CROP PROTECTION PROGRAMME

Development of biorational brassica IPM in Kenya
R No 7449 (ZA No0319)

FINAL TECHNICAL REPORT

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Executive Summary

The project, a collaboration between NRI, the Kenyan Agricultural Research Institute, CAB International Africa Regional Centre and Horticulture Research International was to develop improved non-chemical insecticide approaches for the management of Kenyan vegetable. The project focused on control of the diamondback moth (DBM *Plutella xylostella*) identified as the priority pest problem for vegetable farmers in Kenya. The objective was to develop and evaluate two alternatives to chemical insecticide, a natural pesticide based upon an insect virus and pheromone mating disruption technique.

An socio-economic survey of Kenyan peri-urban farmers collected data from 200 farmers. It confirmed the high profile of DBM as a pest and identified the large potential market for a new control technology and the viable target cost of any product. It also identified aphid control as an important farmer problem.

After extensive development trials the pheromone mating disruption was found not to be effective on the small Brassica plots (less than 0.1 ha) typical in Kenya and the conclusion is that this is not a feasible option for small vegetable farmers in the Kenya.

The development of an endemic *P. xylostella* (*Plxy*GV) granulovirus, a virus first isolated in 1997, as a biological pesticide has made very significant progress towards a commercial product. Field trials were carried out to determine the effective application rate for the technical product. Follow on trials testing cheap local formulation additives have shown that incorporating such additives can improve the efficacy of the PlxyGV enabling the active ingredient rate to be reduced and lowering potential cost. DBM control results with *Plxy*GV are better than pyrethroids such as Karate to which DBM have developed resistance. A drawback with a specific biological such as *Plxy*GV is the absence of activity against other pests, primarily aphids. However, field trials have shown that *Plxy*GV can be successfully combined with a specific aphicide, Pirimicarb if required.

Work on mass production of the *Plxy*GV has improved the productivity fourfold by using incorporating insect juvenile hormone analogue in the culturing diet. This increased production efficiency, combined with formulation improvements should make *Plxy*GV economically competitive with expensive imported insecticides. The Kenyan authorities have adopted a registration protocol for biopesticides covering *Plxy*GV. Kenya is currently harmonising this package with Tanzania, Uganda and Ethiopia so that the same product registration dossier can be used in all countries.

The results of this work have encouraged a commercial partner to come forward to take on further development of *Plxy*GV. A plant to produce *Plxy*GV is now being set up in Kenya under CPP co-funding (R7960) with a commercial partner Dudutech Ltd using the technologies of production and formulation developed by this project. This will be the first viral insecticide to go into commercial production in sub-Saharan Africa. The product will be promoted specifically to smallholder farmers and outgrowers to provide a safe effective alternative to the increasingly expensive and unreliable chemical pesticides. Local *Plxy*GV production will create jobs and may lead to the development of export markets for biopesticides to other African countries, that are interested in adopting *such as* Ghana.

Background

DBM is one of the most serious pests of brassica crops worldwide. In the last decade it has become increasingly difficult to control by conventional chemical means because of the widespread development of resistance to most classes of chemical insecticides including pyrethroid insecticides, insect growth regulators and *Bacillus thuringiensis*. DBM management is a worldwide problem and is estimated to cost up to US$1 billion in control costs and losses (Shelton *et.al.,*1997).

Kale and cabbage are typically the most important vegetable crops grown by smallholders in Kenya. These brassica crops are an important food source for the urban populations in East African and their production a major income generator for 40,000 small scale growers supplying the towns. The development of insecticide resistance by the key pest, DBM currently threatens the sustainability of production by these growers who have no alternative crops that would generate sufficient returns from their small farms (cropping area average < 0.8 ha) to maintain family incomes.

In 1994 the Ministry of Agriculture estimated the total crop area for Kale and cabbage at 25,826 ha with a production of 266,467 tons valued at £126,038,891 (MALDM 1994). Much of this is grown for home and local consumption. The commercial export sector is much less at around 25-30,000 tons per annum (see table 1). This sector has shown a steady increase in production of 15-20% in the last decade (HCDA 2002) so that further expansion can be expected in future providing both an important source of income to Kenya and providing livelihoods for an increasing number of peri-urban and urban families.

Table 1 Brassica production in Kenya 1998-2000 (metric tons).

Crop	1998	1999	2000
Kale's	15,175	21,050	16,450
Cabbages	8,600	8,750	12,300

Source: HCDA Horticultural News Jan/Fed 2002.

The major brassica crops are kales and cabbages, the balance between them varies from year to year probably reflecting relative market prices the previous year and perhaps also recent pest/disease constraints. Kale is the crop preferred by Kenyan consumers and as its leaves are harvested 4-6 times from mid season onwards it also has advantages for growers as it generates income earlier in the crop cycle and reduces exposure to sudden DBM outbreaks in the late season. Many varieties of kale are grown and this plays an important part in the local diet alongside the other staples of Maize and beans for both urban and rural poor

A survey by the KARI/GTZ Horticulture Project identified DBM as a priority pest on brassicas in Kenya and recommended development of appropriate crop protection measures to be implemented (Michalik, 1994). Research in on farm trials also identified DBM as the primary pest with aphids the key secondary pest (Oduor *et*.al., 1997) a picture reflected in other tropical and subtropical brassica cropping systems worldwide (Lim *et*.al., 1997). The GTZ IPM Horticulture Brassica Planning Workshop for East and Southern Africa (Nyambo and Pekke, 1995) similarly identified DBM as the key pest on crucifers and brassicas throughout East and Southern Africa and highlighted the lack of alternative control measures.

Historically DBM as a constraint on vegetable production became much reduced in Kenya in the 1970's as smallholder use of organo-chlorines insecticides spread. The widespread introduction of pyrethroids in the 1980's subsequently ensured effective control of this pest. Michalik (1994) reported extensive use of pyrethroid insecticides on brassicas, and also some use of aldrin and carbosulfan that are not registered for use on vegetables in Kenya. However by the mid 1990's the problem of DBM had shown a widespread resurgence in Kenya and this was associated with results showing decreased effectiveness of chemical control (Kibata 1997). Trials of standard insecticide in the linked CPP vegetable IPM project R6614 have confirmed that Kenyan high level resistance to chemical insecticides and that some insecticides in widespread use are no longer effective against DBM on brassicas (Cooper 2001).

This had occurred in other areas of North America, Asia and Australasia where intensive culture of Crucifers and brassicas was threatened by the development of insecticide resistance by DBM (Roush 1997). Failure of chemical control in several countries in the early 1980's was averted by the widespread adoption of the microbial pesticide *Bacillus thuringiensis (*Bt) as an alternative to synthetic insecticides. However subsequently in the USA, Central America, Hawaii and Malaysia DBM has been shown to rapidly develop resistance to Bt when this is used repeatedly and exclusively (Tabashnik 1994, Wright *et. al.,* 1995) so that again sustainable control of this pest is under threat.

The introduction of new families of chemical insecticides has refilled the chemical armoury in the fight against DBM. However new insecticides such as Bifenthrin are too expensive for many Kenyan farmers (J. Cooper pers. comms.). Recent experience from Hawaii in 1998-2000 also shows that resistance can appear to the newest chemicals in only two years (Mau 2001). Even committed advocates of the importance of chemical insecticides now recognise a clear role for natural biocontrol agents in brassica IPM if resistance is to be avoided and DBM is to be effectively managed (Sparks 2001).

