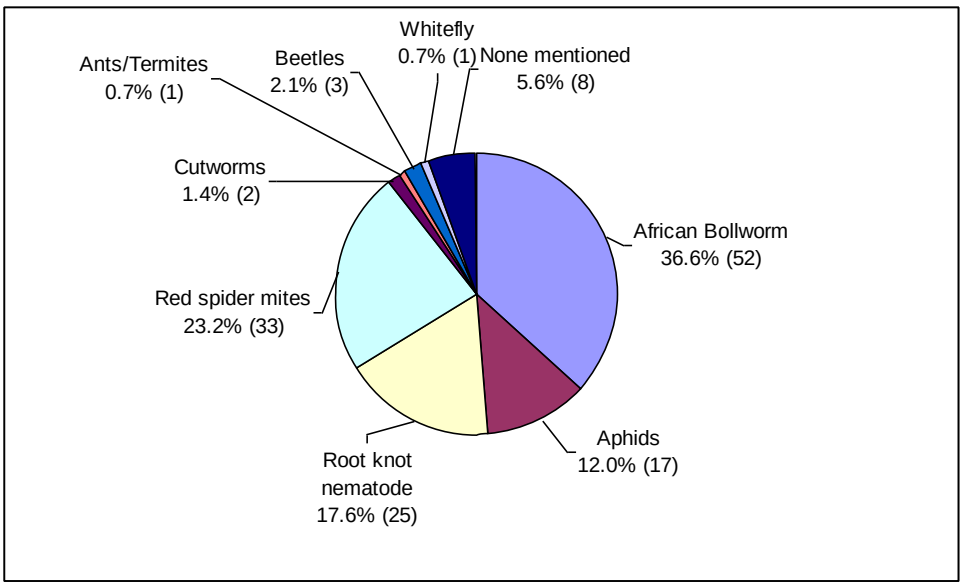
The table below (Table 5.1) identifies the tomato pests mentioned by farmers, and the number of farmers who mentioned each one. The pie-chart (Figure 5.1) shows the percentage of farmers who ranked each pest highest during the ranking exercise. Note that the pests not mentioned in the pie-chart were therefore ranked highest by none of the farmers.

Table 5.1 Tomato pests named by farmers and numbers of farmers mentioning each.

Pest	*Number of farmers who mentioned each pest (percentages shown in brackets)* (n=142)
African bollworm	125 (88.0)
Aphids	92 (64.8)
Root knot nematode	87 (61.3)
Red spider mites	50 (35.2)
Leaf miner	49 (34.5)
Cutworms	17 (12.0)

African bollworm, aphids, nematodes, red spider mites and leaf miner were the most frequently mentioned tomato pests by farmers. When asked to rank their highest pest none of the farmers ranked leaf miner.

Figure 5.1 Percentage of farmers who ranked each tomato pest highest



(number of farmers in brackets)

Table 5.2 Percentage of farmers in each district who ranked each pest highest

Top ranked pest	**District** (numbers of farmers in brackets)			
	Thika	Kiambu	Machakos	Kajiado
African bollworm	32.2 (19)	58.3 (14)	20.6 (7)	48.0 (12)
Aphids	6.8 (4)	20.8 (5)	11.8 (4)	16.0 (4)
Root knot nematode	37.3 (22)	4.2 (1)	5.9 (2)	----
Red spider mites	10.2 (6)	8.3 (2)	52.9 (18)	28.0 (7)

It is interesting to note that root knot nematode was ranked as the most important pest problem by over a third of farmers in Thika District, but only by one farmer in Kiambu, two farmers in Machakos and no farmers in Kajiado. In Kiambu and Kajiado the African bollworm was considered the most important pest problem by a high percentage of farmers (58% and 48%, respectively) and in Machakos the majority of farmers (53%) consider Red spider mites to be their most important pest problem.

Table 5.3 Percentage of farmers who ranked each pest highest – shown for irrigated farms and non-irrigated farms

Top ranked pest	**Irrigation treatment** (numbers of farmers in brackets)	
	Non-irrigated	Irrigated
African bollworm	31.8 (7)	37.5 (45)
Aphids	27.3 (6)	9.2 (11)
Root knot nematode	9.1 (2)	19.2 (23)
Red spider mites	22.7 (5)	23.3 (28)
Cut worms	----	1.7 (2)
Ants/termites	----	0.8 (1)
Beetles	4.5 (1)	1.7 (2)
Whitefly	----	0.8 (1)
None mentioned	4.5 (1)	5.8 (7)

27% of farmers who do not irrigate, rank aphids as their most important pest problem, compared to only 9% of farmers who do irrigate. Conversely only 9% of farmers who do not irrigate rank root knot nematode as their worst pest problem, compared to 19% of farmers who do irrigate. Farmers who do not irrigate did not rank cutworms, ants and termites or whitefly as their worse pest problem, although four farmers who do irrigate gave highest ranking to these pests.

5.2.2 Control of tomato pests

Only 4 out of the 142 farmers growing tomatoes did not apply some form of pest control to their crop. Farmers were asked to name the chemical, botanical, biological and cultural practice controls that they used to control the three most frequently mentioned pests, the African bollworm, aphids and root knot nematode. It was found that the numbers of farmers using botanical, biological or cultural practice controls was very small (1-5 farmers for each pest). The table below (Table 5.4) shows the most frequently used chemical control for the tomato pests.

Table 5.4 Chemicals used by farmers to control tomato insect pests

Pest	**Chemicals most frequently used for control of pest**	**Number of tomato growing farmers using this control (%)**	**Total number of farmers using some form of chemical control (%)**
African Bollworm	Karate *(Lambda – cyhalothrin)* Dimethoate *(Dimethoate)*	25 (17.6) 17 (12.0)	74 (52.1)
Aphids	Karate *(Lambda – cyhalothrin)* Dimethoate*(Dimethoate)* Diazinon *(Diazinon)*	59 (41.5) 31 (21.8) 21 (14.8)	107 (75.4)
Nematodes	Furadan *(Carbofuran)*	16 (11.3)	28 (19.7)

5.2.3 Tomato diseases

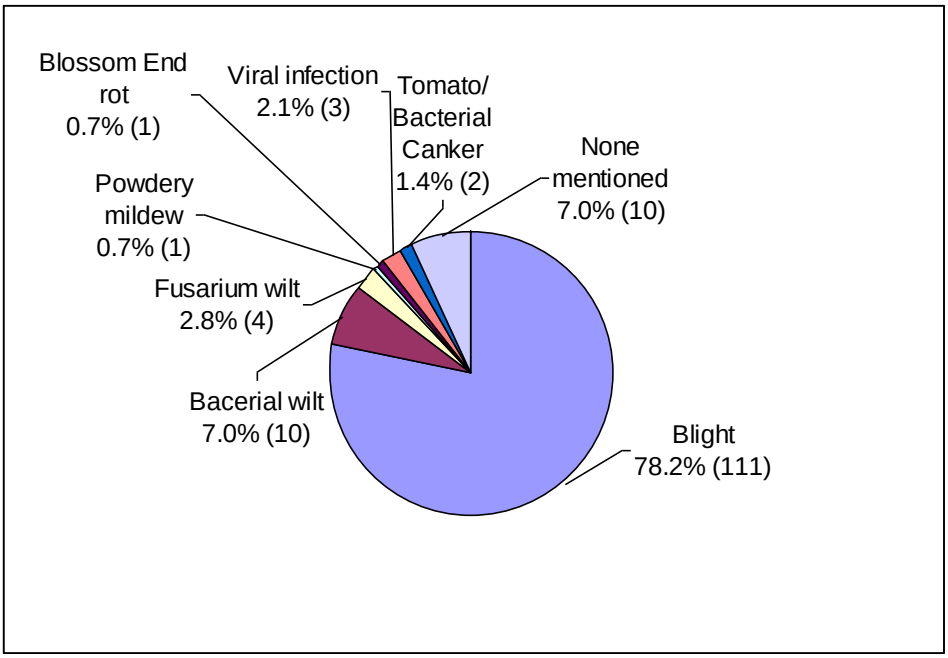
The table below (Table 5.5) identifies the tomato diseases mentioned by farmers, and the number of farmers who mentioned each one. The pie-chart (Figure 5.2) shows the

percentage of farmers who ranked each disease highest. Note that the diseases not mentioned in the chart were therefore ranked highest by none of the farmers.

Table 5.5 Tomato diseases named by farmers and numbers of farmers mentioning each

Disease	*Number of farmers who mentioned each disease (percentages shown in brackets) (n=142)*
Blight	134 (94.4)
Bacterial wilt	47 (33.1)
Fusarium wilt	14 (9.9)
Viral infection	14 (9.9)

Figure 5.2 Percentage of farmers who ranked each tomato disease highest



(number of farmers in brackets)

5.2.4 Control of tomato diseases

The majority of tomato-growing farmers (85%) applied some form of disease control to their crop. They were asked to name the chemical, botanical, biological and cultural practice controls that they used to control three of the most frequently mentioned diseases, blight, fusarium wilt and bacterial wilt. As with the pest control it was found that the numbers of farmers using botanical or biological controls was very small (between 1-5 out of 142 farmers in total for each disease). The number of farmers using cultural practices to control for diseases was also low, although it is worth noting that 14 of the farmers used a variety of cultural practices, predominantly uprooting and crop rotation, to control wilt diseases. In terms of chemical control only five farmers controlled for wilt diseases. The table below (Table 5.6) shows the most frequently used chemical control for tomato blight.

Table 5.6 Chemicals used by farmers to control tomato diseases

Disease	Chemicals most frequently used for control of disease	Number of tomato growing farmers using this control (%)	Total number of farmers using some form of chemical control (%)
Blight	Dithane M45 *(Mancozeb)* Ridomil *(Metalaxyl)*	73 (51.4) 60 (42.3)	122 (85.9)

5.3 Kale

Of the farmers surveyed, 159 grow kale and these farmers were asked to identify the pests and diseases that they observed in their kale crop. They were then asked to rank their 'top 3' most important pest and disease problems.

5.3.1 Kale pests

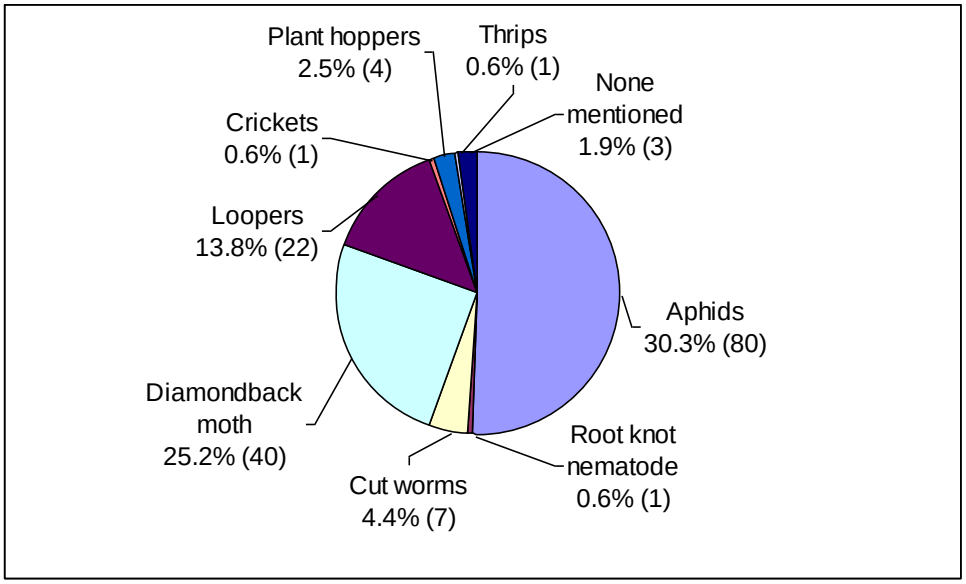
The table below (Table 5.7) identifies the kale pests mentioned by farmers, and the number of farmers who mentioned each one. The pie-chart (Figure 5.3) shows the percentage of farmers who ranked each pest highest during the ranking exercise. Note that the pests not mentioned in the pie-chart were therefore ranked highest by none of the farmers.

Table 5.7 Kale pests named by farmers and numbers of farmers mentioning each

Pest	*Number of farmers who mentioned each pest (percentages shown in brackets) (n=159)*
Aphids	155 (97.5)
Diamondback Moth	120 (75.5)
Loopers	106 (66.7)
Cutworms	19 (11.9)

Aphids, diamondback (DBM) moth and loopers were the most frequently mentioned tomato pests by farmers. They were also the three pests that received the majority of 'highest' rankings from farmers.

Figure 5.3 Percentage of farmers who ranked each kale pest highest



(number of farmers in brackets)

5.3.2 Control of kale pests

Only eleven of the farmers growing kale did not apply some form of pest control to their crop. Farmers were asked to name the chemical, botanical, biological and cultural practice controls that they used to control the three most frequently mentioned pests, the Diamondback moth, aphids and loopers. It was found that the number of farmers using botanical and biological was very small (max. of 2 farmers). Cultural practices were not used to control Diamondback moth, however a few farmers stated that they used cultural practices to control aphids and loopers. For aphids, five farmers used uprooting, two used crop rotation and six used pruning. For loopers, four farmers manured their crops whilst just one farm used uprooting. The table below (Table 5.8) shows the most frequently used chemical control for the kale pests.

Table 5.8 Chemicals used by farmers to control kale pests

Pest	Chemicals most frequently used for control of pest	Number of kale growing farmers using this control (%)	Total number of farmers using some form of chemical control (%)
Diamondback Moth	Karate *(Lambda – cyhalothrin)*	70 (44.0)	128 (80.5)
	Dimethoate*(Dimethoate)*	31 (19.5)	
	Diazinon *(Diazinon)*	26 (16.4)	
Aphids	Karate *(Lambda – cyhalothrin)*	68 (42.8)	116 (73.0)
	Diazinon *(Diazinon)*	28 (17.6)	
Loopers	Karate *(Lambda – cyhalothrin)*	45 (28.3)	75 (47.2)
	Diazinon *(Diazinon)*	18 (11.3)	

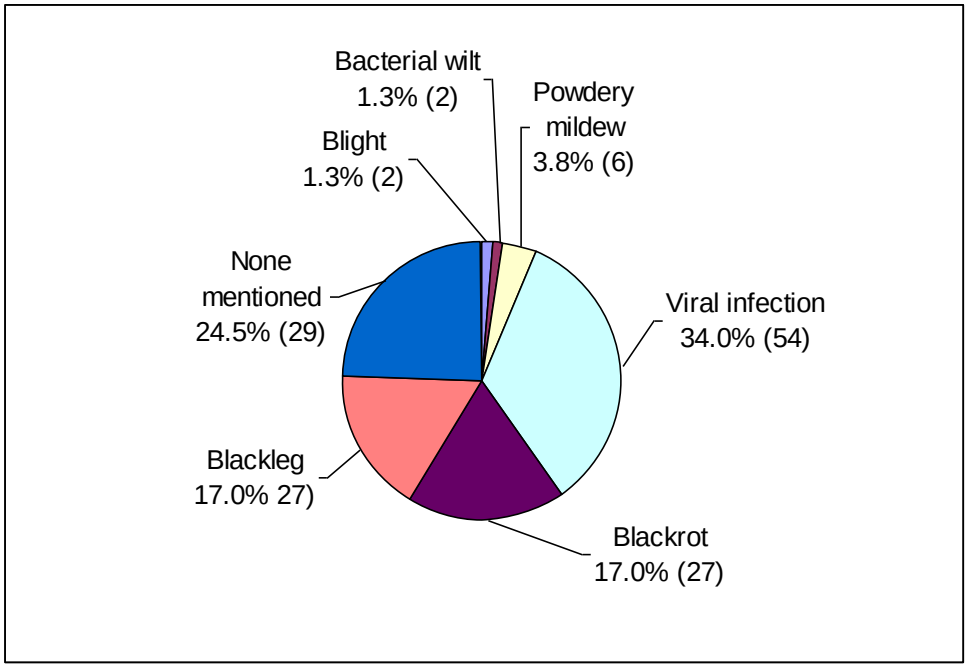
5.3.3 Kale diseases

The table below (Table 5.9) identifies the kale diseases mentioned by farmers, and the number of farmers who mentioned each one. The pie-chart (Figure 5.4) shows the percentage of farmers who ranked each disease highest. Note that the diseases not mentioned in the chart were therefore ranked highest by none of the farmers.

Table 5.9 Kale diseases named by farmers and numbers of farmers mentioning each

Disease	*Number of farmers who mentioned each disease (percentages shown in brackets) (n=159)*
Viral infection	99 (62.3)
Blackrot	62 (39.0)
Blackleg	51 (32.1)
Powdery Mildew	9 (5.7)

Figure 5.4 Percentage of farmers who ranked each kale disease highest



(number of farmers in brackets)

5.1.4 Control of kale diseases

Only 31% of kale farmers said that they apply some form of disease control to their crop. They were asked to name the chemical, botanical, biological and cultural practice controls that they used to control four of the most frequently mentioned diseases, viral infection, blackrot, blackleg and powdery mildew. It was found that no farmers were using botanical or biological controls. The number of farmers using cultural practices to control for diseases was also low, although it is worth noting that 17 of the farmers used a variety of cultural practices, predominantly uprooting and crop rotation, to control for blackrot. Also, 12 farmers stated that they were using these same cultural practices to control viral infection. Eight farmers were using uprooting as a way of controlling for blackleg. In

terms of chemical control only six farmers controlled for blackleg and seven farmers controlled for powdery mildew. The table below (Table 5.10) shows the most frequently used chemical control for viral infection and blackrot.

Table 5.10 Chemicals used by farmers to control kale diseases

Disease	**Chemicals most frequently used for control of disease**	**Number of kale growing farmers using this control (%)**	**Total number of farmers using some form of chemical control (%)**
Viral infection	Karate (Karate *(Lambda – cyhalothrin)*	10 (6.3)	25 (15.7)
Blackrot	Dithane M45 *(Mancozeb)*	13 (8.2)	26 (16.4)

Other chemicals cited for controlling viral infection were Diazinon (3 farmers), Dimethoate (7 farmers), Ambush (2 farmers) and Polythin (2 farmers). Only one farmer used each of the following; Malathion, Kelthane, Decis, Dithane M45, Marshall.

5.4 Cabbage

Of the farmers surveyed, 98 grow cabbages and these were asked to identify the pests and diseases that they observed in their cabbage crop. They were then asked to rank their 'top 3' most important pest and disease problems.