Currently the only alternatives to chemical insecticides are imported biocontrol products based on the *kurstaki* and *aizawai* strains of Bt. These products are however relatively expensive for poor farmers in developing countries such as Africa so they are only adopted as a last resort. However the rapid development of Bt resistance in DBM has been reported in South East Asia, Hawaii and America (Tabashnik 1994). There is therefore an urgent need to develop new alternative tools for control of DBM in East Africa, as well as other parts of the world (Shelton *et.al.,.,* 1997).

There was therefore a clear need to develop new, locally producible and environmentally friendly DBM control technology that could be integrated into a sustainable system of integrated crop management appropriate for small peri-urban farms in Kenya. Local production of such a technology was considered an important factor as this would help to ensure continuity of supply. It would also help to ensure appropriate pricing not subject to disruption from currency fluctuations that effect imported pesticides.

Research under an earlier project (R6615) investigated a number of radically different "biorational" alternative approaches to DBM management. These include:

- pheromone mating disruption,
- an endemic granulovirus of DBM as a biopesticide
- co-formulation of the fungi *Beauveria bassiana* and a granulovirus

- fungal pathogen *Zoopthera radicans* dispersed from a pheromone baited auto-dissemination trap system.

Significant progress was made in developing all of these approaches under the earlier project between 1996-99. However only two, the pheromone mating disruption and the granulovirus, had produced field trial results that were deemed promising. These two approaches were selected for further development in this project phase.

The rational in developing a biological pesticide in Kenya to control DBM is partly derived from experience elsewhere with DBM. In areas of South Asia such as Malaysia where massive overuse of chemical insecticides have created a serious DBM resistance problem daily spraying of cocktails of insecticides is a common practice (D Wright pers comms.). This results in high pesticide residue levels on produce, environmental contamination by insecticides and an often serious but largely unreported problem of farm worker poisoning (Harris 2000). In Kenya as yet such pesticide overuse has not become a serious issue and the development and introduction of a novel non chemical control based upon either pheromones or an endemic pathogen was perceived to be a an important tool to prevent over reliance on chemical insecticides.

Another perceived advantage in developing a control technology based upon a baculovirus is that local production in Africa of such a technology is a viable and sustainable option. Production technologies for a number of microbial pesticides including viruses and fungi are low technology manpower intensive techniques that are ideal for developing countries in contrast to chemical pesticides which are often technically complex and highly capital intensive (Prior 1989). Commercial baculovirus production for pest control has been implemented in a number of developing countries including, India and Thailand (Warburton and Grzywacz 1999), Bolivia and Brazil (Moscardi 1999).

Project Purpose

The purpose of the project and how it addressed the identified development opportunity or identified constraint to development.

To secure and enhance the incomes of small holder Peri-urban vegetable farmers by developing an improved sustainable IPM system for brassica production in Kenya. The aim of the project was to develop, new non-insecticide techniques based upon natural pathogens and pheromone mating disruption, for managing the key pest (Diamondback moth or DBM) of peri-urban vegetable production in East Africa.

Output 1 Socio-economic analysis of brassica production and a cost benefit impact of new IPM technologies

Activity 1 Socio-economic survey

A socio-economic survey funded by this project, in collaboration with other projects in the cluster, of Kenyan peri-urban farmers collected data from 200 farmers in major vegetable growing areas around Nairobi (Thika, Kiambu, Machakos and Kajiado) around Nairobi (Appendix 7) . The survey represents one of the most detailed pictures of peri-urban farming yet collected in Africa. This confirmed the small size of peri-urban farms average 1.6 ha with a 0.8 ha cropping area. The survey showed the importance of vegetables in the peri-urban farming economy. On these farms vegetables accounted for 55% of the cropped area and 80% of farmers grew Kale, 71% tomatoes and 49% cabbage. Brassicas were the largest cropping area with on average 23% of the cropped area down to Kale, 29% to cabbage and 27% to tomatoes. In answer to

questions about constraints to improved production 67% of farmers identified pests and diseases as the primary constraint though inadequate capital for inputs (15%) and inadequate irrigation water (11%) were also significant.

For kale 80.5% of farmers employed chemicals for DBM control but in addition aphid chemical control was practised by 73% of farmers. For cabbage there was a similar picture with 75% of farmers using chemicals for DBM control and 66% for aphid control. It was noticeable that the most popular pesticides were Karate and Diazinon that are broad spectrum and could be used for both pests. Currently farmers expend between £90-240 per ha to protect their brassica crops. A few farmers (5%) knew of the use of botanical insecticides but an overwhelming majority (99%) used commercial chemical products. The cost of chemical plant protection products was between 21-65% of total vegetable production costs. These vegetable crops produce incomes for the growers of between £833-2180 per ha with Kale yielding an average of £1000-£2000 per hectare depending upon the district and tomatoes up to £1500-2000 per hectare.

The survey highlighted that while DBM is a major pest of brassica production in Kenya it was perceived by farmers to be of equal importance to aphids. Prior to this survey and this project it was understood that DBM was the primary pest constraint to brassica production in Kenya (Kibata 1997). The survey though agrees with the findings of small on station trials carried out previously in Kenya (Oduor et. al. 1997). It may be that DBM is the major cause of damage and crop losses to brassicas when and where serious attacks occur. The farmer's perception may reflect their observation that aphid problems and the plant virus diseases they vector occur on a wider scale and with more regularity.

Thus the development of an improved DBM management system will need to be integrated into a growing system that provides the farmer with a solution to aphid problems if it is to achieve widespread acceptance. This will be crucial, as it is appearance of aphids that often stimulates farmers to apply the broad-spectrum insecticides such as Pyrethroids that exacerbate or even initiate DBM outbreaks. This is not merely a problem for Kenya as a forthcoming paper reviewing the status of DBM world-wide identified that serious DBM outbreaks commonly lined to earlier use of broad spectrum insecticides (Wright 2003).

A feature of DBM outbreaks is the timing. In all the trials during this project DBM levels were uniformly low during the two rainy seasons, when brassicas are planted and large DBM population increases only occur after the rains have ceased. It is known that larvae are very susceptible to the physical impact of rain, though the higher humidity during rainy periods may also favour fungal pathogens of DBM. Again this appears not merely to be unique to Kenya but has been described as general for Africa (Lohr 2003) and elsewhere (D. Wright pers. comms.). In Kenya if the rainy season is long a whole cropping cycle can be completed without serious DBM attack, providing early use of broad-spectrum insecticides is eschewed. However in many places in Kenya or in everywhere some years the rains do not persist until the end of the brassica season and DBM becomes a problem.

This suggests that DBM interventions such as PxGV will normally only be necessary only towards the end of the normal growing season or where cabbages are grown as irrigated crops in the dry seasons. This can be used to help predict both the scale of potential need for a new DBM technology and the timing of demand. In Kenya where commercial brassicas are estimated to be grown on 30,000 ha this would suggest a need for 2-4 weekly applications thus an annual need for up to 60-120,000 hectares of DBM control product. The demand for most of this would be concentrated at the end of the long rains in May-July depending upon location in Kenya. Initially market

penetration would be likely to be limited and even a successful product rarely captures >30% of its target market within five years. Thus an initial penetration of 10% by two years rising to maxim 30% by 5 years after release could represent a successful growth profile for a DBM product in Kenya. This would suggest an initial estimate of 20,000 ha equivalent of PlxyGV per annum by 2004 as a reasonable target market share.

This farmer survey generated an extensive data set on Peri-urban farmer crop protection practices. This is available for research and can be accessed by contacting Dr S Simons Kenya project cluster local co-ordinator.

In conclusion the information from the survey is an important resource for understanding the economics of crop protection and the decision processes that guide farmers in their adoption of new pest control technologies. They indicate that farmers overwhelming use commercially sourced insecticides and that they have significant resources with which to purchase them. They provide a guide to the current costs of crop protection and will provide insight into the affordable price that any new technology must compete with in order to be acceptable to farmers. However it must be remembered that this situation is dynamic and any factor such as the appearance of resistance by target pests to current chemicals (such as appeared in DBM to Karate during 1997-2000) will change the picture and may open new market opportunities for the more expensive technologies.

DBM has manifested a greater capacity to develop resistance to new insecticides than any other insect vegetable pest. In the late 1990's in South Asia and the USA the biopesticide based upon Bt has been widely adopted by farmers in place of chemicals for DBM control even though it is more expensive, because DBM resistance has made the chemical alternatives ineffective. A recent survey in Ghana has shown that 48% of vegetable farmers their have adopted the use of Bt on similar grounds (A.C.Cherry pers. comms.). However while Bt is a safe and effective biopesticide with some similarities in mode of action to *Plxy*GV DBM can also develop resistance to Bt when it is used as the only control technique.

Activity 1.2 Determine the acceptability of the new virus and pheromone systems

As the rate of progress in the technical development of the *Plxy*GV virus system was slower than hoped for, mainly due to low DBM pest pressures in the field trials. As a result the rate application and formulation trials took longer than expected and on farm acceptability trials of a pilot formulation could not be completed as planned. The project was able to carryout a farmer demonstration trial of the *Plxy*GV before the end of the project. The 30 farmers who attended gave a very positive response (Kimani 2002), but there was not the time to carry out a structured market analysis of farmer acceptability as part of farmer run on farm trials.

As the technical assessment of the pheromone system showed that this was not a system appropriate for small holder farmers in Kenya this activity fell by default.

Activity 1.3 Identify promotion methodologies for locally sustainable uptake of virus.

Again due to slower than expected progress of field trial activities under output 2 there was no time left to undertake this activity. However this activity will be undertaken by Dudutech, the company that is commercialising the *Plxy*GV, which will use materials prepared by the project to promote the *Plxy*GV to smallholder farmers. This project is

committed to providing farmers with on farm demonstrations and training as part of its promotion strategy.

Activity 1.4 Identify producer of pheromone.

As the technical assessment of the pheromone system showed that this was not a system appropriate for small holder farmers in Kenya this activity fell by default.

Activity 1.5 Identify promotion pathways

This is to be addressed by KARI and Dudutech the company who are setting up commercial *Plxy*GV production. Dudutech will take over the promotion of the *Plxy*GV using their existing commercial supply chain and farmer training network. Dudutech and KARI have already developed a close collaboration in developing new biological control agents. This is a novel partnership in the Kenyan national programme and would be a model for commercialising other KARI/CPP the crop protection research outputs.

Dudutech will initially promote *Plxy*GV product alongside its existing range of biocontrol agents (BCA) which currently includes predators and parasites. These will initially be promoted with a training package to commercial export growers and their associated out growers while building market confidence and acceptance of the product. But it is the intent of the company to move on to market products directly to smallholder farmers not linked to the export sector through Dudutech's marketing network.

Output 2 A system of DBM control based upon endemic Kenyan *P.xylostella* viruses developed and evaluated for use on Kenyan farms

The virus component of the previous project, to identify a biorational technology for DBM control, had isolated an identified *Plxy*GV as endemic in Kenya in 1997, this was the first recorded finding of a DBM granulovirus in Kenya or sub-Saharan Africa. Fourteen genetically distinguishable isolates were collected and identified (Parnell *et*.al., 2002). The most common of these (NYA-01) was selected for field evaluation in 1998-99. Two field trials in 1998-99 had confirmed that this *Plxy*GV was highly infectious and when applied in the field at 3 $x10^{13}$ and 3 $x10^{14}$ occlusion bodies (OB) per hectare would typically result in DBM infections in 40-90% of the DBM. It could reduce DBM damage more effectively than the standard farmer insecticide lambda-cyhalothrin (Grzywacz 1999). However it had not been possible to collect yield data on *Plxy*GV so confirm its true efficacy.

Genetic studies on the Kenyan cluster of *Plxy*GV strains has continued in parallel to this project in collaboration with HRI as part of a University of Greenwich funded PhD project. This study has cloned the five most genetically diverse of these isolates, sequenced a number of genes and confirmed that Kenya isolates are genetically different from the previously reported Asian strains. Investigations into the pathogenicity of the strains, the genetics of pathogenicity and the biological significance of the genetic differences continue (Woodward *et*.al., 2002).

The first important objective of this project was to confirm that *Plxy*GV could effectively control DBM. There was also a need to establish the minimum effective application rate. It was decided to use the two application rates previously shown to reduce DBM, damage (3 $x10^{14}$ and 3 $x10^{13}$ OB ha^{-1}) plus another one 10 times lower. Correctly identifying the minimum effective application rate would be an important step towards

determining the likely feasibility and economics of using *Plxy*GV as a DBM control technology.

Activity 2.1 Select and produce *Plxy*GV strains for use in field trials.

Based upon laboratory assays described in Parnell *et.al.,.,* (2002) the Nya-01 (Nyathuna farm isolate number 01) was selected and used in the field trials to determine efficacy and optimum application. This isolate was selected during the previous project (R6615) as it had been the most pathogenic of the 14 genetically different strains when screened with discriminant single dose assays (Grzywacz 1999) . Subsequently precise LC50s were determined at HRI for 8 of the strains and these showed no significant differences in LC50s between strains (Woodward *et*.al., 2002). Average LC_{50} values for first instar DBM larvae ranged from 1.1 OB for Nya-01 to 2,2 OB ml^{-1} for isolate Nya-10. In comparison the LC_{50} for the *Plxy*GV-Taiwanese isolate was 3.29 OB. As the NYA-01 strain was the one with the lowest LC50 and had been used previously in the field it was decided to continue the field trials with this strain. It can be seen that with an LC50 of 1 viral particle per insect *Plxy*GV is extremely pathogenic as it means that ingesting a single infectious virus particle (OB) will kill on average 50% of insects. To bring this result into context a single virus killed insect can produce up to 4 $x10^{10}$ new infectious particles (Parnell 1999).

The *Plxy*GV was produced at CABI ARC laboratories in Nairobi using insects mass reared at the KARI NARL laboratories in Nairobi. This was an *in vivo* rearing method that involved multiplying the *Plxy*GV in 2nd instar larvae that had been infected by feeding them leaves painted with an inoculating dose of *Plxy*GV. Insects were then reared at 27 C for 5-7 days, and harvested. The *Plxy*GV were processed using standard methods described in detail elsewhere (Parnell 1999).

Activity 2.2 Determine optimum application for *Plxy*GV

Application rate field trials.

Fieldwork was carried out 2000-2002 at two field stations around Nairobi. It had been planned that trials would be carried out on farm during the project. However, the Kenyan phytosanitary inspection service (KEPHIS) allowed experimental work with *Plxy*GV only on Government research stations during the period of the project so on farm research trials could not be undertaken before the end of the project.