5.4.1 Cabbage pests

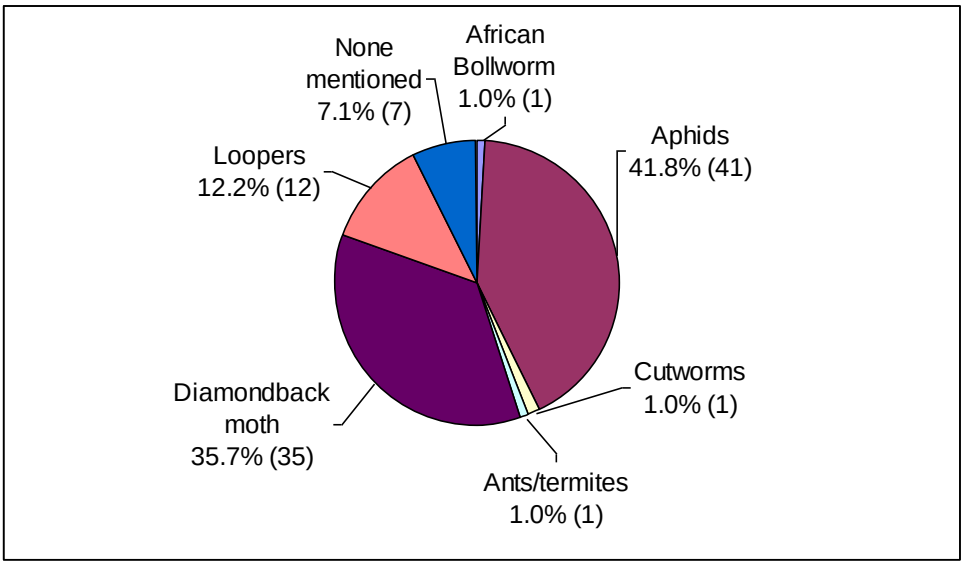
The table below (Table 5.11) identifies the cabbage pests mentioned by farmers, and the number of farmers who mentioned each one. The pie-chart (Figure 5.5) shows the percentage of farmers who ranked each pest highest during the ranking exercise. Note that the pests not mentioned in the pie-chart were therefore ranked highest by none of the farmers.

Table 5.11 Cabbage pests named by farmers and numbers of farmers mentioning each.

Pest	*Number of farmers who mentioned each pest (percentages shown in brackets) (n=98)*
Aphids	88 (89.9)
Diamondback moth	68 (69.4)
Loopers	63 (64.3)
Cutworms	12 (12.2)

Aphids, diamondback moth and loopers were the most frequently mentioned cabbage pests by farmers.

Figure 5.5 Percentage of farmers who ranked each cabbage pest highest



(number of farmers in brackets)

5.4.2 Control of cabbage pests

Of the 98 farmers growing cabbage, 85 (87%) applied some form of pest control to their crop. Farmers were asked to name the chemical, botanical, biological and cultural practice controls that they used to control the three most frequently mentioned pests, diamondback moth, aphids and loopers. It was found that the number of farmers using botanical and biological was very small (max. of 2 farmers). A few farmers used cultural practices only (3 farmers used uprooting to control diamondback moth and 2 farmers used it to control aphids). Table 5.12 shows the most frequently used chemical control for the cabbage pests.

Table 5.12 Chemicals used by farmers to control cabbage insect pests

Pest	Chemicals most frequently used for control of pest	Number of cabbage growing farmers using this control (%)	Total number of farmers using some form of chemical control (%)
Diamondback Moth	Karate *(Lambda – cyhalothrin)* Dimethoate *(Dimethoate)*	36 (36.7) 23 (23.5)	74 (75.5)
Aphids	Karate *(Lambda – cyhalothrin)*	33 (33.7)	66 (67.3)
Loopers	Karate *(Lambda – cyhalothrin)*	24 (24.5)	46 (46.9)

5.4.3 Cabbage diseases

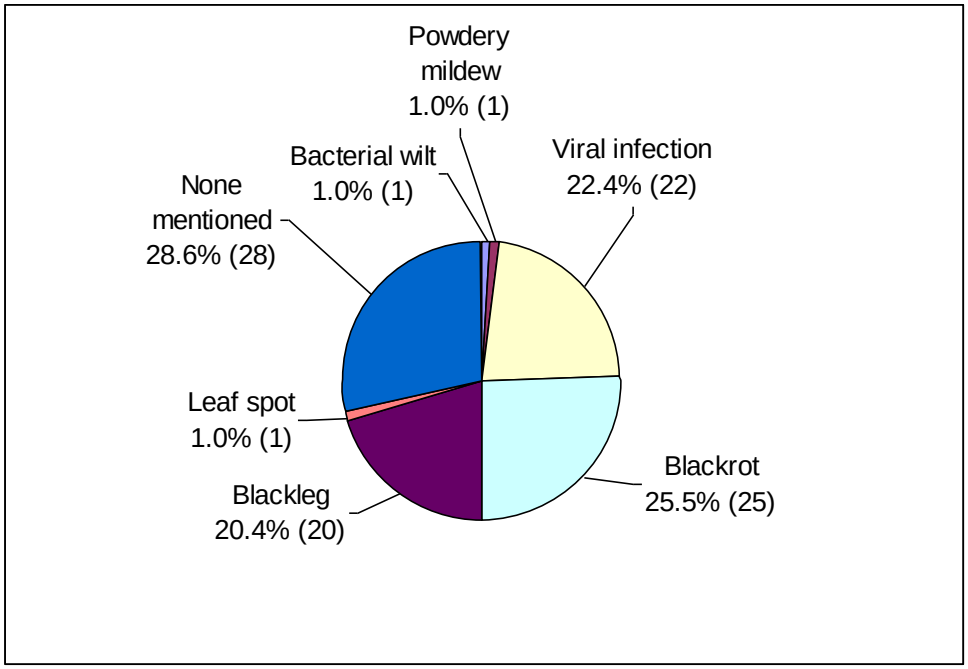
Table 5.13 identifies the cabbage diseases mentioned by farmers, and the number of farmers who mentioned each one. The pie-chart (Figure 5.6) shows the percentage of

farmers who ranked each disease highest. Note that the diseases not mentioned in the chart were therefore ranked highest by none of the farmers.

Table 5.13 Cabbage diseases named by farmers and numbers of farmers mentioning each

Disease	*Number of farmers who mentioned each disease (percentages shown in brackets)* (n=98)
Viral infection	47 (48.0)
Blackleg	43 (43.9)
Blackrot	40 (40.8)
Powdery Mildew	3 (3.1)

Figure 5.6 Percentage of farmers who ranked each cabbage disease highest



(number of farmers in brackets)

5.4.4 Control of cabbage diseases

Only 54% of cabbage farmers said that they apply some form of disease control to their crop. They were asked to name the chemical, botanical, biological and cultural practice controls that they used to control four of the most frequently mentioned diseases, viral infection, blackrot, blackleg and powdery mildew. It was found that few farmers (max. 2) were using botanical or biological controls. The numbers of farmers using cultural practices to control for diseases was also low, although it is worth noting that 7 of the farmers used uprooting to control for blackrot and 3 farmers used this practice to control viral infection. In terms of chemical control only seven farmers controlled for blackleg and ten farmers controlled for powdery mildew. The table below (Table 5.14) shows the most frequently used chemical control for viral infection and blackrot.

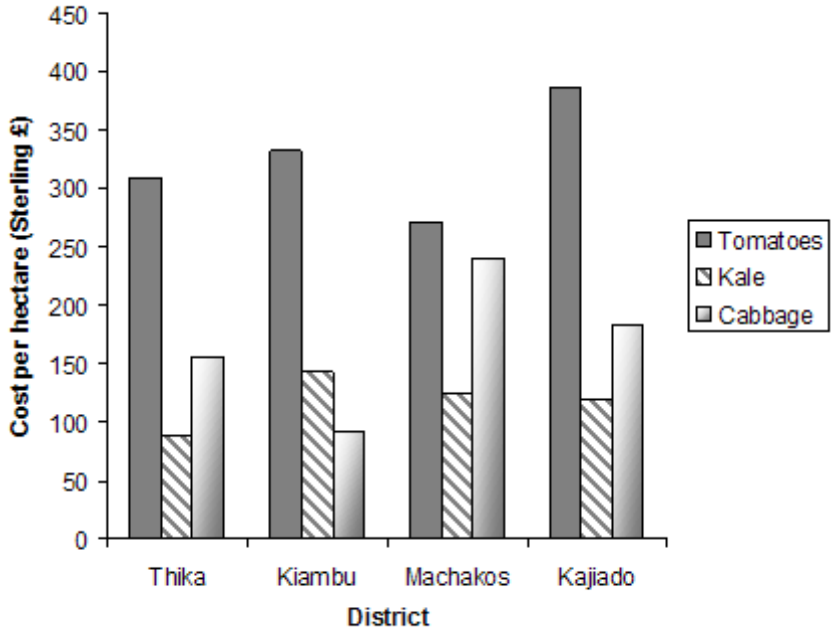
Table 5.14 Chemicals used by farmers to control cabbage diseases

Disease	**Chemicals most frequently used for control of**	**Number of cabbage growing farmers using this control (%)**	**Total number of farmers using some form of chemical control (%)**
Blackrot	Dithane M45 *(Mancozeb)*	11 (11.2)	24 (24.5)

5.5 Cost of crop protection

Farmers were asked to estimate the amount of money they spent on crop protection for the last crop of tomato kale and cabbage. Figure 5.7 shows the average cost per hectare of crop protection for each crop in each district. The cost of crop protection varied by crop and location (district). The cost of protecting a tomato crop is generally much higher (£270-390) than the cost of protecting brassicas (£90-240).

Figure 5.7 Cost of crop protection for the last crop by district (£/hectare)



5.6 Conclusions

African bollworm, aphids and Root knot nematodes were the priority pests in tomatoes while blight was the highest ranked disease by the majority of farmers. Other pests and diseases included Red spider mites, leaf minors, cutworms, bacterial wilt, fusarium wilt and viral infections. Application of chemical pesticides was the main control strategy for these pests. Farmers used Karate and Dimethoate to control African bollworm and Aphids. In addition to the 2 chemicals named above, Diazinon was also used to control Aphids while Furadan is used to control nematodes. To control blights, farmers spray Dithane and Ridomil.

Aphids, DBM and loopers are the most important pests of kale and cabbage. In addition, viral infection and blackrot are the most frequently mentioned diseases of kale and cabbage. In addition to the above, cutworms, African bollworm, spider mites and root knot nematodes are also mentioned by farmers. Notably, viral infection is ranked highest by the majority of kale farmers. Most farmers spray Karate to control DBM aphids and loopers.

Out of the 200 farmers surveyed, only 1-5 cited use of botanicals to control vegetable pests. 99% of the peri-urban vegetable farmers employ chemical control strategies for pests and diseases. Surprisingly farmers use Karate to control viral infection. This possibly reflects farmer ability to link viral infection with arthropod pests, which are vectors of the same. Black rot is controlled by Dithane M45. The other surprising result was that weeds were not specifically mentioned as a pest or constraint. Being a labour requiring activity, this finding perhaps reflects availability of affordable labour throughout the cropping season in the peri-urban vegetable production system.

Costs and Returns to Vegetable Farming

6.1 Revenue

Although different methods for assessing farm productivity exist depending on the data available, yield is the most commonly used indicator. In the present study however, physical output was measured by farmers in a variety of units. In addition, the survey respondents could not estimate quantities consumed at home. However, the respondents were able to recall the quantities sold and the price per unit. In the case of kale, farmers reported sales in terms of bunches whereas in the case of cabbage, the produce was sold in bags. Tomatoes were sold in crates of either 60kg or 30kg.

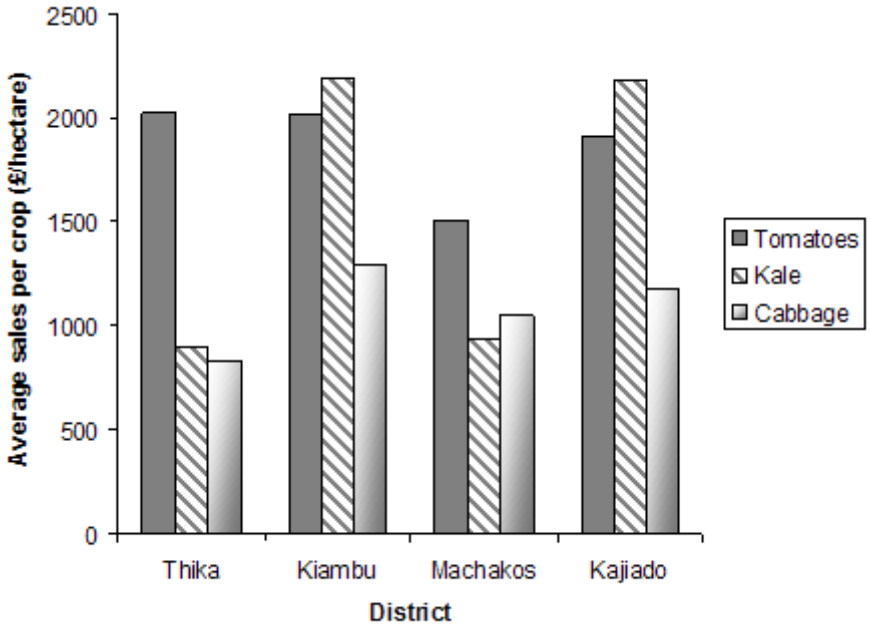
In order to make an assessment of farm productivity and relative competitiveness of each region in the production of a given vegetable crop, the value of sold output per hectare was computed for each farm and summarised by district (Table 6.1). Sales in Kenyan shillings per acre have been converted and are presented in Sterling pounds per hectare.

Table 6.1 Value of marketed produce from last vegetable crop (£/hectare)

Vegetable	District				All districts
	Thika	Kiambu	Machakos	Kajiado	
Tomatoes	2,019 (3,165)	2,015 (2,860)	1,509 (1,756)	1,909 (2,300)	1,874 (2,661)
Kale	903 (1,367)	2,188 (2,673)	938 (865)	2,182 (2,431)	1,515 (2,064)
Cabbage	833 (1,696)	1,294 (2,530)	1,046 (1,974)	1,174 (1,535)	1,077 (2,008)

(standard deviations in brackets)

Figure 6.1 Value of marketed produce from last vegetable crop (£/hectare)



Revenue from kale sales varies between districts with sample farms in Thika and Machakos generating around £1000 per hectare. This value doubles to £2000 in the case of Kiambu and Kajiado farms. Revenue per hectare of tomatoes varies from £1500 in Machakos to £2000 Thika, Kiambu and Kajiado. Compared to kale and tomatoes, revenues per hectare from cabbage are lowest in all districts except Machakos. Noteworthy however, are the large standard deviations indicating variations within districts (Table 6.1).

6.2 Returns to vegetable farming

The value of sold products gives an indication of productivity. However, gross margin (total revenue - total variable cost) provides a clearer picture of the relative profitability of a given enterprise. Gross margins can also be used to assess the relative competitiveness of a given enterprise. Of more relevance to the present study is the proportion of total variable cost attributable to pest control in vegetables given that farmers rank pests and diseases as the main constraint to expanded vegetable production. In order to delineate the variable costs, the whole production process is analysed. Prior to the farm household survey, focus group interviews were conducted in Kiambu and Machakos where farm enterprise budgets were developed for tomatoes and the brassicas, through a participatory budgeting exercise. The production process for each crop was therefore studied in detail and monetary values for activities not captured in the individual household survey were documented. Both the focus group interviews and the individual farm surveys revealed that peri-urban vegetable farmers employ both hired and family labour in most farm operations. Labour requiring activities include nursery preparation and management, seedbed and basin preparation, irrigation, weeding, fertilizer application, spraying, pruning, staking, mulching, shading and harvesting.

6.2.1 Variable inputs and outputs

- The hired labour wage rate is used as the opportunity cost of family labour
- Value of seed at the point of purchase is used to estimate the cost of seed
- Cost of irrigation includes pump, pipe and labour costs during irrigation
- Manure and fertilizer costs are estimated at farm gate
- Foliar feed, cost of chemical pesticides are estimated at point of purchase
- Outputs measured in terms of value of sold outputs

Different scenarios are presented to reflect different production systems and intensity of input use. Kiambu area reflects cool highland regions (LH3 and UM4). Machakos and Kajiado on the other hand lie in the hereafter referred to as “lowland” lie in the hotter and more arid upper midland 6 (UM 6). Accordingly, Kiambu is selected to represent high input scenario for tomatoes while Machakos represents a high input system for kale and cabbage. In this scenario, producers follow all the recommended practices. The variable costs and revenues indicated are those reported during the participatory budgeting exercise.

The “lowland” system represents both Machakos and Kajiado. The costs of crop protection and revenue for each crop are those reported in the individual household survey. The same applies for Mbagathi area in Thika, which is a unique low input zone with minimal irrigation cost.

Variable cost per hectare (C)

$$\sum_{i}^{j}(a..q)$$

summation from i to j farmer

a=seedbed preparation cost
b=cost of seed
c=cost of ploughing
d=cost of basin construction
e=cost of transplanting
f=cost of manure for seedbed preparation
g=cost of manure for field
h=cost of seedbed fertilizer
k=cost of foliar feed
m=cost of seedbed irrigation
n=cost of irrigating seedbed
p=cost of crop protection
q=harvesting, pruning, staking

Gross margins were estimated for high input scenario (Kiambu and Machakos) lowland regions and low input Mbagathi zone (Table 6.2).

6.2.2 Results

From the analysis, it is evident that the cost of crop protection as a proportion of total variable cost ranges from 21-65%. The other important variable cost is that of irrigation (Table 6.2). In the "lowland" areas, irrigation costs include labour and pump hire costs while in parts of Kiambu and Thika, the costs would involve labour costs for fetching water and actual irrigation. In cases where the opportunity cost for family labour =0 and all the returns apportioned to capital and family labour, the gross margins nearly double. Likewise, where the cost of pump hire is reduced to reflect pump ownership and family labour thereby accounting for depreciation and fuel costs only, the gross margins rise appreciably (Table 6.3). From the scenarios presented, high input systems reflects highly optimistic revenue projections compared to the other scenarios. This is not surprising however, given the large standard deviations (Table 6.1). The gross margins are highest for tomatoes irrespective of the District. This would explain the intensity of crop protection practices in the peri-urban system especially cosmetic spraying for quality.