The field trials were conducted at two stations in and around Nairobi during two growing seasons. These sites were the National Horticulture Research Centre, Thika, which lies 60 Km north of Nairobi just outside Thika town and the National Agricultural Research Laboratories (NARL), at Kabete. In these trials the crop chosen was Kale as this is the most common crop grown by peri-urban vegetable farmers. Three application rates of *Plxy*GV were tested 3 x 10^{12}, 3 x 10^{13}, and 3 x 10^{14} OB ha^{-1} against an insecticide control lambda cyhalothrin (Karate®) at the recommended rate of 17.5 g active ingredient (a.i.) per hectare and an unsprayed control. Karate was chosen for these trials as it was the insecticide used by the largest proportion of growers surveyed (55% of those using DBM chemical control, Oruko & Ndun'gu 2001).

The treatment applications were on a weekly basis as this reflected local farmer practice and a realistic evaluation of the resources available to farmers. In fact farmers vary widely in application practice from biweekly sprays to spraying every few days in areas where resistance has become a serious problem. In this Kenya is not unique and in many brassica areas of South Asia such as Malaysia where

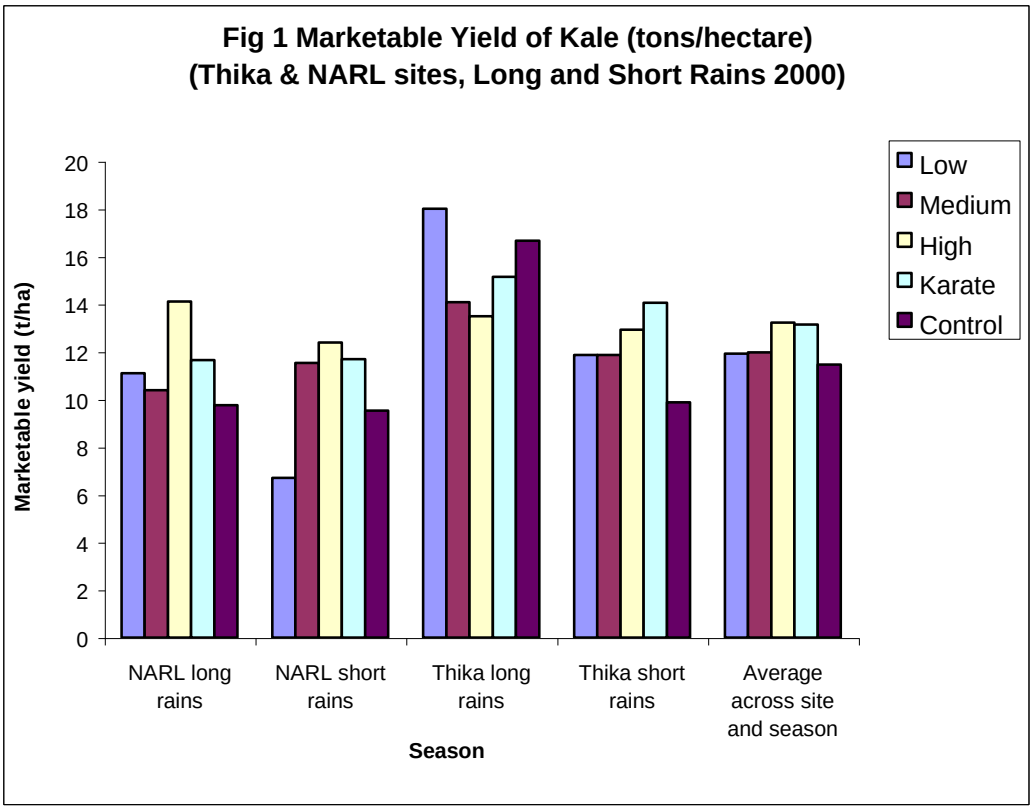
massive overuse of chemicals have created a serious problem daily spraying of insecticides has become common practice.

In the case of the virus a study of the pest life cycle and PlxyGV biology also made weekly spraying a logical outcome. It was known from laboratory studies that the insect is most sensitive to PlxyGV during its first and second instar but that later instars were more resistant and adults and eggs not effected. In brassica growing areas of Kenya, which tend to be the higher cooler locations the pest life cycle lasts 12-15 days and the duration of the L1 & L2 is 5.8 days for DBM larva at an average of 22 ° (Lui. et. al., 2002). It was expected that the persistence of the PlxyGV could be as short at 1-3 days based upon earlier work with Chinese strain of PlxyGV and other baculoviruses in the tropics (Su 1991, Jones 1998). Thus weekly sprays would ensure that no larvae could hatch and pass through the sensitive L1 and L2 without the chance of encountering an infective dose of PlxyGV.

Trials were designed to incorporate both recommended application practices (Matthew's 1992) and best field practices for field trials on Brassicas (Vandeberg *et*.al., 1999). The statistical methods used to analyse data were standard ANOVA parametric techniques carried out on the GENSTAT or SIGMASTAT statistical packages and were checked by the CABI statistician Dr J Poole. In some cases where data sets on preliminary analysis were found to have non-normal or non homogeneous variances the data was transformed using logarithmic transformation. In ANOVA analyses only "a priori" hypotheses were tested in multiple comparison tests, as per statistical recommendations for the analysis of microbial control field experiments (Campbell 2000).

These five treatments were replicated four times in a randomised block design. The gross plot size was 3.6 x 4.2 m, with a net plot of 2.4 x 3.0 m where sampling was being undertaken. In addition to the treated plot area a guard row of kale was planted around each plot, which was left untreated. The same trial design was used during the two seasons. All the treatments were applied using lever operated knapsack sprayers at a volume application rate of 1000 litres/hectare, and applied weekly as this most closely resembled farmer schedules. Weekly sampling was done on 10 plants in the net plot. During each sampling session the numbers of DBM pupae, DBM larvae, *Plxy*GV infected DBM, and damage caused by DBM were noted. Yield data was collected fortnightly by harvesting kale leaves in the net plot. After harvesting the leaves were sorted out into marketable and unmarketable and the numbers and weights of each group noted. Full trial details are in Parnell (2001) attached in appendix 6.

The summary results for the yields of these four trials are shown in Figure 1. The yield figures varied significantly with site ($p = 0.001$) and season ($p = 0.006$). In only one case were their significant treatment yield differences in the Short rains at Thika where both the high *Plxy*GV treatment and the Karate gave significantly higher yields than the controls (Table 5).

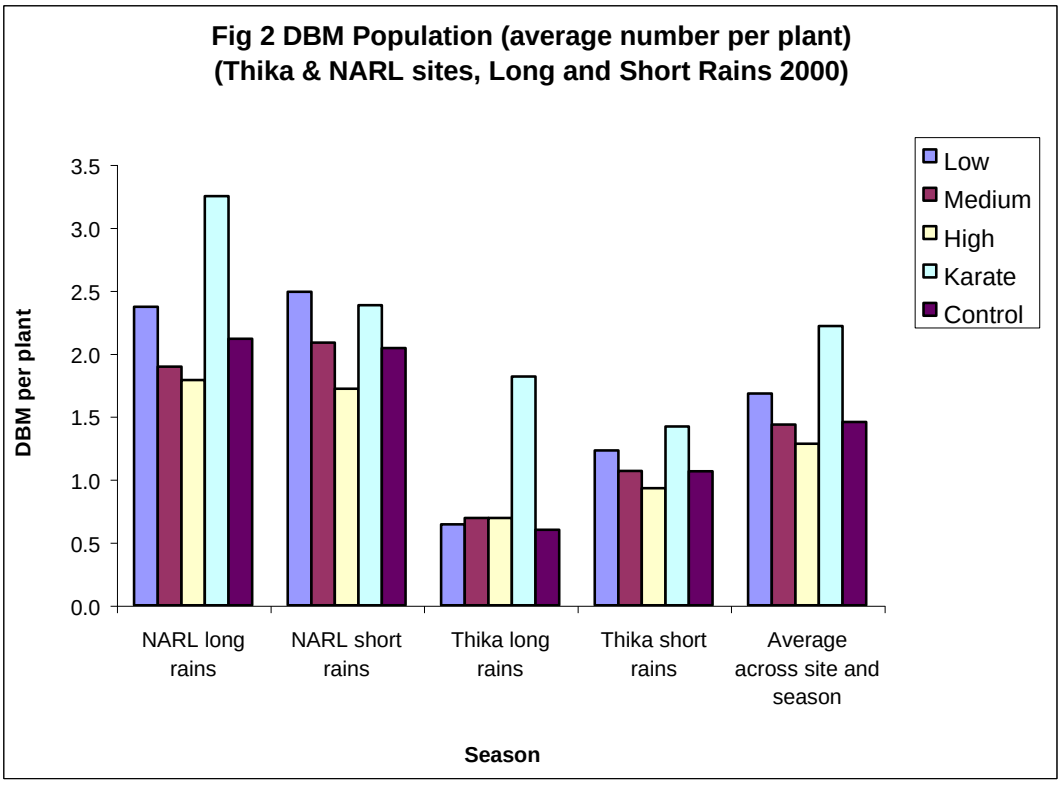


The yields at Thika were higher than at NARL in both seasons (Thika 15 & 12 tha^{-1}, NARL 11 and 9 t ha^{-1}). An explanation of the higher yields at Thika could be related to the finding that average DBM numbers (Figure 2) at Thika are about half at NARL. In all treatments were always lower average numbers of DBM at Thika than at NARL. This data though also shows that average DBM numbers appear to be correlated negatively with increasing application rates of *Plxy*GV at NARL.

One apparent contradiction is that DBM numbers were frequently highest in insecticide treated plots, in three out of four trials significantly so (Tables 2-5). This is probably an indication that DBM resistance to Karate was a problem in these trials and indeed Karate ceased to be recommended as a standard DBM control by KARI after 2001.

The numbers of DBM were in these two seasons low compared to previous years (G Kibata pers. comms.). This could be one reason why no treatment differences in yield or DBM numbers were observed in the Thika trials. Even in the NARL trials DBM numbers were much lower than was considered usual at an average of between 0.5-2.0 DBM per plant. In on farm trials in Kenya yield reductions were seen where mean DBM numbers on Kale exceeded 4-9 per plant depending upon growth stage (Kibata pers comms.) and can reach >18 per plant which was associated with 90% reduction in marketable yield (Kibata 1997). The much lower levels of infestation seen in all these application rate trials in 2000 & 2001 would account for lack of significant yield effects in the trials reported here as DBM damage was light to minimal.

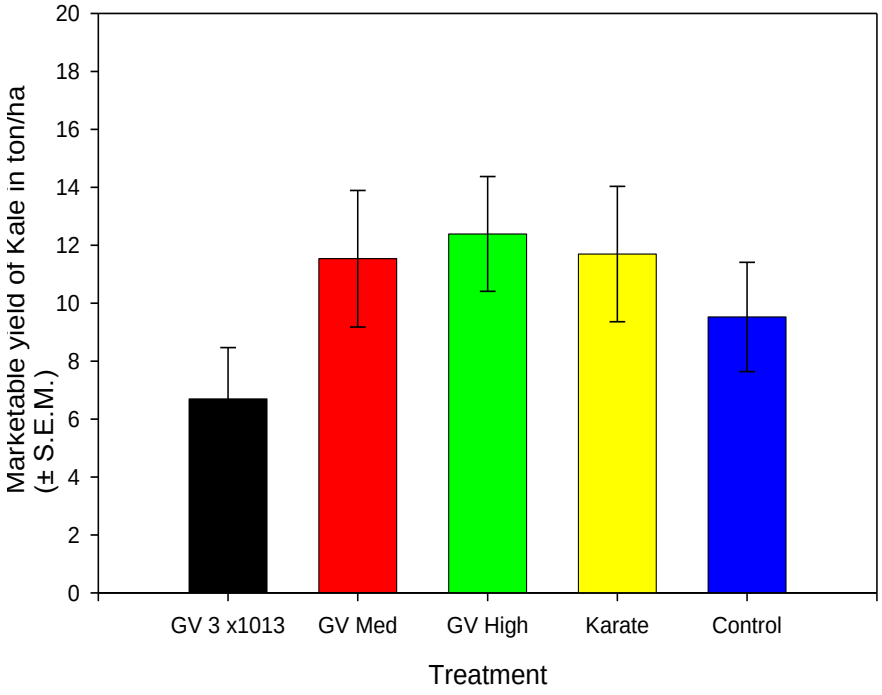
It is possible that in both seasons that unusual pattern of rains created adverse conditions for DBM that reduced pest pressure to below that seen in “normal “ years or it could reflect the variable status of this pest from year to year.



It had been hoped to move research to alternative sites more prone to DBM attack. However the Kenyan authorities would only allow the use of the PlxyGV on the two specified research station sites, until such time as trials produced data on the impact of the PlxyGV on non-target fauna (S.Simons pers. comms.).

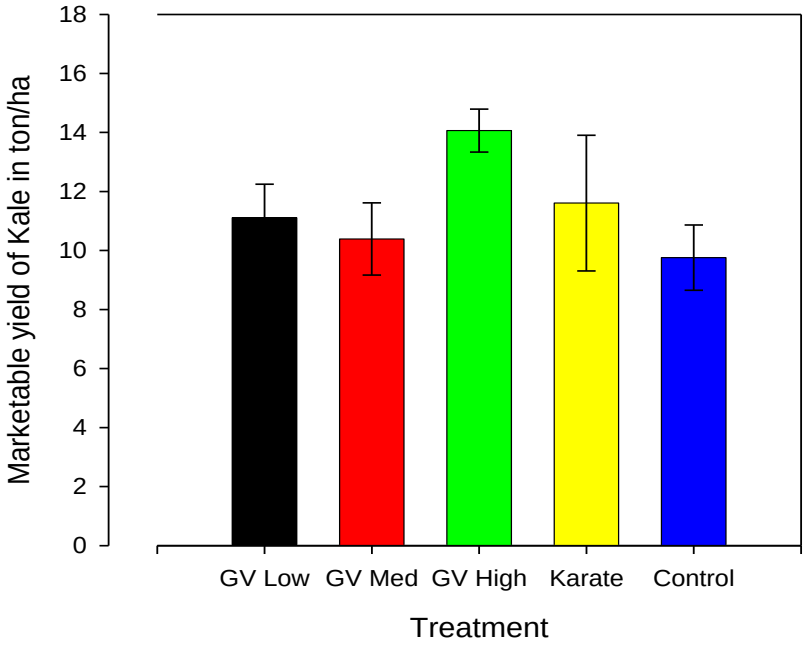
If we examine in more detail the yield results for NARL where DBM pressures were highest it was the highest *Plxy*GV application rate that gave the highest yield in the short rains season (Fig 3). This was found not to be statistically significantly different from the medium application rate or the insecticide.

Fig 3 Marketable yield of Kale with different PlxyGV application rates compared to insecticide (Karate) and control, Field trial at NARL short rains 2000.



In the long rains trial at NARL (Fig 4) the 3 x10^{14} OB ha^{-1} high rate gave a yield 30% higher than the unsprayed control and 17% higher than the insecticide but again this was not statistically significant.

Fig 4 Marketable yield of Kale with different PlxyGV application rates compared to insecticide (Karate) and control, Field trial at NARL long rains 2000.