Table 6.2 Gross margins analysis for Vegetable production (£/hectare)

	CABBAGE			KALE			TOMATO		
Activity	*Machakos*	*Lowland areas*	*Mbagathi*	*Machakos*	*Lowland areas*	*Mbagathi*	*Kiambu*	*Lowland areas*	*Mbagathi*
Seedbed preparation cost	1.72	1.72	1.72	1.72	1.72	1.72	4.3	4.3	4.3
Cost of seed	5.8	5.8	5.8	5.8	5.8	5.8	10.31	10.31	10.31
Cost of ploughing	32.22	32.22	32.22	32.22	32.22	32.22	21.48	21.48	21.48
Cost of basin construction	171.83	41.88	41.88	171.83	41.88	41.88	0	0	0
Cost of transplanting	137.46	34.37	34.37	137.46	34.37	34.37	0	0	0
Cost of manure for seedbed preparation	0.43	0.43	0.43	0.43	0.43	0.43	1.07	1.07	1.07
Cost of manure for field	15.03	0	15.03	15.03	0	15.03	0	0	0
Cost of seedbed fertilizer	1.72	1.72	1.72	1.72	1.72	1.72	0.64	0.64	0.64
Cost of foliar feed	40.59	40.6	40.59	40.59	40.6	40.59	0	0	0
Cost of seedbed irrigation	622.87	622.87	214.78	622.87	622.87	214.78	859.13	644.35	214.78
Cost of crop protection	270.63	190	150	270.63	270.63	270.63	1640.94	343.65	343.65
Variable cost	1300.3	971.61	538.54	1300.3	1052.24	659.17	2537.87	1025.8	596.23
Revenue	4000	1755	903	5000	1560	923.57	8591.3	2028	3065
Cost of protection as a % of total variable cost	21	20	28	21	26	41	65	34	58
Gross margin	2699.7	783.39	364.46	3699.7	507.76	264.4	6053.43	1002.2	2468.77

Table 6.3 Changes in gross margin when irrigation costs are reduced

Activity	*Machakos*	*Lowland areas*	*Mbagathi*	*Machakos*	*Lowland areas*	*Mbagathi*	*Kiambu*	*Lowland areas*	*Mbagathi*
Cost of seedbed irrigation	622.87	300	0	622.87	300	0	859.13	300	0
Gross margin	2699.7	1106.26	579.24	3699.7	830.63	479.18	6053.43	1346.55	2683.55

6.3 Modelling Determinants of Cost of Pest Control

During the survey, farmers reported expenditure on pest control for the last crop of kale cabbage and tomatoes. There are wide variations in the reported costs of crop protection. Nonetheless, the reported costs reflect smallholder vegetable farmer expenditure patterns on crop protection. These costs exclude the cost of weeding and other transaction cost elements associated with obtaining crop protection products.

6.3.1 The explanatory variables

Experience in vegetable farming

By and large, experienced vegetable growers should allocate their resources optimally. On the one hand, they may use more costly and effective pest management strategies in order to avoid pest resistance. On the other hand, they may adopt cost cutting strategies as long as they meet product quality dictated by market demand. The expected influence of experience on pest control expenditures is therefore either positive or negative.

Value of marketed output

In order to capture the price effects, value of output per acre is incorporated as an explanatory variable. The higher the value of marketed output, the greater the attention to pest management problems, which translates to higher expenditure given the importance of pests and diseases.

Total farm area

Total farm area refers to the cropped and non-cropped areas. Owners of large land holdings tend to be wealthier and may spend more on pest management practices.

Level of formal education

Previous adoption studies have shown that education level influences adoption of new innovations especially complex technologies. Furthermore, the majority of survey respondents apply chemical control strategies. It is hypothesised that a high level of formal education has similar influence on pest management expenditure to that of experience.

Region of production

Agro-climatic factors are also hypothesised to influence the cost of pest management. Previous studies in the peri-urban vegetable systems indicated that Kajiado and Machakos areas have a high incidence of vegetable pests and diseases.

6.3.2 Estimation

It was hypothesised that expenditure on crop protection is influenced by a number of factors, generally expressed as follows.

COST ƒ (PERIOD, YIELD, TERTIARY, REGION, FARM)

COST= expenditure on crop protection for the last kale, cabbage or tomato crop in Kenya shillings
PERIOD= the period for which the farmer has been growing vegetables in years
YIELD=value of marketed output per acre grown of kale, cabbage or tomato respectively
FARM =Total farm area in acres
TERTIARY= Level of formal education; 1= at least secondary level of education; 0 otherwise
REGION= Production zone; "Lowland"=1; 0 otherwise

The model was estimated for kale, cabbage and tomato using Ordinary Least Squares Regression procedure in the Statistical Package for Social Sciences (SPSS).

The reported cost of crop protection for tomato kale and cabbage for the last crop was taken as the dependent variable. This model explains 21%, 26% and 37% of the variations in expenditure on crop protection on tomato, kale and cabbage respectively. In the case of tomatoes, the "lowland" region (comprising Kajiado and Machakos), at least secondary school level of education, total farm area and period of growing vegetables have a significant positive influence on crop protection expenditure. In the case of kale, only region and education level have a significant positive influence while for cabbage, total farm area, formal education level and region all have a positive and significant effect (Table 6.5).

6.3.4 Discussion

These findings indicate that location of farms in Kajiado and Machakos has a positive influence on crop protection expenditure for kale, cabbage and tomatoes compared to farms in Thika and Kiambu. A possible reason could be higher incidence of pests and diseases in these areas or the nature of pests and diseases that require more costly chemical pesticides to control.

Although farm operators in Machakos have attained higher levels of formal education than the rest of the sample, this effect is not significant when they are combined with farm operators from Kajiado. A test for multicollinearity between education level and region revealed a weak linear association (correlation coefficient of 0.1). Across the sample farms therefore, farm operators educated to secondary school and above spend more on crop protection than their counterparts with lower level of education. These operators are probably more aware of the crop protection needs and therefore employ more costly strategies.

Total farm area can be used as a proxy for wealth status especially in the peri-urban Nairobi. Large farm owners are better endowed with resources and can afford timely purchase of chemical pesticides used by the majority of the peri-urban farmers. This could be more relevant in the case of tomatoes where the proportion of variable cost attributable to pest management is generally higher. In the case of cabbage, a crop that is harvested once as compared to tomatoes and kale that are harvested over a period of time, there would seem to be a minimum area of land below which it is uneconomical to plant cabbage. Table.4.3. indicates that the cropped area for cabbage is on average higher than

that for tomato and kale and kale. The larger the area planted therefore, the greater the cost of crop production.

Table 6.4 Descriptive statistics of factors influencing expenditure on crop protection

	Tomato N=107		Kale N=110		Cabbage N=56	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
COST (Dependent variable)	5742.83	5150.37	1728.87	2779.45	3325.57	4906.22
FARM	3.50	3.14			3.91	3.42
PERIOD	9.14	7.85			9.72	8.11
TERTIARY (Binary variable)	.47	.59	.42	.50	.48	.50
REGION (Binary variable)	.41	.49	.32	.47	.29	.46
YIELD	93059.63	13027563	65559.29	89502.43	53158.22	100071.80

Table 6.5 Regression results of factors influencing expenditure on crop protection

	Tomato Adjusted R^2 = .21 F-statistic=6.72 (p<0.001) N=109			Kale Adjusted R^2 = .26 F-statistic=8.59 (p<0.001) N=112			Cabbage Adjusted R^2 = .37 F-statistic 7.8 (p<0.001) N= 58		
	Coefficient estimate	**t-statistic**	**P value**	**Coefficient estimate**	**t-statistic**	**P value**	**Coefficient estimate** □	**t-statistic**	**P value**
Constant	561.66	.540	.537	-541.92	-.13	.263	-1694	-1.44	.157
PERIOD	109.44	1.95	.054	34.21	1.24	.220	105.74	1.64	.108
YIELD	.01	1.64	.104	0.00	-.23	.770	.01	-1.53	.132
FARM	315.20	2.19	.031	224.56	3.04	.220	279.60	1.18	.076
TERTIARY	2873.14	3.22	.002	1815.53	3.84	.000	4252.62	3.96	.000
REGION	2973.32	3.27	.001	1569.55	3.19	.003	4364.69	3.83	.000

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Annex 1: Questionnaire for vegetable grower characteristics survey July-August 2000

ANNEX 1: QUESTIONNAIRE FOR VEGETABLE GROWER CHARACTERISTICS SURVEY JULY AUGUST 2000

1.Survey code _____ 2. Date of interview _____ 3. I

4. District _____ 5. Location _____ 6.V

7. Distance to all weather road (km)_____ 8. Name of farm owner (FO) _____ 9. C

10. Age of FO _____ 11. Education level of FO_____

12. Name farm manager _____ 13.Gender FM _____ 14.

15. Age FM _____ 16. Relationship FM to owner _____

Wealth status

17. Residential housing type: **if the farm is rented or a plot indicate house at place of residence.**

a. Permanent stone walls with tile / iron roof [ ] b. semi-permanent with iron roof [ ] c. Mud walls with iron roof …. [ ] d. Mud walls wi grass roof [

Assets

18. Do you have a water tank Yes { } No { } 19. Do you have a milk cow Yes { } No { } 20.

Land

21. Total farm size (acres)_____ 22. Total land owned (acres) _____ 23.

24.Are there members of this household on full time salaried employment? Yes[ ] No[ ]

25.Are there members of this household engaged in off farm self employment ?Yes[ ] No[ ]

26. Estimate the average annual income from the following

Source	Less than Ksh.10000	Ksh.10000–20000	Ksh.20000–50000	Ksh.50
Sale of crops				
Sale of livestock				

products				
Off farm salaried employment				
Off farm self employment				

27. Do you get remittances from off-farm activities for investment on farm activities? Yes[ ] No[ ]

28. If yes for which farming operations

Farm operation, activity	**Period of the year**	**Am**

Crops and land use

29. How long have you grown vegetables? __ 30 No. acres under all crops _____ 31. Ac

32. If you do not grow vegetable, have you ever grown vegetables ? Yes [ ]No =-[ ]

33. If Yes, Why did you give up ? _____

34. Do you rent land for vegetables? Yes [ ] No[ ] 35. If Yes area in acres _____ 37. W

38. What are the main vegetable crops you have grown in the last year ?

Vegetable	Month/year planted	Area (acres)	No. plants*	Start harvest End Harvest month	Total output**
Tomato					
Kale					
Cabbage					
Spinach					

*** Cabbages, tomatoes in particular. Not necessary for kale or beans and others**
****specify units e.g. cartons of 3 kg, bunches =250g**

	Tomatoes	Kale
39. Do you sell at farm gate (F) or in market (M)		

40. Distance to market? _____km

41. Journey time to market hours _____ (door

42. What constraints do you have :

a) with vegetable production

b) with marketing?

c) Considering bo constraints

1. 1, 1.
2. 2. 2.
3. 3. 3.
4. 4. 4.

43. Vegetable seed variety and seed source used in the last year

Vegetable	**Seed source**	**Variety**
Tomato	1 2	1 Cal-J 2. RIO
Kale	1 2	1 2
Cabbage	1 2	1 2
Spinach	1 2	1 2

44. Do you grow any of your own seed ? Yes { } No { }

45. If yes, for which vegetable (s) _____

46. Why? _____

Pest and diseases control (show a picture or sample of the pest, or disease)

47 a. What are the main pests you have in Kale cabbage and tomatoes?

b. Which are the main diseases you have in Kale cabbage and tomatoes?

…..c For each crop (cabbage, kale and tomatoes) considering both pests and diseases rank the 3 most important problems

Vegetable	**Pest**	**Rank**	**Disease**
Tomato			
	1.African bollworm		1 Blight
	2.Aphids		2 Bacterial wilt
	3.Leaf miner		3 Fusarium wilt
	4. Nematodes		
Kales			
	1.Diamondback moth		1. Blackrot
	2. Aphids		2. Virus
	3.Looper		3. Blackleg

Cabbage			
	1.Diamondback moth		1. Blackrot
	2. Aphids		2. Virus
	3.Looper		3. Blackleg
Other			
	1		1
	2		2
	3		3

48. Do you follow any vegetable crop rotation? If yes for which crops?_____

49.
Why?_____
_____

50. Did you control for these pests and diseases in Tomatoes in the last year? (1=yes) (0=No)

Enter name of product below

Pest	**Chemical used**	**Botanical used**	**Biological used**	**Cultural pra**
1.African bollworm				
2.Aphids				
3.Leaf miner				
4. Nematodes				

51. By entry: Why do you use the product: 1. Cheapness 2. Most effective 3. Most reliable 4. Health reasons 5. Yield reason

6. Only know this one 7. Only available 8. Other

_____

52. Did you control for these diseases in Tomatoes? (1=yes) (0=No). Enter name of product below

Disease	Chemical used	Botanical used	Biological used	Cultural pra
Blight				
Fusarium wilt				
Bacterial wilt				

53.By entry: Why do you use the product: 1. Cheapness 2. Most effective 3. Most reliable 4. Health reasons 5. Yield reason 6. Only know this one 7. Only available 8. Other

_____

54. Did you control for these pests in Kale? (1=yes) (0=No) . Enter name of product below

Pest	Chemical used	Botanical used	Biological used	Cultural pra
Diamondback moth				
Aphids				
Looper				

55.By entry: Why do you use the product: 1. Cheapness 2. Most effective 3. Most reliable 4. Health reasons 5. Yield reason 6. Only know this one 7. Only available 8. Other

_____

56. Did you control for these diseases in kale? (1=yes) (0=No) . Enter name of product below

Disease	Chemical used	Botanical used	Biological used	Cultural pra
Blackrot				
Virus				
Blackleg				
Powdery mildew				

57.By entry: Why do you use the product: 1. Cheapness 2. Most effective 3. Most reliable 4. Health reasons 5. Yield reason 6. Only know this one 7. Only available 8. Other

58. Did you control for these pests in cabbage? (1=yes) (0=No). Enter name of product below

Pest	Chemical used	Botanical used	Biological used	Cultural pra
Diamondback moth				
Aphids				
Looper				

59. By entry: Why do you use the product: 1. Cheapness 2. Most effective 3. Most reliable 4. Health reasons 5. Yield reason 6. Only know this one 7. Only available 8. Other

60. Did you control for these diseases in cabbages? (1=yes) (0=No). Enter name of product below

Disease	Chemical used	Botanical used	Biological used	Cultural pra
Blackrot				
Virus				

Blackleg				
Downy mildew				

61.By entry: Why do you use the product: 1. Cheapness 2. Most effective 3. Most reliable 4. Health reasons 5. Yield reason 6. Only know this one 7. Only available 8. Other

_____

62. Have you changed any of the following practices in the last 3 years ? If yes why?

Practice	No / Yes	If yes, which ?	Why? Give rea
Stopped growing a particular crop			
Stopped using any product			
Stopped a rotation			
Stopped other cultural method			
Started growing a particular crop			
Started using any different product			
Started a different rotation			
Started using a new cultural method			

	Tomato	Kale	C
63. Do you know how much you spent on each crop on crop protection last crop ?* Enter Ksh			

* If producers do not know these then record not known

64. Do you own a knapsack sprayer Yes { } No{ } 65 If No, how do you apply chemicals or botanicals __

66. Do you irrigate Yes{ }No {….}

67. If Yes, how did you irrigate?

1. From river, pumped, overhead irrigation	
2. From river, pumped, hose / basin irrigation	
3. From river, channels and gravity	
4. From own well, by hand	
5. From communal well, by hand	
6. From river, by hand	

7. Other	

68. What is the distance to your source of water ? _____ meters 69. Do you own a pump Yes=1 No

71. Did you use hired labour in the last year for tomato, kale or cabbage?

	Tomato	Kale	Cabbage
Land preparation			
Transplanting			
Watering			
Crop spraying			
Weeding			
Picking			
Other			

72. If you used hired labour which months (if any) is it difficult to obtain sufficient labour

_____

73. Which months do you need most labour?

74. How many people from your household worked on the vegetable plot in the last year?

75. Which months is it most difficult to find money for chemicals (or other inputs)?

76. Why is this? a. No cash available [ ] b. More chemicals / inputs required [ ] c. Other (specify)_____

77. Do you improve your soil fertility ? Yes { } No{. } 78. If yes indicate the method

name the fertilizer or foliar feed

	Tomatoes	**Kale**	**Cabbage**
Manure	1 2		
Fertiliser	1 2		
Foliar feed	1 2		
Other : e.g. organic means , specify			

FIELD TRIAL REPORT MARK PARNELL DRAFT

Field trials of *Plutella xylostella* granulovirus against diamondback moth on kale, carried out in Kenya 2000 and 2001.

Summary

Brassica crops are important foods source for the urban populations in Kenya and their production is a major income generator for many small scale growers around towns. The development of insecticide resistance by the key pest, diamond back moth (DBM), currently threatens the sustainability of production thus new and improved technologies are required. *Plutella xylostella* granulovirus (PlxyGV) has shown promise for DBM control in other countries and in past field trials in Kenya and may be an important alternative to chemical control in the future.

Field trials were carried out from February to August 2000 (long rains) and September 2000 to January 2001 (short rains) in order to ascertain the lowest dose of PlxyGV required to control DBM on small plots of kale established on research station ground at two sites in and around Nairobi, Kenya. Results showed that PlxyGV performed significantly better than chemical control in reducing DBM populations and increasing kale yield over a growing season. Up to 30% increase in yield was observed in PlxyGV plots when compared to the control or chemical over the growing seasons, a very encouraging initial result for an un-formulated, crude extract. The results have also shown that to provide adequate control of DBM populations a dose rate of $3.0x10^{14}$ occlusion bodies per hectare would be required. Such a dose-rate is equivalent to 7500 larvae per hectare.

Introduction

Project R7449 evaluation and promotion of biorational control of *Plutella xylostella*, the diamond back moth (DBM), is a three year follow-on project from project R6615 that was set up to develop alternatives to chemical control of DBM in Kenya. The specific target group of the project is smallholder Kenyan farmers growing vegetables belonging to the *Brassica* family, the preferred diet of DBM. The project is being carried out in collaboration with the Kenyan Agricultural Research Institute (KARI), CAB International Africa Regional Centre (CABI-ARC) and Horticulture Research International (HRI) based in the UK. This report specifically concerns trials to develop the use of *Plutella xylostella* granulovirus (PlxyGV) against DBM on kale and discusses work carried out during two growing seasons, the long rains season of February to August 2000 and the short rains season of September 2000 to January 2001.