A problem with these trials was the relatively high variability in yield observed between plots. This made detecting statistical differences between treatments difficult even when mean treatment yields differed by up to 30%. The loss of plants to causes other than DBM, such as bacterial disease was a source of some of this variation. The small number of plants per plot 18-30 also meant that such losses had a greater impact on the analysis that if the plant numbers had been much larger. In later experiments the number of replicates was increased to six to improve the sensitivity of the statistical analysis.

A confounding factor was that the Karate had a broad-spectrum action against both DBM and the aphids that are also an important pest. In contrast the *Plxy*GV is specific to DBM and has no effect on aphids. Thus yield assessment comparisons to the chosen insecticide had an important potential limitation in providing unambiguous data on the efficacy of *Plxy*GV to control DBM.

More detailed analysis of DBM numbers, broken down into larvae and pupae plus yields is presented for all four trials in Tables 2, 3, 4 and 5. Analysis of total DBM larval counts and pupal counts at both sites did show a clear treatment effect in all of the trials.

Table 2 DBM cumulative counts and marketable yields from NARL 2000 long rains on Kale

Treatment	Rate active ingredient (per hectare)	Larval DBM numbers per 10 plants*	Pupal DBM numbers per 10 plants (log**)	Marketable yield (ton hectare)
PlxyGV low rate	3 x10^{12} OB	534.0c	1.62b	11.1 a
PlxyGV medium	3 x10^{13} OB	446.0a	1.36a	10.39 a
PlxyGV high	3 x10^{14} OB	459.8a	1.27a	14.11 a
Karate	17.5 g a.i.	725.8b	1.81b	11.66 a
Untreated Control		511.0c	1.65b	9.76 a

*Treatments significant at P = 0.0419 F = 3.24 Df = 4 & 19
**Treatments significant at P = 0.001 F = 12.4 Df = 4 & 19. (analysis used log values to normalise variance)
Means within column followed by same letter not significantly different at 5% level.

Table 3 DBM cumulative counts and marketable yields from NARL 2000 short rains on Kale

Treatment	Rate (per hectare)	Larval DBM numbers per 10 plants	Pupal DBM numbers per 10 plants (**)	Mean Marketable yield (ton hectare)
PlxyGV low rate	3 x10^{12} OB	572.3a	47.5^{c}	6.71a
PlxyGV medium	3 x10^{13} OB	479.5a	28^{b}	11.53a
PlxyGV high	3 x10^{14} OB	395.3a	14^{b}	12.53a
Karate	17.5g a.i.	547.5a	72.5^{a}	11.69a
Untreated Control		469.5a	72.8^{a}	9.52a

**Treatments significant at P = <0.0001F = 19.4Df = 4 & 19.
Means within column followed by same letter not significantly different at 5% level.

Table 4 DBM cumulative counts and Marketable yields from Trial at Thika long rains 2000 (11/02/2000 -13/07/2000) on Kale

Treatment	Rate (per hectare)	Larval DBM numbers per 10 plants*	Pupal DBM numbers per 10 plants**	Marketable yield
PlxyGV low rate	3 x10^{12} OB	147.8c	11.0c	18.01 a
PlxyGV medium	3 x10^{13} OB	159.3a	6.0a	14.09 a
PlxyGV high	3 x10^{14} OB	159.3a	2.25a	13.49 a
Karate	17.5g a.i.	417.8b	19.5b	15.15 a
Untreated Control		137.8c	13.75c	16.67 a

*Treatments significant at P = 0.0001 F = 37.7 Df = 4 & 19.
**Treatments significant at P = 0.008 F = 8.54 Df = 4 & 19.
Means within column followed by same letter not significantly different at 5% level.

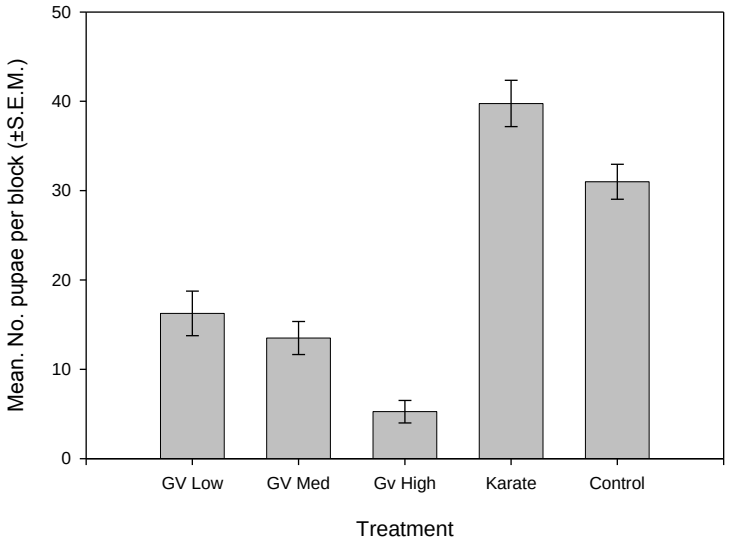
Table 5 DBM cumulative counts and marketable yields from Thika short rains 2000 on Kale

Treatment	Rate (per hectare)	Larval DBM numbers per 10 plants throughout the season*	Pupal DBM numbers per 10 plants throughout the season(**)	Marketable yield (ton hectare)***
PlxyGV low rate	3 x10^{12} OB	283.0^{ab}	16.25^{a}	11.87ab
PlxyGV medium	3 x10^{13} OB	245.5^{b}	13.5^{a}	11.87ab
PlxyGV high	3 x10^{14} OB	214.0^{b}	5.25^{b}	12.39a
Karate	17.5g a.i.	326.5^{a}	39.75^{c}	14.06a
Untreated Control		244.8^{b}	31.0^{d}	9.52b

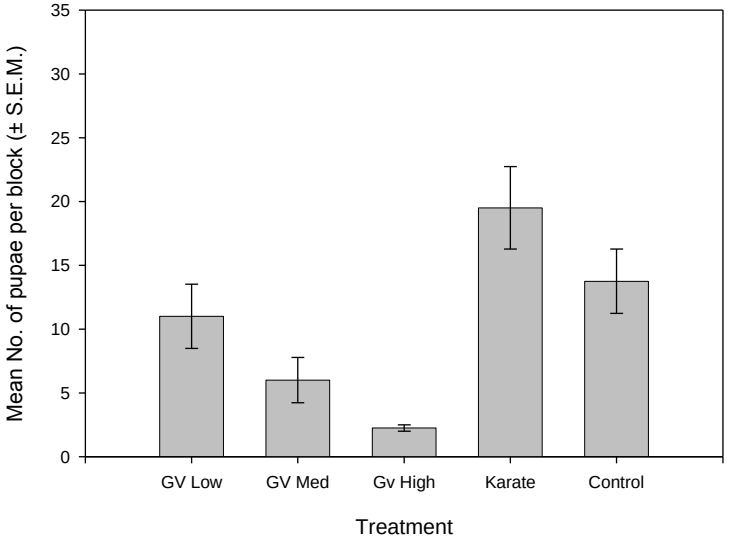
*Treatments significant at P = 0.004 F = 6.86 Df = 4 & 19
**Treatments significant at P = <0.0001F = 36.2 Df = 4 & 19. ***Treatments significant at P= 0.01 F=5.15 df 4 & 19
Means within column followed by same letter not significantly different at 5% level.

Fig 5 Graphs of DBM pupal numbers in PlxyGV application rate trials at Thika and NARL long and short rains trials in 2000.