PlxyGV can be applied to crops in much the same was as conventional insecticides using existing spray application technology of the small or large-scale farmer. To this end it has great advantages for adoption by farming communities. Its mode of action is by stomach poisoning and successful infection requires the high pH environment of its host's midgut in order to release virus particles from protective occlusion bodies. PlxyGV is host specific and has shown no effect on organisms other than DBM.

There are no commercially available PlxyGV-based products. Therefore the material used in the trials was produced in country by CABI-ARC staff in laboratories at their regional centre in the ICRAF Complex, Nairobi. The virus produced had been sent to the UK and bioassayed at NRI to check its efficacy in lab culture insects using a leaf-dip bioassay method.

Studies carried out in 1998 under the previous phase of the project to identify alternatives to chemical control of DBM (R6615) had shown that PlxyGV was significantly better than chemical pesticide (Karate) in controlling pest populations and damage level in kale (Parnell 1999). In that study PlxyGV had been applied at dose rates of $3.0x10^{13}$ and $3.0x10^{14}$ OB/ha. Although significant differences occurred between PlxyGV, control and chemical, no significant differences were observed in damage or DBM population between the two dose rates of PlxyGV. The purpose of the current study was therefore to identify the lowest dose possible to achieve adequate control and also to obtain data on the effect of treatments on harvest levels throughout a growing season of kale.

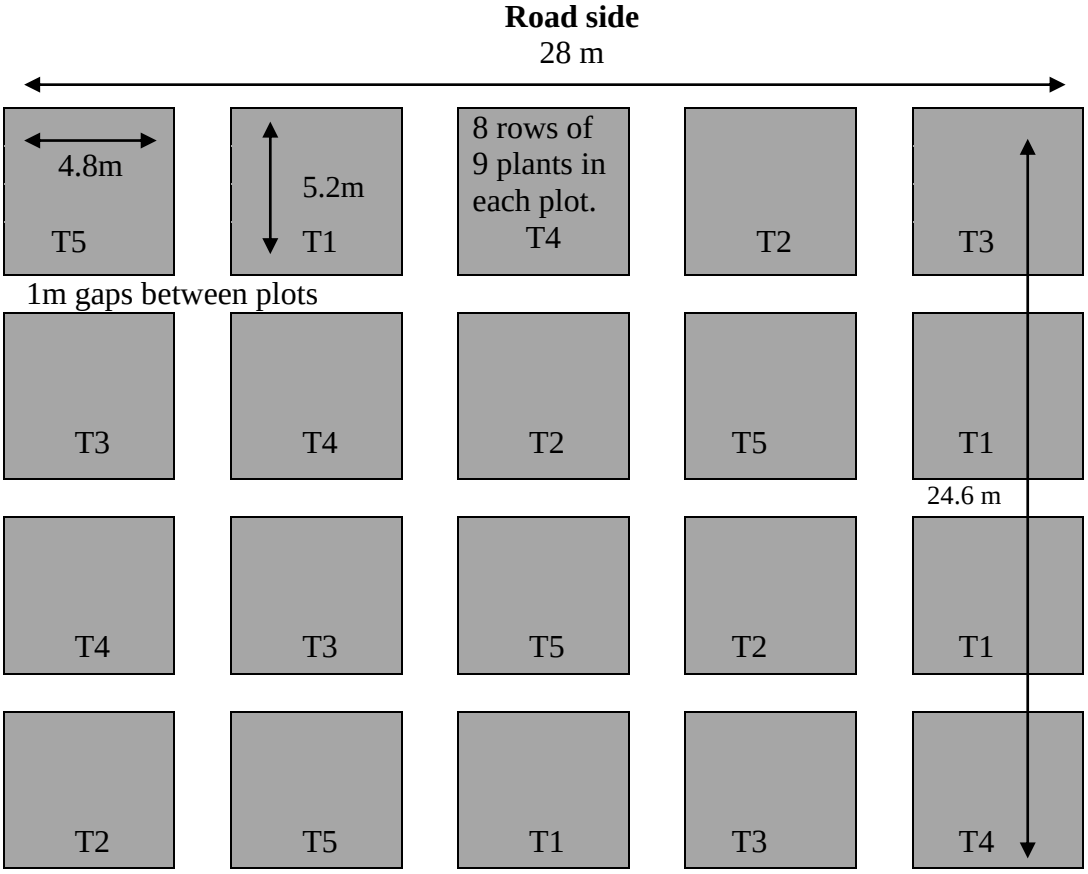
Materials and Methods

The PlxyGV dose rate trials consisting of five treatments replicated four times were set-up at two separate locations in and around Nairobi in two different growing seasons. At both times one trial site was established at the National Agricultural Research Laboratories (NARL) in Nairobi and the other was on research land owned by KARI-Thika 60 km north of Nairobi just outside Thika Town. The test crop for the trial was kale at both sites and treatments were allocated in a complete random block design. The following treatments were included in the trial

- T1: 3×10^{12} of GV (in 0.02% Triton X100)
- T2: 3×10^{13} of GV (in 0.02% Triton X100)
- T3: 3×10^{14} of GV (in 0.02% Triton X100)
- T4: Karate
- T5: Control (0.02% Triton X100)

All were applied using lever operated knapsack sprayers at a volume application rate of 1000 litres/hectare. Figure 1 shows the site design for both field sites.

Figure 1. Field site design for field trials in Kenya, February to August 2000.



Each treatment was applied following good spraying practice guidelines set out by IPARC (Matthews, 1992). Before spraying commenced, all equipment was calibrated to ensure accurate application of NPV and spray procedures followed standard practice. Volume application rate of all treatments was 2 litres per plot.

Monitoring of the trial was carried out in the following way

- 10 plants in the central area of the plot (guard rows at edges) were sampled weekly for numbers of DBM larvae present, damage caused by DBM and number of GV infected DBM.
- At sampling, a number of apparently infected dead larvae were collected for later confirmation that they were GV killed.
- Yield data was collected for weight of harvest and the percent marketable at regular intervals and a clear note was made of the days harvesting was done in order to account for sudden drops in DBM population due to removal.

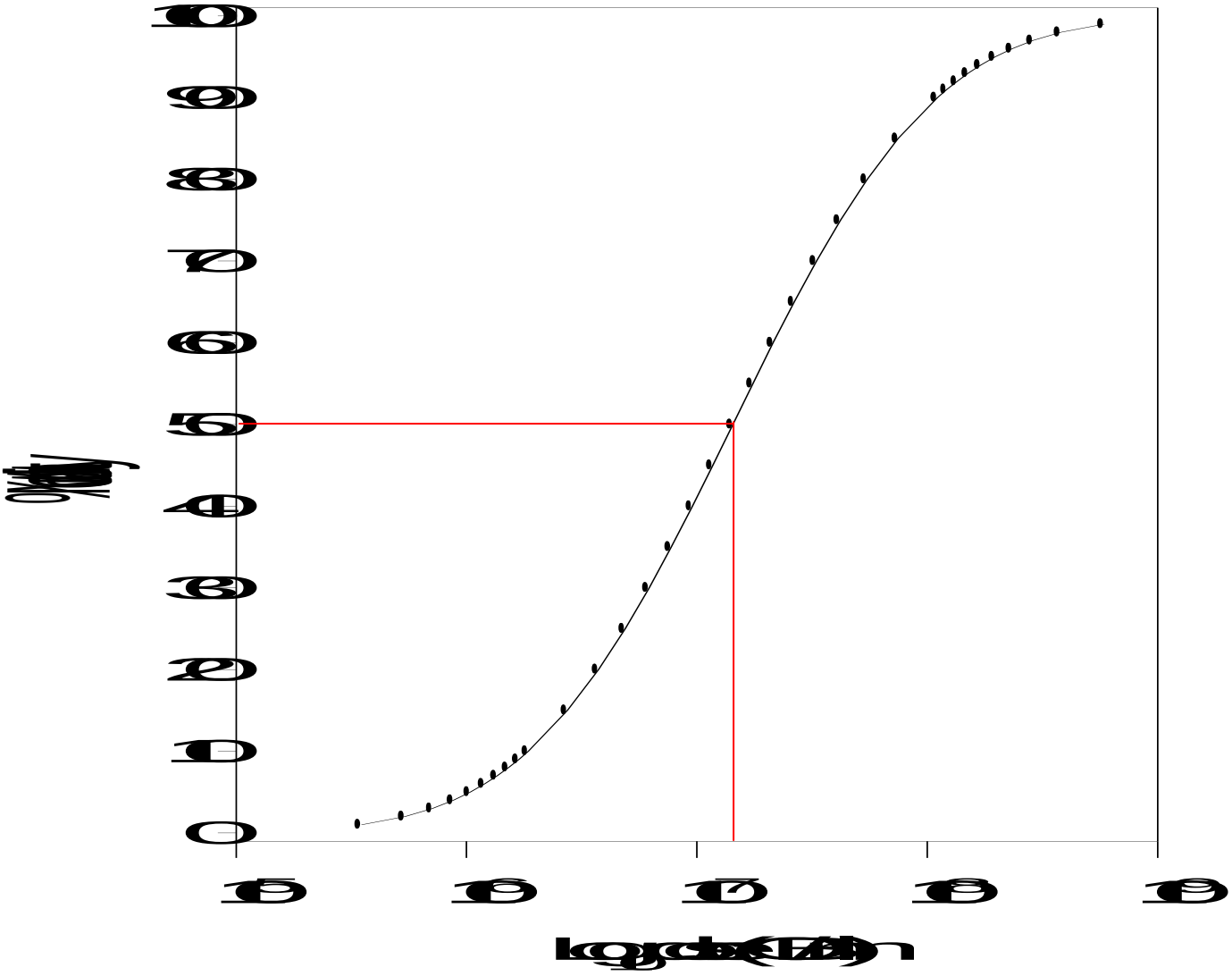
In an independent experiment to compare observed infection in the field to final infection of observed larvae, one plot of kale 5m x 5m was sprayed with the medium dose virus. After four days, between 30 and 50 larvae of 1st 2nd, 3rd, and 4th instar were collected from the plants and taken to the lab for rearing on to observe level of infection. A record was made of the number of collected larvae showing virus symptoms at collection and subsequently GV infection was recorded 5, 7 and 9 days post collection.

Results

Field Inoculum

The LC_{50} value of the field inoculum produced by CABI-ARC staff was $1.90x10^7$ OB/ml. See Figure 2.

Figure 2. Dose response curve of second instar DBM to PlxyGV produced at CABI-ARC for the year 2000 field trials.

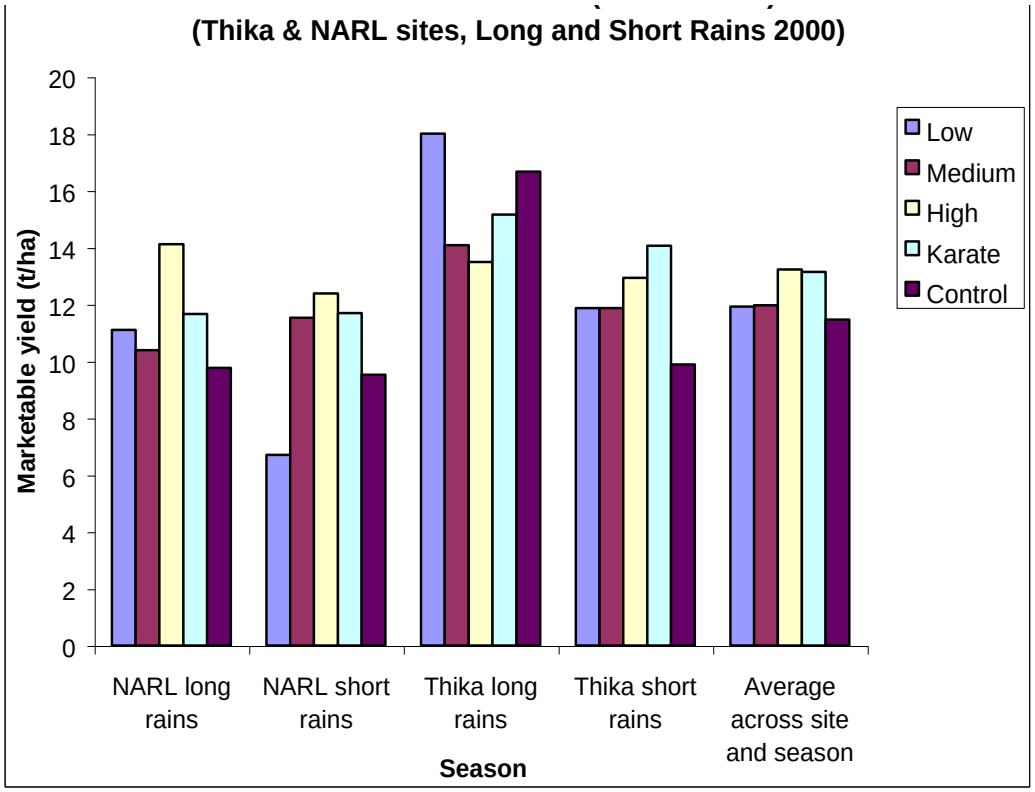


DBM population

The field trials were conducted over a period of weeks during two different growing seasons. From February 2000 to the end of August 2000 covered the long rains and from September 2000 to January 2001 covered the short rains. Weekly pest populations were recorded and eight harvests gathered. In both seasons, the results showed there to be a large difference in pest populations between the two sites ranging from 18.5 to 53.5 thousand DBM/hectare at Thika to 45 to 95 thousand DBM/hectare at NARL (Figure 3). Average DBM populations across treatments for Thika was 26 thousand per hectare while for the NARL site it was 66 thousand.

Figure 3. Average DBM population per hectare at the NARL and Thika field sites from both seasons of the trials.

Control
9.76
9.52
16.67
9.88
11.46

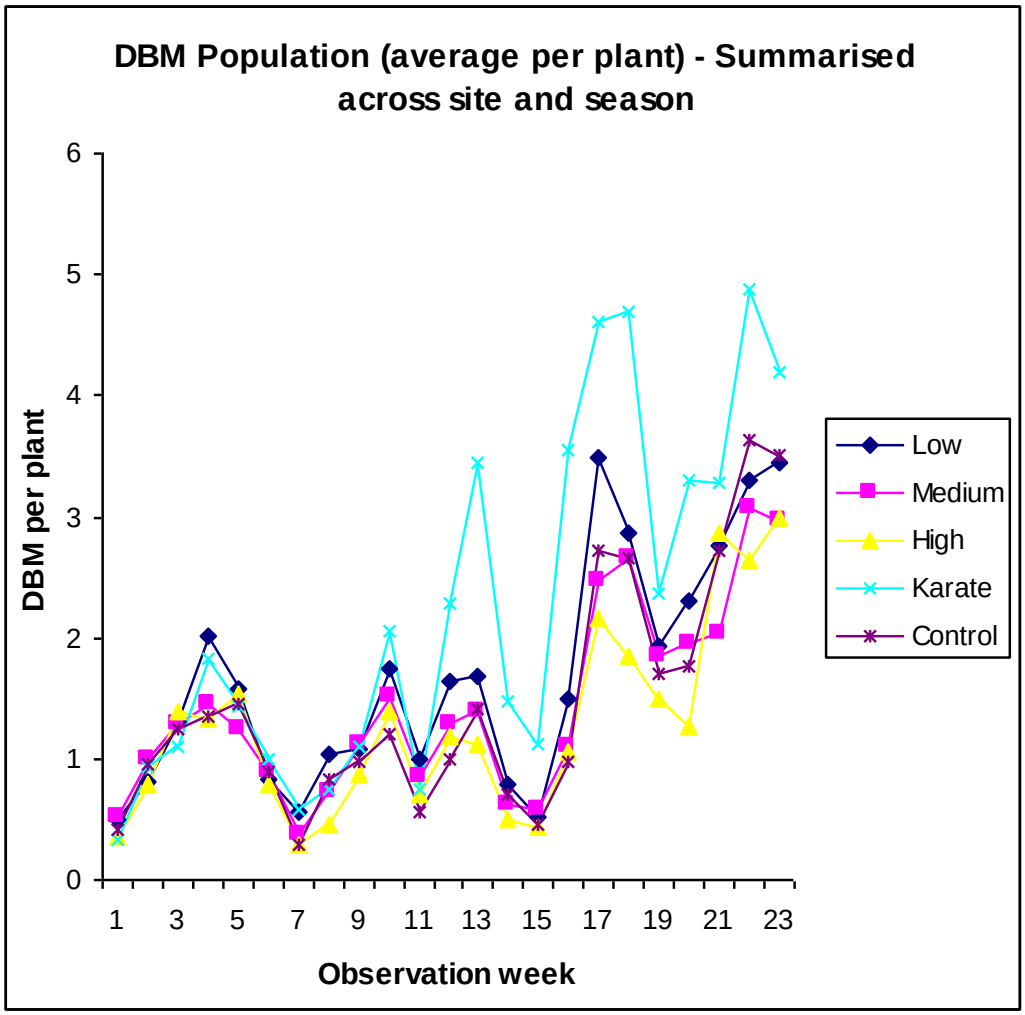


Where the average population per plant exceeded one DBM, a definite dose response was apparent in DBM numbers from low to high dose PlxyGV. The most striking result is that Karate caused high population increases in plots sprayed at both sites and both years. The Karate treatment had significantly higher populations of DBM than any other treatment at all sites, P= <0.0001 (df= 4 and 92, F= 11.1). This provides strong additional support to the suspicion that Karate is no longer effective in controlling DBM on kale. DBM populations of the control, low, medium and high-dose were not significantly different to each other either at NARL or at Thika and it is suspected that the reason for this was due to the generally low DBM population prevailing throughout the experimental period.

The DBM population fluctuated in all treatments throughout the trials. The only clear trend in DBM population between treatments was that from Week 9 there was a

consistently higher DBM population in the Karate treated plots compared to any other. This trend was apparent at all site in both seasons of the trial.

Figure 4. Number of DBM per week for each treatment averaged across both growing seasons.

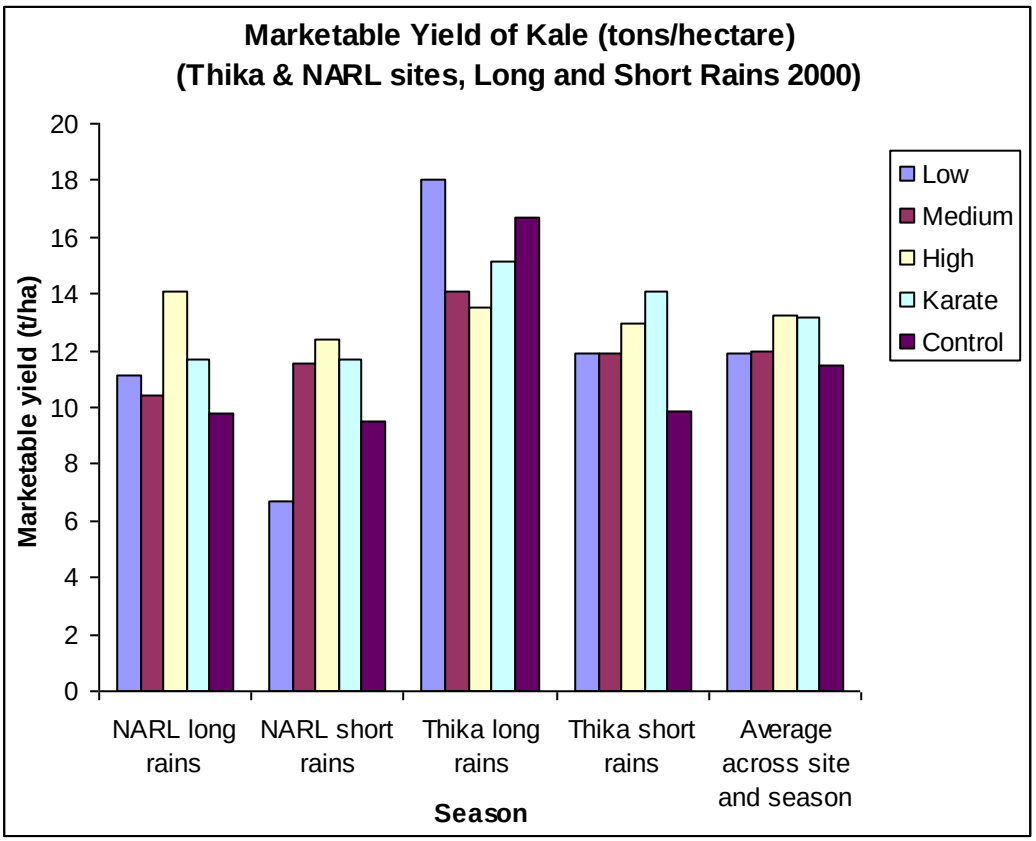


Yield data

Yield at the two sites differed in accordance with the difference in pest pressure with the NARL site generally producing less marketable kale-yield than the Thika site (Figure 5). The reduction in yield varied from 7 to 42% with pest-increases in Karate treated plots giving rise to larger reductions in yield than those in high-dose PlxyGV treated plots. For an equivalent increase in pest pressure, Karate treated plots produced a three times greater reduction in yield than did the PlxyGV ones.

Two way analysis of variance of the yield data from both seasons across both sites revealed the high dose provided significantly higher yields than the control or the low dose (P= 0.0019, df = 4, F = 4.49) but not the Karate or medium dose. It also showed Karate did not provide significantly higher yields than the untreated control.

Figure 5. Average kale-yield per hectare at the NARL and Thika field sites from February to August 2000.



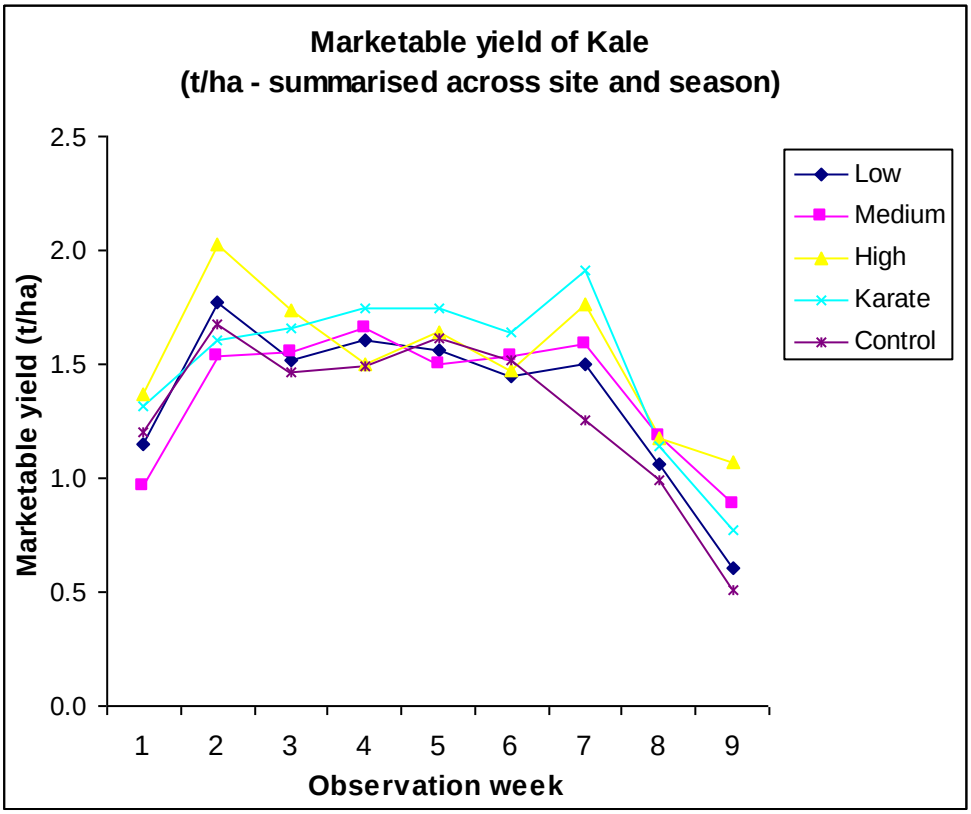
During the long rains, at the NARL site, where pest pressure was highest, the high-dose PlxyGV treatment produced a significantly higher kale-yield than any other treatment, P=<0.001 (df = 4 and 28, F = 6.25). There were no significant differences between any of the other treatments at that site though. The increase in yield of the high-dose over the others ranged from 17 to 30%. In the short rains period at NARL the low dose treatment provided a significantly lower yield than the high & medium doses and the karate (P= 0.005, df =4, F = 3.89) but no other significance's were observed.

At the Thika site, during the long rains the low-dose treatment produced a significantly higher yield than the high and medium-dose treatments (P= <0.0001, df = 4 and 28, F= 5.02) but results were not significantly different to the Karate or control. There were no other significant differences between any of the treatments.

During the shorts rains period there were no significant differences in yield between any of the treatments at all.

An overview of harvest over time using the average yield per hectare from all seasons and sites showed that the high dose-treatment produced higher yields in the initial weeks of the trial but from the fourth harvest to the eighth, Karate produced higher yields. From Figure 5 it can be seen that when averaged over the entire trial, neither treatment provided significantly higher yields to the other.

Figure 6. Average marketable yield from each treatment on each of the eight harvests.



Observed infection rate

The mean infection rate of DBM populations overall ranged from 22.5% in the low dose PlxyGV to 41.0% in the high dose PlxyGV. Maximum infection rate reached 100% and once infection had become established (by the third week) PlxyGV symptoms were observed in every assessment. Figure 7 below shows the infection rate over time for each of the treatments as an average taken from both seasons and sites. It can be seen that as PlxyGV dose rate increased, so did the observed GV infection rate (Figure 8). Analysis of data from the whole trial shows there were significantly higher levels of infection in the medium and high dose than there were in the low dose, (P= <0.001, df = 4, F = 94.9), but the difference in infection levels between the medium and high-dose was not significant. The analysis also showed levels of infection in the control and Karate were significantly lower than in all PlxyGV treatments. Figure 8 shows that this trend was apparent at all levels of the trial with dada from all sites and seasons showing the increase in infection level as dose increased.

Figure 7. Average observed infection rate in DBM larvae over time.

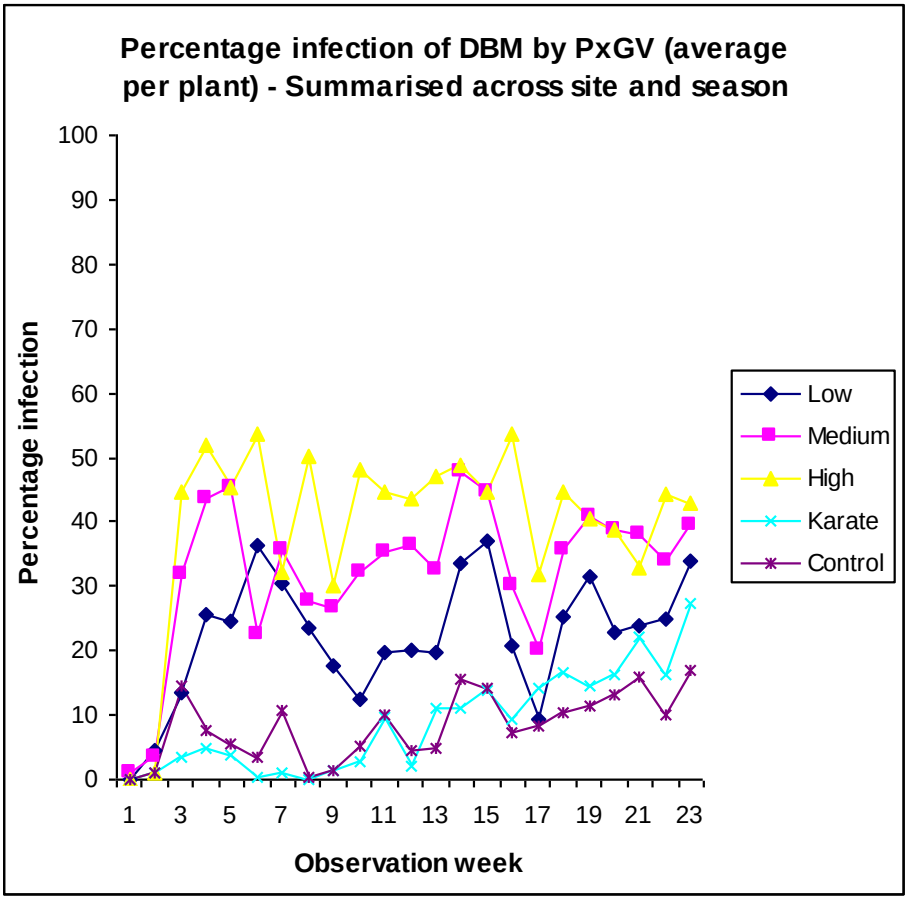
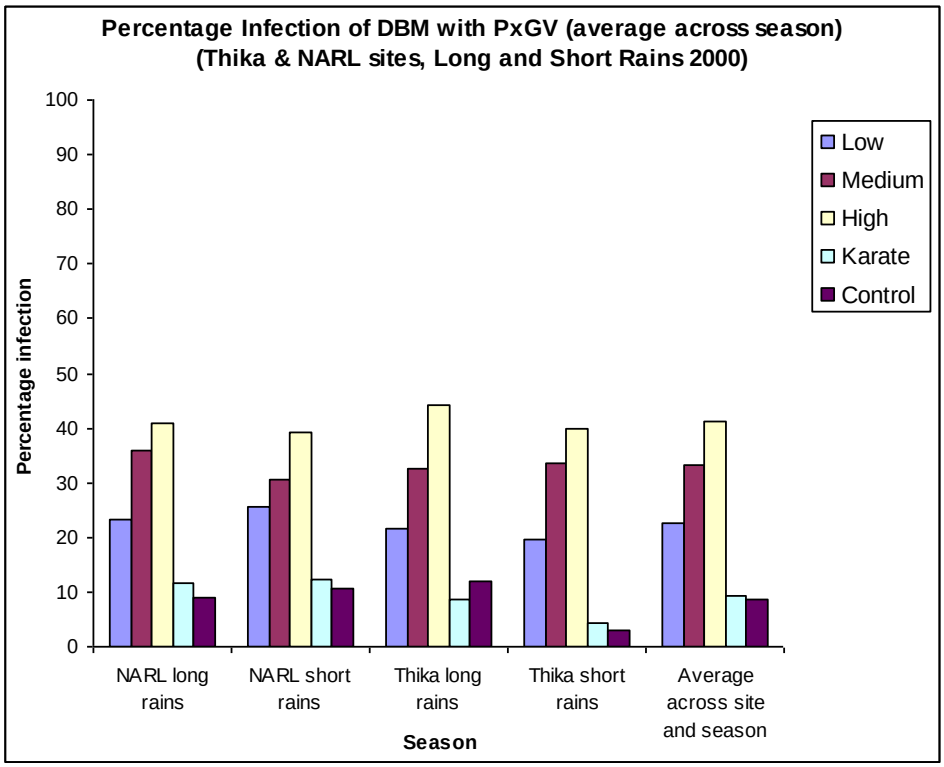
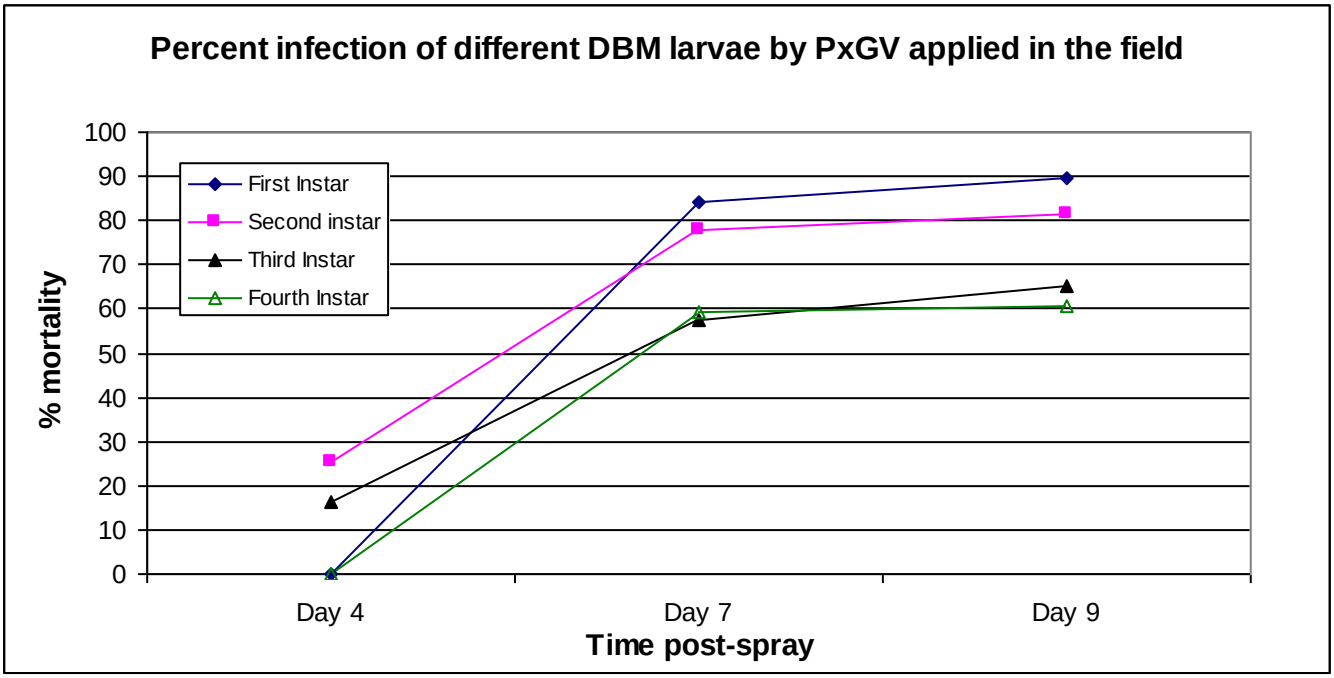


Figure 8. Overall average of % infection for each treatment.



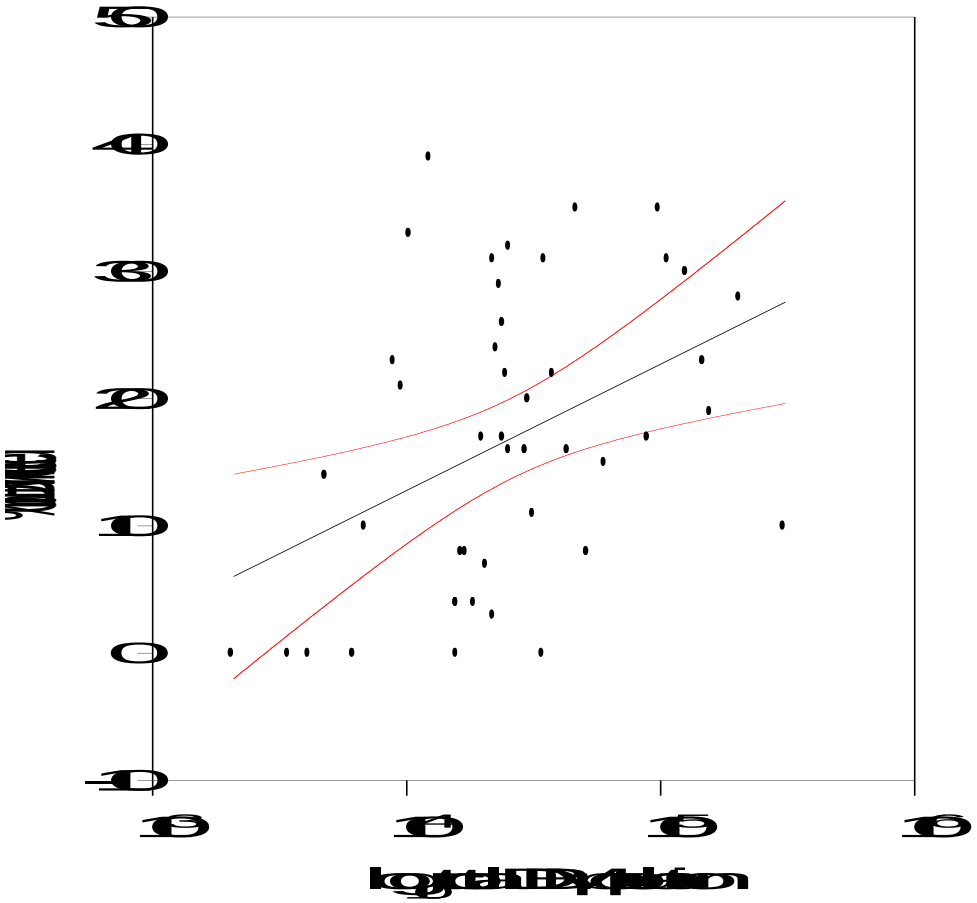
The independent experiment to compare levels of observed GV infection in he field at any particular time to the final GV infection in observed larvae showed that larvae from all instars collected succumbed to GV infection. In 4th instars the level of infection reached a maximum of 60.6% infection (death) whereas in the 1st and 2nd instars, infection levels reached up to 90% (figure 9).