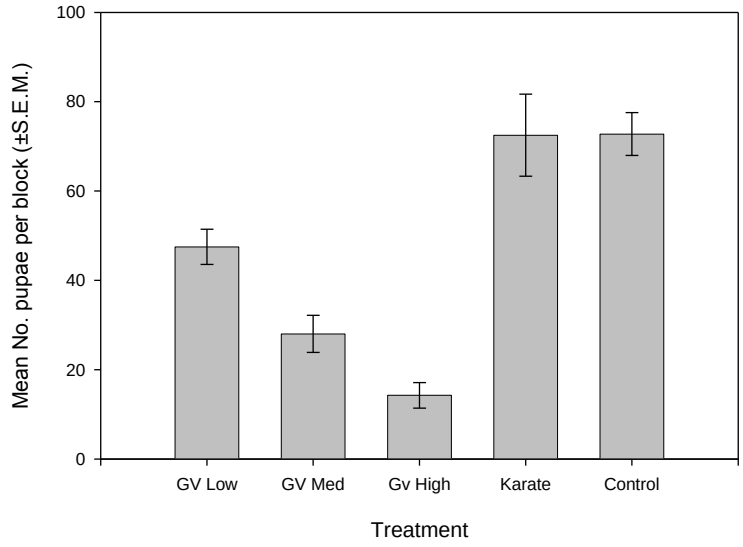
Effect of treatment on total pupal numbers.
application rate trial, Thika 2000, Short Rains.



Effect of treatment on pupal numbers.
Application rate trial. Thika 2000, Long Rains.



Effect of treatment on pupal numbers.
Application rate trials. NARL 2000, Short Rains.



Effect of treatment on pupal numbers.
Application rate trial. NARL 2000, Long Rains.

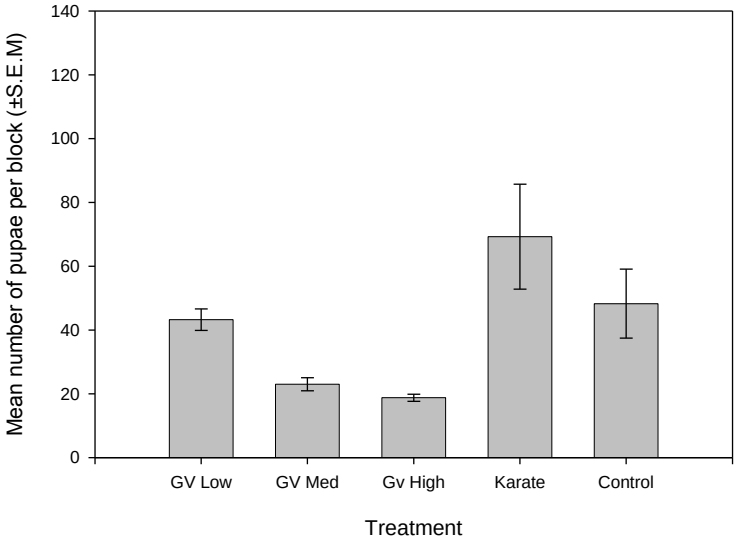
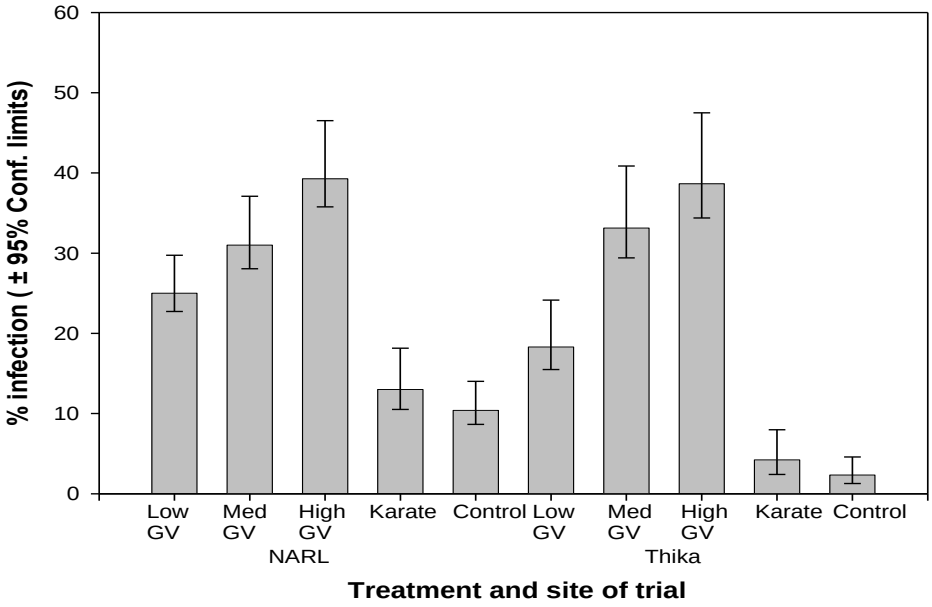


Fig 6 Observed average infection rates in field trials testing different PlxyGV rates compared to Insecticide (Karate) and unsprayed control.

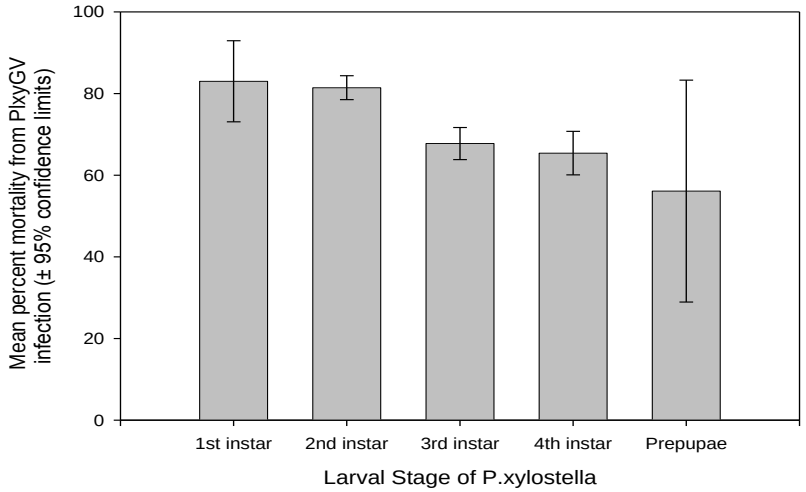


In Figure 5 a summary of the data on rate of *Plxy*GV infection from two trials both at NARL and Thika in the long rains are shown. This figure shows the average percentage of larvae collected on the weekly plot sampling that were observed to be showing overt *Plxy*GV infection. The overall average infection rate ranged between 25-40% in *Plxy*GV treatments with the highest mean infection rate of 40% seen in the high application rate plots. Background levels in unsprayed and insecticide plots are much lower. This could represent natural background infection rate but in small plot trials some movement of virus from the virus treated plots through the movement of infected insects cannot be eliminated as a possibility. What this clearly shows is application rate has an effect that the low and intermediate application rates do not produce the highest infection rate observed.

A mean observed infection rate of 40% would appear not to be high enough to give acceptable DBM control. However the symptoms of infection by *Plxy*GV are only apparent in the last two days prior to death of the 4-6 day infection cycle in DBM larvae. An observed rate of 40% would represent a much higher real rate of infection. To quantify the true rate samples of larvae were collected from sprayed plots and reared in the laboratory to disclose the real infection rate. The results for a batch of insects, characterised by instar, taken from plots sprayed with 3 $x10^{13}$ OB ha^{-1} as part of the rate trials are presented in Figure 6. This sample group of larvae was recorded as having an observed infection rate of 28% so is representative of larvae from plots sprayed at the medium rate in which mean infection rates were 28-35% (Fig 6).