Figure 9. Infection levels in DBM larvae reared in the lab after being collected from a virus-sprayed plot.



The infection rate in DBM larvae only appears to be density dependent for low dose application. In this treatment, infection rate generally increased as the population density grew (Figure 10), r^2 =0.165.

Figure 10. Density dependence of PlxyGV infection rate in low-dose treatments of Kenyan field trials, February-August 2000.



In the medium and high-dose treatments, infection rate was not population density dependant (Figures 11 and 12).

Figure 11. Density dependence of PlxyGV infection rate in medium-dose treatments of Kenyan field trials, February-August 2000.

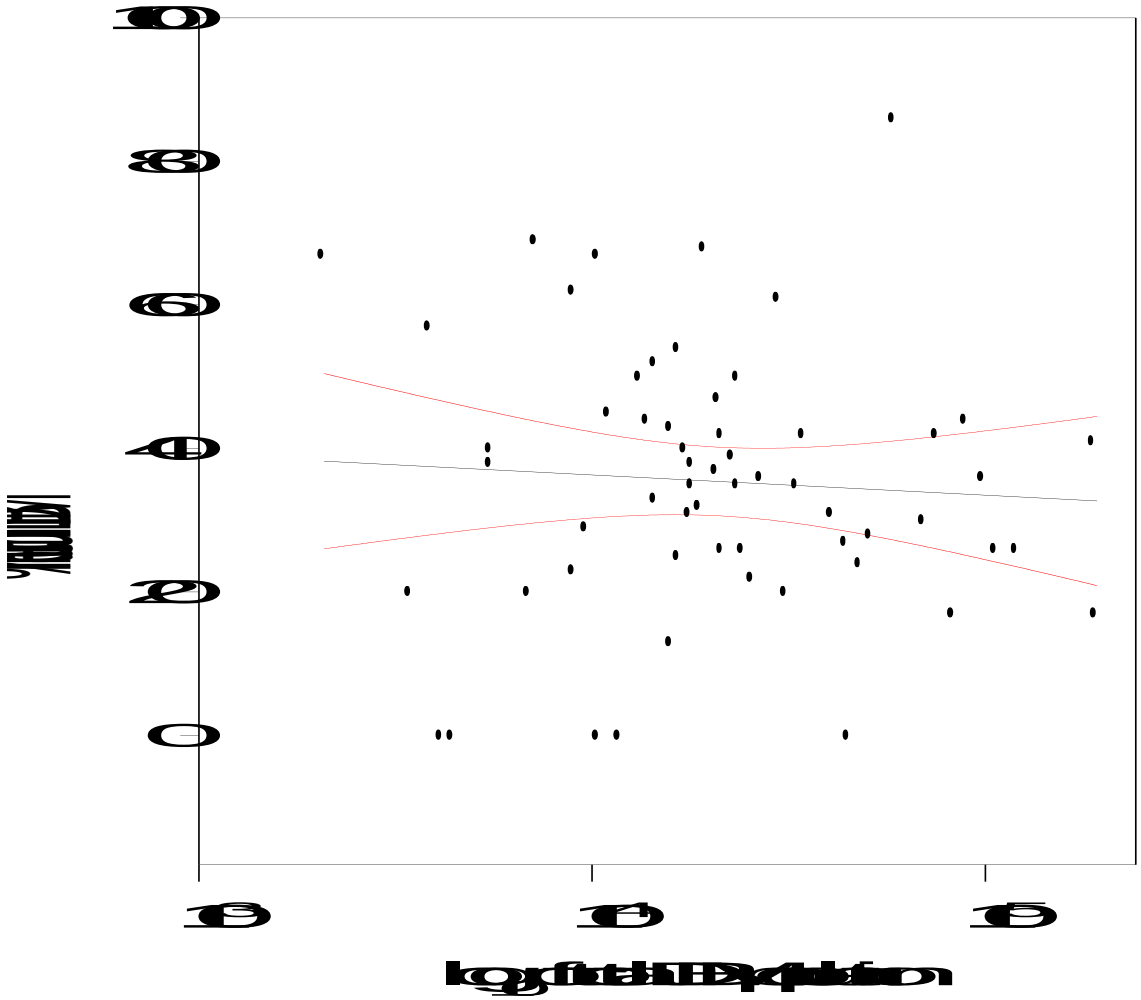
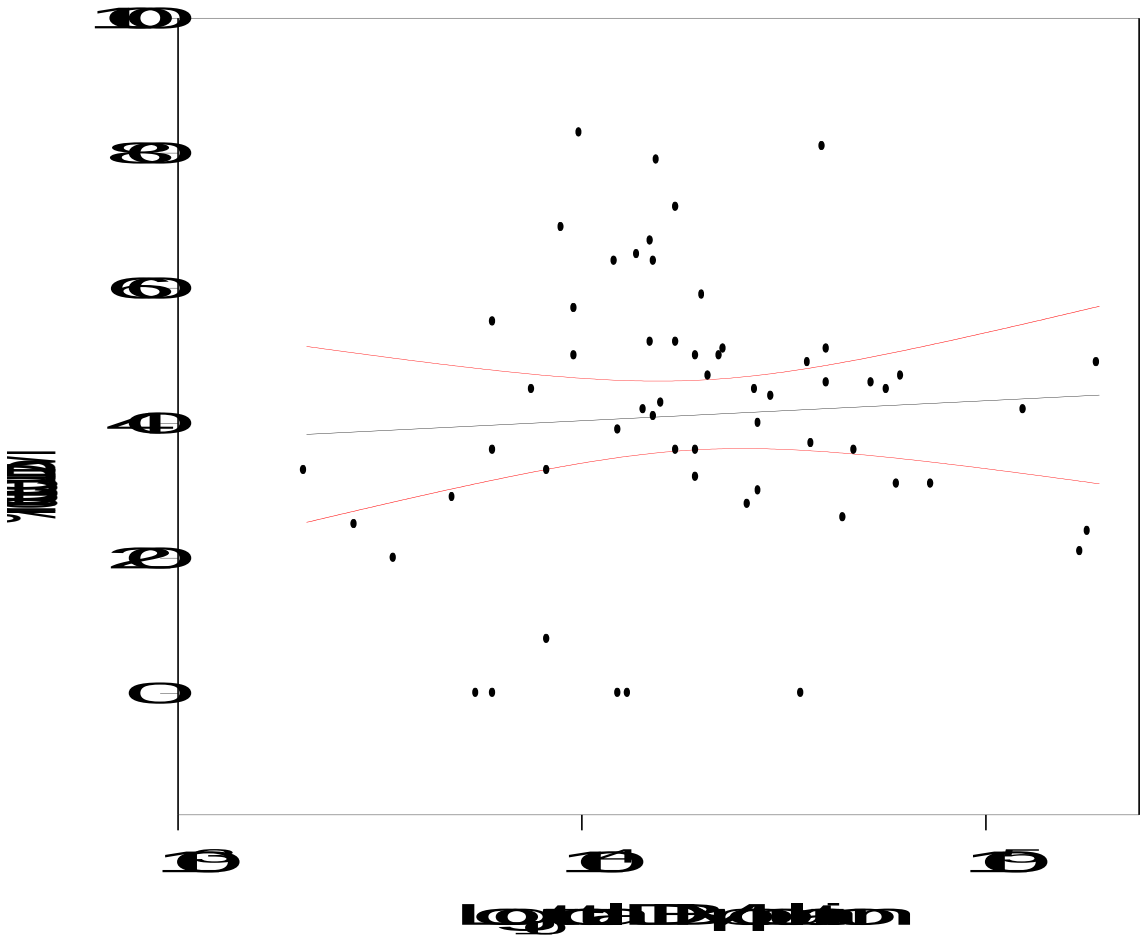


Figure 12. Density dependence of PlxyGV infection rate in high-dose treatments of Kenyan field trials, February-August 2000.



Discussion

Potency of PlxyGV field inoculum

The LC_{50} value of the field inoculum used in the current trial was not significantly different to that of the inoculum used in the previous trial of 1998. Kenyan-based local staff produced the batch used in the 2000 trial having previously received training and the batch produced in 1998 was done under the supervision of an NRI Insect Pathology expert. The inoculum of the previous trial had an LC_{50} value of 7.2 $x10^6$ OB/ml (Parnell, 1999) and that used here was 1.90 $x10^7$ OB/ml. Such differences are within the normal variation seen in bioassays of baculovirus when performed using the leaf-dip method and are often observed between replicates of the same sample. Therefore the 2.6 fold difference in potency between two different batches (samples) of PlxyGV is perfectly acceptable and is a good indication that the PlxyGV production run was of high quality.

DBM Population

DBM populations were generally low throughout the season at both sites. This was most marked at Thika where, on average, pest pressure was less than 1 larva per plant. The highest population was recorded at only 2 larvae per plant and in many cases (especially early on in the trial) no larvae at all were recorded. At NARL where pest pressure was highest there were no more than 3 larvae per plant on average with a maximum average population of 3.5 larvae per plant. Kenyan experts from KARI and CABI-ARC expressed great surprise at such low infestation levels suggesting the unusual rainfall patterns of two previous years may have been the cause.

The results obtained from the Karate treatment provide additional support to existing evidence that this product causes significant increases in DBM populations on kale in Kenya. The level of evidence is such that the Kenyan authorities are advising local farmers against the use of Karate for DBM control and in farm visits made, it is apparent that local farmers have begun to stop applying the current formulation of Karate for DBM control already. As a trial average the Karate treatment caused increases in DBM population of between 25 and 42%. All other treatments recorded average populations per plant that were within 18% of each other indicating that the known effect Karate has on reducing natural enemies may have been the cause of the increase in those plots.

At the NARL site, difference in DBM population between treatments was more pronounced than at Thika although the only significantly higher population was still only observed in the Karate treatment. However a treatment effect was observed in differences in DBM population between the control and PlxyGV doses and between the different PlxyGV doses. The effect showed that even in exceptionally low populations an increase in dose reduced the average DBM population. The only occasion when this was not shown was in the long rains at Thika where, in general, average DBM population per plant was less than 1 larva.

None of the PlxyGV treatments caused significantly lower DBM populations than the control. It is likely that population levels were so low that a significant treatment effect (other than for Karate) was undetectable given the size of plots used and the

number of replicates included in the trial. On many occasions, zero larvae were recorded on many plants sampled in all treatments therefore at such low and relatively variable pest pressure far larger plots or many more replicates were required to generate enough data to show up significant trends.

Evidence for this lies in a previous trial carried out in 1998 under the earlier project R6615. PlxyGV doses of 3.0 $x10^{13}$ and 3.0 x 10^{14} both resulted in DBM populations that were significantly lower than in the control or Karate treated plots (Parnell 1999). However, the average DBM populations at field sites during the 1998 trial were no greater than those seen at the NARL site in the present one but general level of infestation in 1998 was more consistent throughout the trial period. In the current study one or two weeks of high pest populations inflated the overall average of the NARL site that would otherwise have shown similar pest pressure to that of Thika.

Yield data

At the NARL site in the long rains, the high-dose PlxyGV treatment provided significantly higher yield than any other treatment but there were no significant differences between the control, chemical, low or medium-dose treatments. This result does not reflect that of the DBM population data. That data showed the karate treatment to have significantly higher DBM populations than any other treatment but showed no significant differences elsewhere. In that case, one might have expected the yield of the Karate to be lower than the other treatments and all other treatments to be equal, which is not the case here. However, the population data does show that the high-dose treatment did have the lowest number of DBM of all treatments and it may be that although the difference wasn't large enough to show up as significant in that analysis, it was large enough to produce a significant effect on harvest. If data on larval instar had been collected, it would possibly have shown that DBM present in the plots were of differently proportioned across instar, with certain treatment having more or less large instars than others. The early instars cause an insignificant level of damage compared to the level caused by late instars therefore the difference in yield could have been explained. In future experiments, data recording will be done on an instar dependent level.

At the Thika site during the long rains, the low-dose PlxyGV treatment produced a significantly higher kale yield than the medium and high-doses but there were no other significant differences observed between any other treatments. The population data from this site showed DBM levels in all but the Karate treatment to be virtually identical and less than one larva per plant on average at that time. At such low pest pressure, the level of DBM are highly unlikely to have any effect what so ever on yield thus it is suspected the observed differences in yield were probably due to pests other than DBM. During the short rains there where no significant differences at all were observed, the pest pressure was still below 2 larvae per plant. It is highly suspected that these pest pressures simply aren't high enough to incur levels of DBM damage significant enough to effect yield therefore any treatment at all was irrelevant.

Observed infection rate

In the initial stages of the trial it became apparent that a maximum of no more than 42% infection was being observed in the field. From laboratory experiments we knew

this was a very low infection rate so we conducted a small trial to ascertain if observed infection rate on a single day of assessment was not a true indication of final infection levels. The results showed that from initial infection rates of 0 to 28% observed on what would have been a field assessment day, the final virus induced mortality was between 60 and 90% depending on instar. The experiment was replicated three times and a similar pattern emerged from each replicate leading to the conclusion that an observed infection level of 40% on a single day in the field actually represented up to a 90% infection level the generation of larvae present.

These results showed that infection rate in DBM larvae was only density dependant when populations were treated with low-dose PlxyGV. It is the opinion of the researchers that the increased infection rate with increased population was possibly due to secondary cycling of PlxyGV released from larvae originally infected by the applied dose. At medium and high-dose treatment where infection was not shown to be density dependent, it is suspected that GV levels in the environment were such that the effects of secondary infection were negligible, a marked increase in infection rate was therefore not apparent.

Conclusion

From the results of this trial it is possible to say that at the low dose of $3.0x10^{12}$ OB/ha, control of DBM on kale would not be sufficient to make the application effort worthwhile. It can also be said that it's highly likely that Karate is not able to control DBM on kale and probably causes significant increases in population levels.

The pest pressure over all was uncharacteristically low compared to the last decade, which confounded efforts to draw statistically valid conclusions. Where pest pressure was highest the indication is that PlxyGV at the high dose of $3.0x10^{14}$ OB/ha would provide significantly better control than any other treatment tested. However, at the current time such an application rate would be uneconomically viable for DBM control.

The work to follow on from these trials will be to investigate the effect of formulating the PlxyGV to increase efficacy and thus lower the effective dose to an economically viable application rate.

Reference

Matthews, G.A., (Ed) 1992. Pesticide Application Methods, 2nd Edition. Longman Singapore Publishers Ltd., Singapore.

EVALUATION OF A NATURALLY OCCURRING BACULOVIRUS FOR THE MANAGEMENT OF DIAMONDBACK MOTH, *Plutella xylostella* L. IN KENYA[4]

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Introduction

Diamondback moth (DBM) *Plutella xylostella* L. is a major pest of brassica crops in Kenya, causing reduction in produce quality and yield. The most commonly practiced management strategy for this pest in Kenya is the use of chemical insecticides. This use has generated concerns regarding the cost, development of resistance to the most commonly used insecticides, residues on produce and environmental contamination (Michalik, 1994, Kibata, 1997). A project to develop non-chemical methods of DBM control in Kenya has been exploring the use of indigenous pathogens as potential control agents. Surveys in farmers' fields identified a baculovirus *Plutella xylostella* Granulovirus (*Plxy*GV) (Grzywacz *et al.*, 2002). Results from initial field trials indicated that *Plxy*GV was highly infectious to DBM (Parnell, 1999). This paper reports on additional laboratory and field studies conducted at Kabete and Thika, areas of contrasting ecologies close to Nairobi, with a view to incorporating this naturally occurring baculovirus into a broader IPM package for DBM management in Kenya.

[4] Poster to be presented during the International Symposium – Improving Biocontrol of *Plutella xylostella*, Montpellier, France, 21-24 October 2002.

Materials and methods

Persistence of *Plxy*GV on potted cabbage plants

The virus used in the present study was produced using the protocols outlined by Parnell (1999). A standard unpurified suspension of *Plxy*GV at a rate of 3.0 x 10^{13} OB/ ha was used to spray 42 two-month-old plants, after which they were split into 6 groups of 7 plants. Three groups were placed in three different shaded areas and three placed in open areas. Once dry, one plant from each group was collected for immediate bioassay. The bioassay was repeated on plants collected 7 hours, 1 day, 3, 7 and 9 days after spraying. At the time of collection, fifteen 2^{nd} instar DBM larvae were placed on each plant. The larvae were monitored daily until all had either died or developed to adulthood, and those showing virus symptoms or death were recorded on the 4^{th}, 7^{th} and 9^{th} day.

Evaluation of adjuvants to improve the efficacy of *Plxy*GV in the field

The effect of a combination of the most promising adjuvants (molasses and neem) on the efficacy of *Plxy*GV against DBM larvae, assessed in laboratory studies (Grywacz D *et al*., 2002**),** was evaluated in the field at Kabete and Thika. The unformulated *Plxy*GV, formulated *Plxy*GV, Brigade® (Bifenthrin) and an unsprayed control were compared in field plots of kale at two sites. Each treatment was replicated 6 times in a randomized complete block (RCB) design in plots of sizes 4.2 x 3.6 m. A polythene sheet was used to surround each plot during spraying time to act as a barrier against spray drift. Sampling was done weekly on 10 randomly selected plants in each net plot. Numbers of pupae, larvae and *Plxy*GV infected DBM, as well as damage caused by DBM were recorded. Yield data was collected fortnightly by harvesting kale leaves in the net plot. After harvesting the leaves were sorted into two groups, consisting of marketable and unmarketable leaves, (based on grading criterion used by local farmers and also at the urban markets) and the numbers and

weights for each group recorded. These trials were conducted during the 2001 short rains season (October 2001 – January 2002) and the 2002 long rains season (January –April).

Laboratory and field assessments of the effect of mixing *Plxy*GV with Pirimor®

The efficacy against DBM of the standard *Plxy*GV was compared to a mixture of standard *Plxy*GV and Pirimor®, and an unsprayed control in the laboratory. The treatments were prepared by thoroughly mixing the ingredients in a glass beaker. Twenty leaves of kale were washed with moist cotton wool and left to dry. Five dry leaves were placed in each test solution and left in the beaker for 1 hour. After drying, each leaf was placed in a round plastic tub measuring 5cm in diameter and 7cm high and into each tub five 2nd instar DBM larvae were introduced. The larvae were monitored daily until all individuals had either died or developed to adulthood. The number of larvae alive, infected and dead was recorded on 4, 7 and 10 days after exposure. The efficacies of these same treatments were also compared on field populations of DBM. Each treatment was replicated 4 times in a RCB Design, each gross plot measuring 4.2 x 3.6 m. Observations were made as outlined previously.

Effect of using a Conventional or V lance on the efficacy of *Plxy*GV against DBM

The efficacy of *Plxy*GV using a conventional and V lance was investigated in field trial plots at two sites. For comparison a treatments using both lances and a chemical insecticide, fipronil (Regent®), and an untreated control were included. The trial consisted of 5 treatments, *Plxy*GV standard dose and fipronil sprayed using either the conventional or V lance and untreated control. Each treatment was replicated four times in a RCB Design, each gross plot measuring 4.2 x 3.6 m. The trials were conducted over two growing seasons, the 2001 long rains and short rains respectively. Observations were made as outlined previously.

Data were entered and summarised in Microsoft Excel 2000 and then transferred to GenStat (Release 4.2) for analysis. Analysis of Variance was applied to summaries of insect counts and yield (means and totals, respectively). Generalised Linear Modelling assuming a binomial distribution was used to analyse percent distribution.

Results and Discussions

Persistence of *Plxy*GV on potted cabbage plants

The activity of the *Plxy*GV on unshaded plants declines rapidly, by two thirds, within 7 hours of spraying corresponding to daylight time (Fig 1). Plants in the shade also show a decline in efficacy but this is less rapid and the virus retains significant activity even after 5 days (120 hours). The slight rise in activity seen on the unshaded plants between 7 and 24 hours is intriguing but other authors have reported that after exposure to daylight some recovery of activity may be seen in other baculoviruses after overnight darkness (Jones et al 1993). Thus the *Plxy*GV retains significant activity in unshaded plants for up to 72 hours post spraying.

These results indicate that the inactivation of the GV particles is not only due to UV radiation but as well as other abiotic factors e.g. physical loss of particles and chemical inactivation by plant exudates. Jones *et al*., (1993) made similar observations in their studies on the effect of natural sunlight on *Spodoptera littoralis* Nuclear Polyhedrosis virus (*Sl*NPV). They noted that the rate of sunlight inactivation varies with season, this being especially reduced with increasing latitude. Richards and Payne, (1982) noted that the biological half-life of *Pieris brassicae* GV (*Pb*GV) deposits on cabbage leaves in England differed by a factor of more than two between June and October and correlated well with integrated monthly UV flux data. Further studies are required to establish the seasonal variability in the persistence of the virus.

Evaluation of adjuvants to improve the efficacy of *Plxy*GV in the field

It has been shown that the addition of molasses to a GV formulation can increase efficacy of the GV and reduce the damage caused to the plants (Kadir 1990, Ballard *et al*., 2000). Results from the present studies showed that although both formulated and unformulated *Plxy*GV produced higher marketable yields than the unsprayed control only the formulated application showed a significant increase in yield ($p=0.03$) (Fig 2). There was no significant difference on the overall infection level between the formulated (37%) and unformulated crude extract of *Plxy*GV (35%).

Significant differences between treatments were observed in the marketable yield ($p=0.002$), with higher yields recorded in Brigade® plots than in the other treatments at both sites ($p<0.001$). However as Brigade is a broad spectrum insecticide with both aphicidal and acaricidal activity it cannot be determined if this higher yield was due to better DBM control or a reflection of its ability to reduce secondary pest damage.

Laboratory and field assessments of the effect of mixing *Plxy*GV with Pirimor®

The results from laboratory studies showed that an application of a tank mix of crude *Plxy*GV inoculum and Pirimor® (a selective pesticide that is specific to aphids) caused a reduction in efficacy on DBM larvae by the *Plxy*GV (Fig 3). Jacques and Morris (1981) indicated that a tank mix including an insecticide would usually not affect the activity of baculoviruses and few direct actions of insecticide on baculovirus activity have been reported (Durand, 1989). It is suspected that Pirimor®) could be acting as a feeding deterrent to DBM. In the field however there was no significant difference in the level of infectivity whether *Plxy*GV was applied alone or mixed with Pirimor® ($p=0.108$). The overall marketable yields at both sites were comparable, Kabete (11.0 t/ha) and Thika (12.0 t/ ha). There was no effect of either ingredients, *Plxy*GV and Pirimor®, on the yield ($p=0.681$ and $p=0.773$ respectively). Therefore it was concluded that Pirimor® did not affect the efficacy of *Plxy*GV against DBM.

Effect of using a conventional or V lance on the efficacy of *Plxy*GV against DBM

The results have shown that spray application using a V lance gives a small but significantly higher infection of DBM than using conventional lance ($p=0.05$) (Fig 4). These results were observed more clearly at Thika than at Kabete. These results support the earlier report that stated that V-lance proved to give good delivery of insecticides especially to the lower surface of broad-leaved and tall crops such as kale (Kibata, *et al*., 2002). DBM numbers during the both growing seasons were generally low. Fipronil was shown to be the most effective product giving higher yields (32.7-42.3 t/ha) than the other treatments (28.8-33.7 t/ha) ($p<0.001$). However, this might reflect to some extent its broader spectrum activity, rather than its control of DBM. The type of lance did not influence the final yield of kale for either *Plxy*GV or fipronil (Fig 5).

Conclusion

*Plxy*GV was not observed to infect other lepidopteran pests of kale or any beneficials such as syrphid and spiders in the field. This observation and the above results show promising potential for the use of *Plxy*GV in the management of DBM. A number of researchable issues still remain that could improve the efficacy and utility of *Plxy*GV. These include the development of new formulations to improve shelf life, persistence and optimization of infectivity of this baculovirus.

ACKNOWLEDGEMENTS

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Table 1. Percent infection of DBM in *Plxy*GV and *Plxy*GV + Pirimor® treated plots at Kabete and Thika sites over two seasons

	Percent (%) infection (95% confidence interval, shown in brackets)				
	Sites (Summaries of two seasons)			Seasons (Summaries of two sites)	
Treatment	Kabete	Thika	Mean	SR 2001	LR 2002
*Plxy*GV alone	32 (29-35)	33 (30-36)	33 (31-34)	34 (32-37)	29 (26-32)
*Plxy*GV + Pirimor®	36 (33-38)	34 (32-36)	35 (33-37)	36 34-38)	33 (30-36)
Pirimor®	5 (4-7)	2 (1-3)	3 (2-4)	4 (3-6)	1 (0-3)
Unsprayed Control	6 (4-7)	2 (1-3)	3 (2-4)	4 (3-5)	2 (1-3)
Mean	**15 (14-17)**	**9 (8-11)**	**-**	**14 (13-15)**	**9 (7-10)**

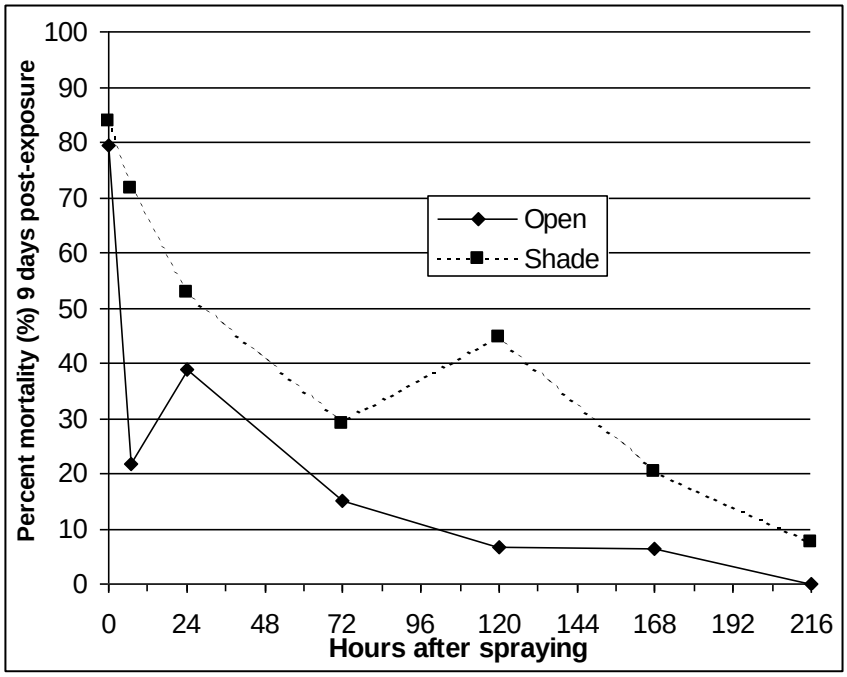

Figure 1. Effect of time on the persistence and efficacy of *Plxy*GV on cabbage plants

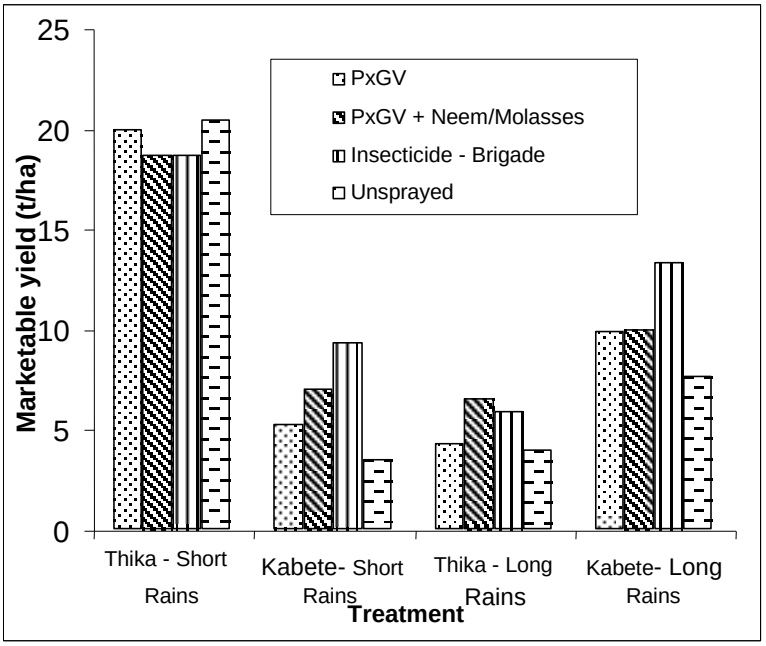

Figure 2. Marketable yield of kale during the two seasons at Kabete and Thika for the trial investigating the effect of adjuvants on the efficacy of *Plxy*GV

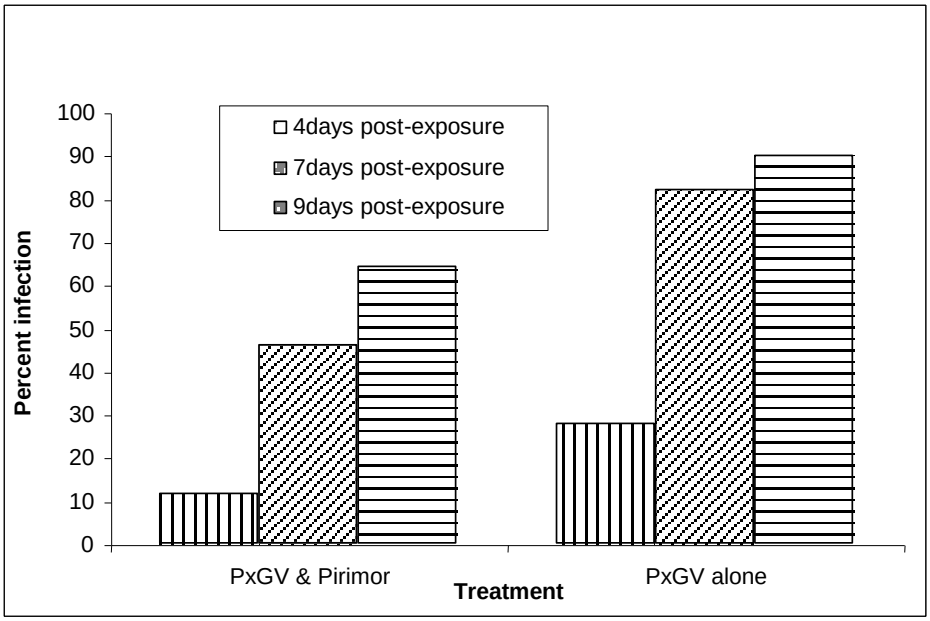

Figure 3. Effect of mixing *Plxy*GV with Pirimor® in the laboratory

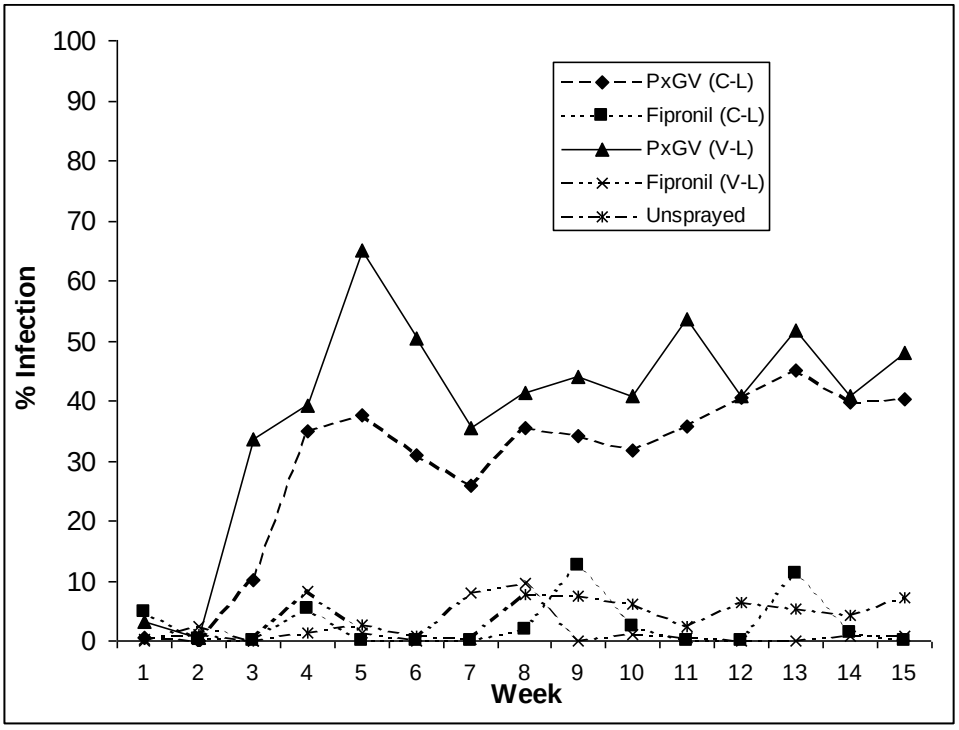

Figure 4. Overall percent infection level of DBM in treated plots using V-lance and conventional lance at the two sites over the two seasons

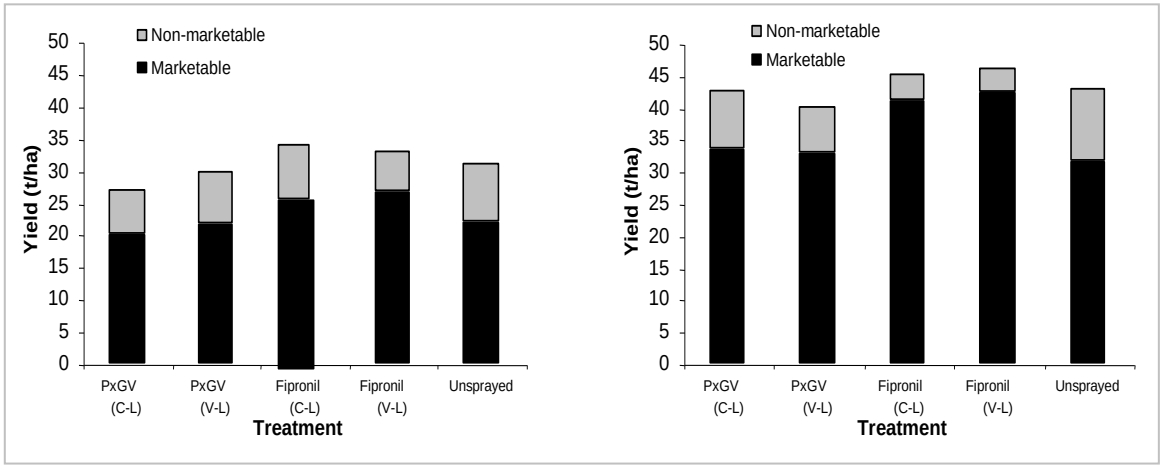

Figure 5. Effect of pesticide application method and pesticide on the yield of kale (marketable and non-marketable) at Thika (left), and Kabete (right).

The granulovirus of *Plutella xylostella* (diamond back moth ,DBM) and its potential for control of DBM in Kenya.

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Introduction

DBM is a major pest of brassica vegetables in Kenya (Michalik 1994). In East Africa, control of DBM is becoming an increasing problem due to escalating resistance to chemical insecticides. A project to develop non-chemical methods of DBM control on brassica crops was initiated in Kenya in 1996 to explore the use of endemic pathogens for DBM control. Prior to this study granuloviruses (GV) of *P.xylostella* had been reported from Japan (Asayama and Osaki 1970) Taiwan and China (Kadir *et al* 1999) and India (Rabindra *et al*, 1997), but there were no previous published records of *Plxy*GV isolates from Africa.

Materials and methods

A survey of 27 brassica farms was conducted within a radius of 170 km around Nairobi to identify endemic pathogens of DBM. Larvae showing signs of baculovirus infection, were collected for later examination and the presence of GV confirmed. Restriction endonuclease analysis (REN) of the baculovirus isolates was performed on each of the GV isolates found, following the protocol of Smith and Summers (1978). The pathogenicity of eight of the fourteen isolates showing different REN profiles were determined by discriminate dose

assays. LC_{50} bioassays were then carried out on five of those eight isolates and an isolate of *Plxy*GV from Taiwan, (*Plxy*GV-Tw), using methods described elsewhere (Parnell *et al* 2002). To evaluate the potential of the Kenyan *Plxy*GV to control DBM, small-plot field trials were conducted on the PlxyGV isolate Nya-01 in 1999. The virus was applied as a simple unformulated suspension using standard hydraulic backpack sprayers. The first field trial was on Kale using a replicated randomised-block design trial carried out on small plots (5m x 5m). This trial compared two virus treatments; a weekly application of either 3.0×10^{14} occlusion bodies (OB) ha^{-1} or 3.0×10^{13} OB ha^{-1}. There was a no treatment control and a standard insecticide treatment based upon weekly application of the local standard pyrethroid insecticide (Karate®- lamda-cyhalothrin). Further field trials were carried out at two sites around Nairobi in 2000. In these trials there were five treatments arranged in randomised replicated small plot design including three weekly virus application rates (3×10^{14}, 3×10^{13} and 3×10^{12} OB ha^{-1}), a no treatment control and a standard insecticide treatment with Karate® as before. In all trials numbers of DBM larvae present, numbers showing symptoms of GV infection and damage caused by DBM were monitored weekly. In the second trial yield data was also collected. To assess more precisely the disease incidence in the plots samples of larvae from virus treated plots were collected from each treatment and reared individually. Laboratory bioassays to test various formulations of *Plxy*GV were also carried out using previously described methods on 2nd instar DBM (Parnell *et al* 2002). The data was analysed using the Genstat statistical analysis package.

Results

During the field survey, 127 larvae with disease symptoms were collected and examination confirmed that 95 of these larvae were suffering from GV infection. The REN analysis of the 95 *Plxy*GV isolates showed that 14 had fragment profiles that could be distinguished from any other with both *Eco*R1 and *Pst*1 cuts. Comparison of these 14 Kenyan *Plxy*GV isolates to the *Plxy*GV-Tw isolate revealed that, although the profiles had many similarities, there were major band differences between all isolates. In the

dose response bioassays no significant differences in LC_{50} values between Kenyan isolates and the *Plxy*GV-Tw isolate were observed. Average LC_{50} values for second instar DBM larvae ranged from $2.36x10^6$ OB ml^{-1} for Nya-01 to $3.95x10^7$ OB ml^{-1} for isolate Nya-40. In comparison the LC_{50} for the *Plxy*GV-Tw was $1.55x10^7$ OB ml^{-1}. Bioassays of simple formulations of *Plxy*GV showed the LC_{50} for the formulated virus to be $3.62 x10^5$ OB ml^{-1} compared to $3.65 x10^7$ OB ml^{-1} with the unformulated control. The productivity study showed that the maximum production of *Plxy*GV was $4.0 \pm 0.44 x10^{10}$ OB per larva from 2nd instars inoculated with $2 x10^8$ OB $ml^{-1.}$

The field trials showed that the *Plxy*GV was highly infectious to DBM, spreading rapidly in trial plots within two to three weeks of application. Both the high application rate of $3.0 x 10^{14}$ OB $ha^{-1}$and the lower application rate of $3.0 x 10^{13}$ OB ha^{-1} reduced DBM damage to crops to below that seen in either unsprayed controls or insecticide treated plots (Figure 1). In the second series of trials the yield data (Figure 2) showed that the highest application rate of virus gave a mean yield 37% higher than the control and 17% higher than the insecticide treatment, although this was not statistically significant. The average observed DBM infection rates in virus treated plots also showed a clear application-rate trend with the highest rate producing an average of 40% infection in DBM larvae (Figure 3). A study of insects sampled from the *Plxy*GV application-rate plots indicated that the true infection rate was much higher than that observed in the field and Table 1 shows the percent virus mortality recorded from insects taken from the plot treated at $3 x10^{13}$ OB ha^{-1}.

Discussion

The discovery of numerous genetic isolates (14) in the small number of infected larvae collected is an interesting result. Previously reported work (Kadir *et al* 1999) has characterised two genetically distinct isolates, one from China and one from Taiwan. Other studies of DBM pathogens have also only reported finding a single genetically distinct isolate from India (Rabindra et al 1997) and Japan (Yamada & Yamaguchi 1985). The GV isolates

from Kenya are genetically similar to, though distinct from the previously reported Taiwanese isolate. The genetic variation in *Plxy*GV isolates discovered in Kenya might indicate a long relationship between host and virus and could be interpreted as providing additional support to the theory of Kfir (1998) that the origin of DBM lies in Sub-Saharan Africa.

The first field trial showed that application of *Plxy*GV at $3x10^{13}$ OB ha^{-1} could reduce DBM damage much better than Karate the standard chemical insecticide. In the second trial the yield results showed that again the *Plxy*GV performed as well as the chemical insecticide at the highest application rate used ($3x10^{14}$ OB ha^{-1}). The lower application rates did not result in effective DBM control. The yield data for the *Plxy*GV at $3x10^{14}$ OB ha^{-1} , while higher was not significantly better than the insecticide. This may be because *Plxy*GV does not control aphids while the chemical insecticide does. In many seasons Kenya aphids are a serious secondary pests of brassicas (Oruko and Ndun'gu 2001) and if *Plxy*Gv is to be successfully promoted to farmers there will be a need to identify an effective compatible aphid control. system.

The productivity of the Kenyan isolates is high at 2.0-4.0 ± 0.44 $x10^{10}$ OB per larva. At this rate of production the highest application rate used in the field trials, 3.0 $x10^{14}$ OB ha^{-1} would be equivalent to 7,500 infected larvae per ha. In comparison most existing commercial baculovirus products are applied at rates of between 50-500 larval equivalents per ha (Moscardi 1999). The two granuloviruses that have been commercialised to date, *Cydia pomonella* GV and *Adoxophyes orana* GV, both have application at rates of $1x10^{13}$ OB ha^{-1}. Thus an an application rate of 3.0 $x10^{14}$ OB ha^{-1} could be higher than is economically viable. It has been shown that the addition of molasses to a GV formulation can increase the efficacy of the GV by a factor of ten, and allow for a consequent reduction in the application rate of (Kadir 1992, Ballard *et al* 2000). Laboratory bioassays showed that a molasses formulation of *Plxy*GV indeed reduces the LC_{50} by a factor of 50. Thus formulation could significantly lower the potential cost of *Plxy*GV. Successful field trials of such a formulation have recently

been completed in Kenya to evaluate the efficacy of reduced rate formulated *Plxy*GV and are reported elsewhere at this meeting (Ogutu *et al* 2002).

In conclusion, from the results of these trials it may be concluded that *Plxy*GV has considerable potential as a biopesticide for controlling DBM, and further work to evaluate its potential commercial use in Kenya is now underway.

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Table 1. Percentage insects killed by *Plxy*GV infections that developed in laboratory reared larvae sampled from field plots sprayed with *Plxy*GV at $3x10^{13}$ OB ha^{-1}.

Larval instar	% PlxyGV mortality
1st Instar	90
2nd Instar	82
3rd Instar	64
4th Instar	60

Figure 1. The level of crop damage observed in treatments from the first field trial of *Plxy*GV in Kenya. Comparison of damage from 2 virus treatments, 1 insecticide treatment (Karate) and 1 unsprayed control.

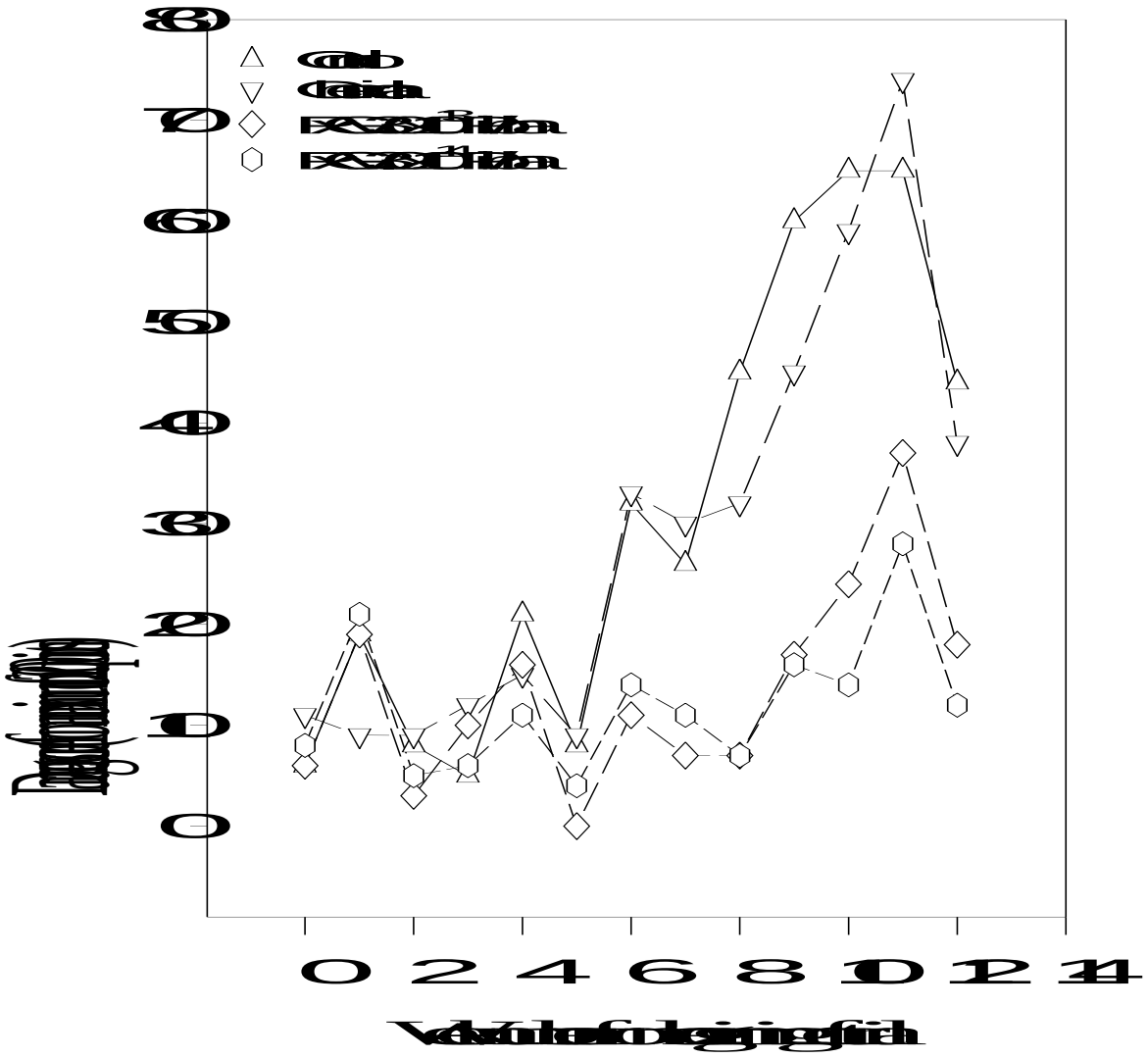


Figure 2. Effect of three *Plxy*GV application rates on marketable average kale yield in comparison with insecticide and no spray control (ton per hectare).

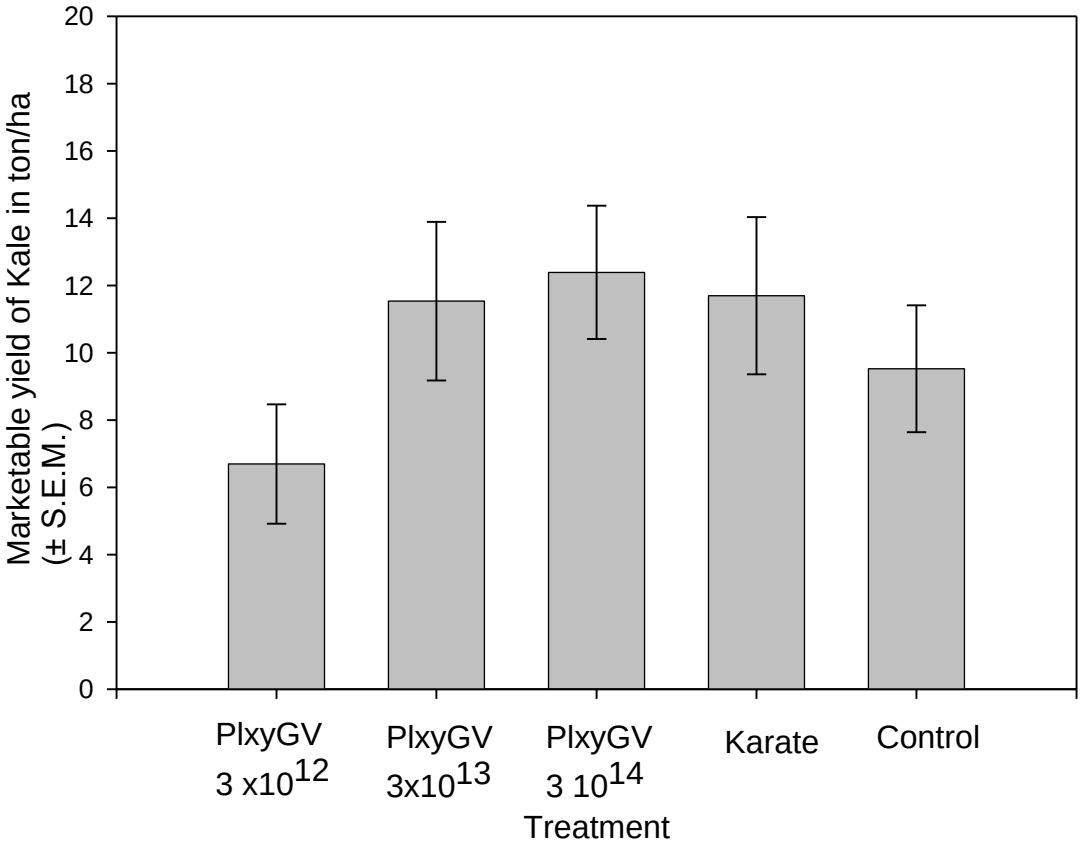
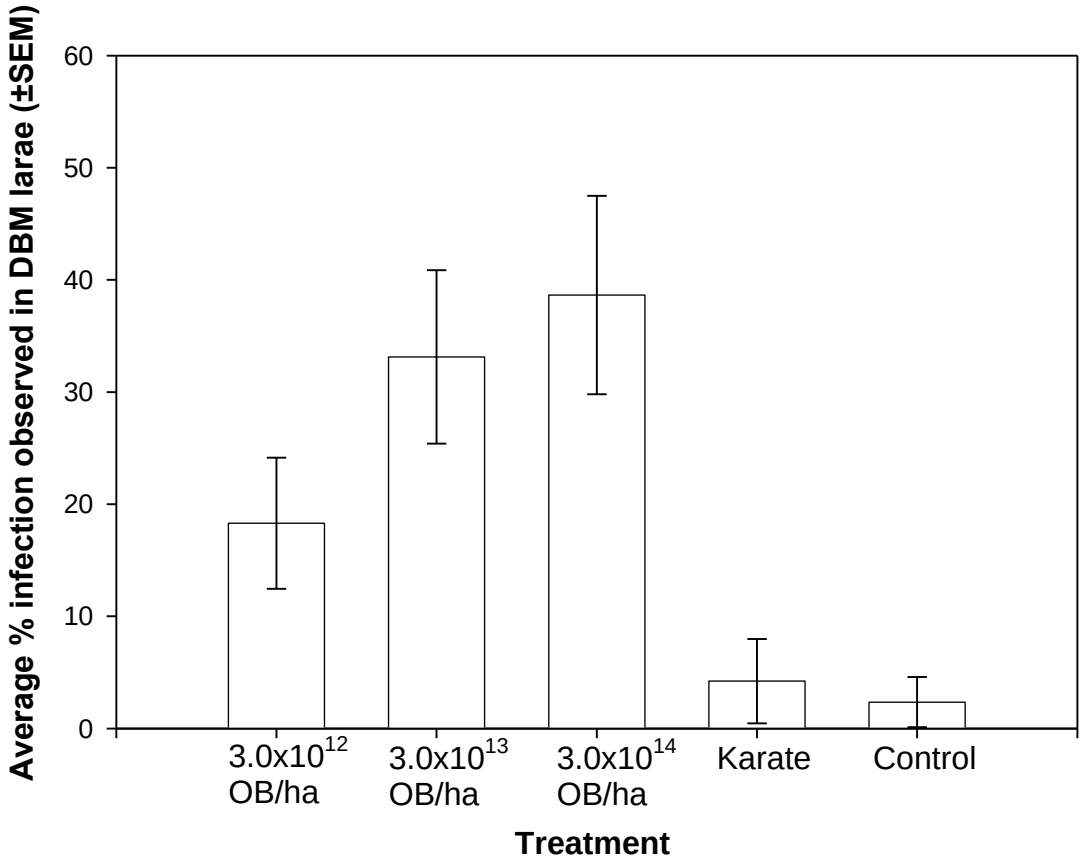


Figure 3. Average *Plxy*GV infection-rate observed in DBM larvae from the field trial treatments



REPUBLIC OF KENYA
PEST CONTROL PRODUCTS ACT, CAP 346, 1982.

APPLICATION FOR THE REGISTRATION OF A BIOPESTICIDE

INTRODUCTION

1. These guidelines are for any propose use of naturally occurring bacteria, protozoa, fungi, viruses, plants and or their products (growth regulators, pheromones, botanical products) for the control of invertebrate pests, weeds or microbial pathogens of crops**. The use of microbial agents for the control of vertebrate pests is not contemplated.**
2. Information in support of a request for registration, both published should be supplied in the form of a summary data sheet laid out according to the format given in Form A2.

Information for Applicants

1. The application form must be completed by a duly authorized person.
2. The application must be submitted in triplicate to: **The Secretary, Pest control Products Board (PCPB) P.O. Box13794, Nairobi, e-mail address pcpboard@todays.co.ke or pcpbboard@nbnet.co.ke Tel.254-2-446115, Fax 254-2-449072.**
3. Every application must be accompanied by:-
   (a) registration fee as prescribed
   (b) 3 copies of the draft label as per PCPB requirements
4. The applicant may be required to submit:-
   (a) a sample of the pest control product;
   (b) a sample of the technical grade of its active ingredients/agent;
   (c) a sample of the laboratory standard of its active ingredients/agent;
   (d) any other sample as may be required by PCPB.
5. All applicants intending to import/export live organisms into or out of the country should seek clearance from the Kenya Standing Technical Committee on Imports and Exports on live organisms (KSTCIE).
6. The use of genetically modified organisms 9GMOs) and living modified organisms (LMOs) as biopesticides should be cleared by the National Biosafety Commttee on GMOs before an application is made.
7. List 1 and II are supplied as check lists and an index to ensure that the applicant has provided all relevant data.
8. The application must be accompanied by a technical dossier as per PCPB data requirements i.e. Lists I and II.
9. An applicant who is not a resident in Kenya must appoint an agent permanently resident in Kenya.