The data presented in Fig 7 show that true infection rates are much higher with infection rates within instars ranging from 83-58%. Early instars are more susceptible while mean rates of infection of >80% but even in later instars that are known to be more resistant infection rates of 58-65% were found in this sample.

Fig 7 Percent mean mortatity from PlxyGV in different larval instars collected from field trials sprayed with 3 x10^{13} OB/ha PlxyGV



From this and we may conclude that the *Plxy*GV is indeed infecting a high proportion of larvae and that infection rates in larvae sprayed with the medium and high application rates is reaching more than 80% for early (1st & 2nd) instars. This result may be considered in relation to the laboratory finding that the first three instars of DBM larvae had susceptibilities to a Taiwanese *Plxy*GV that were relatively similar with LC50 of 3.29, 24.9 and 46.4 OB per insect (Abdul Kadir et al 1999). Whereas with many other baculoviruses, LC50s for later instars are several orders of magnitude higher than for 1st instar (Jones 2000) which is why successful control with some of these baculoviruses is more problematical. This relative similarity in different instar LC50s led Kadir et al (1999) to suggest that a single dose rate of a virus could in theory achieve a similar level of control in all these instars (Adbul Kadir *et*.al., 1999). A suggestion that the findings reported here appear to support.

This data indicates that increasing application rates of *Plxy*GV progressively reduces pupal numbers below that seen in the control but has relatively little impact on larval numbers as monitored in these trials. These observations would be consistent with what we know of the pathology of *Plxy*GV. Thus infection takes 4-5 days to kill so that even in populations with a high degree of successful infection, some early instars are still seen in the crop but later instars and pupa are largely absent. The results for all trials show no significant difference between larval and pupal numbers between the medium rate of *Plxy*GV 3 x10^{13} OB ha and the high rate of 3 x10^{14} OB ha.

The failure to see major reductions in larval numbers on the plant when *Plxy*GV is sprayed even at high rates when pupal numbers are reduced by 84% in comparison to controls may not be that surprising. Larval life is 10 days at ambient temperatures in Kenya and *Plxy*GV takes 4-5 days to kill. So even with 100% infection of L1 and L2 no reduction in L1 or L2 numbers would be seen as 90% of DBM larvae pass through this stage within four days (D Woodward pers. comms.) Deaths due to *Plxy*GV only occur during the L3 and L4 stages so that the only observable reduction in larvae is among the L3 and L4. As there is natural mortality at all stages and the size of the instar populations reduces progressively the L3 and L4 groups may only represent a small proportion (20-30% of the total larval population). Even therefore with a highly effective baculovirus total larval reduction of 10-20% would be all that could be expected to be observed given the weekly monitoring protocol employed in these trials. For example if the survival different instars L1: L2: L3: L4 is

represented by arbitrary populations of 100:70:40:20. If then by applying a delayed action biopesticide such as a virus 100% die before L4 and 50% die as L3 the total larval population would be reduced by (20+20)/(100+70+40+20) = 17% reduction in overall larval numbers. Published life tables on DBM from Canada show that pupae form only 12% of the total population of larvae. Data from India, which may be more comparable to Kenya, showed that less than 7% of total DBM hatching reach the pupal stage (Chelliah and Srinivasan 1986) .

The potential impact of a crop protection agent that changes population structure by reducing recruitment to later instars will in practice be much greater than the mere percentage change in the population structure would at first suggest. In a lepidopteran the final instar is the largest and can account for >80% of the total food consumed during the whole development cycle while the first two instars rarely account for >5-10% of the total feeding. Thus a slow acting crop protection agent that kills >90% of larvae before they enter this stage might, with a defoliating pest such as DBM prevent 80% of feeding damage while only reducing the standing population of total larvae by 20%.

In fact food consumption per day by 2,3 and 4th instar DBM has been estimated at 0.20, 0.50 and 0.85 cm^2 per day at an ambient temperature of 15-25 $^{\circ}$. C. (Chen and Su 1978) thus here the final 4th instar consumes up to 54% of the food consumed in the entire growth cycle. It is not only the death of larvae that may reduce damage by DBM. Work on the Chinese *Plxy*GV has shown that infected larvae eat much less food than healthy larvae by up to 46% for infected final instar larva (Lu. *et.al.,* 2003). In these trials the population sampling protocol (once a week non destructive sampling of >5% of the crop) does not allow us to state categorically that such a mechanism operating or more importantly define its numerical parameters. What can be said is that the larval/pupal population proportions are consistent with such a hypothesis, that are knowledge of larval susceptibilities and actual recorded infection rates in insects sampled from the field is also consistent with such a model.

However whether *Plxy*GV will be an effective crop protection agent will be determined both by the efficiency it operates at, and the economic injury threshold of the crop that will be crucial. Under some scenarios such as a low cosmetic damage threshold and minimal natural enemy pressure even a 95% reduction in late instars through pathogen induced mortality may not prevent levels of damage unacceptable to the farmer.

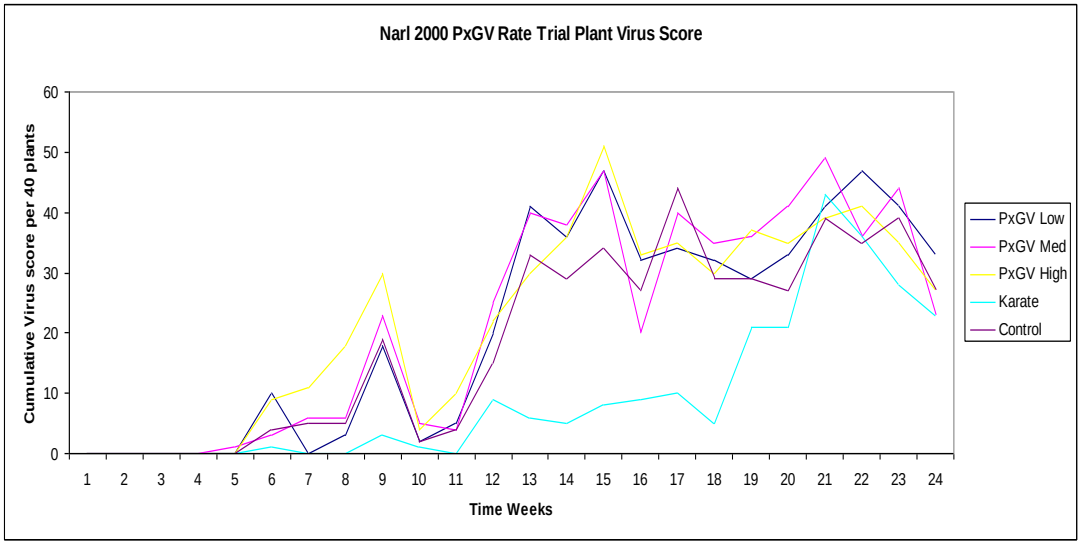
The key question for microbial pathogens such as baculoviruses, fungi, nematodes or infective (as opposed to toxic) bacteria is, can such agents that take time to kill provide effective crop protection agents? In the final instance this determination can only be completed in pilot field trials on actual farms using farmer crop acceptability criteria as the final determinant. Such trials are planned as the earliest field activity of the follow on commercialisation project (R8217) being lead by Dudutech and should be underway by December 2002.

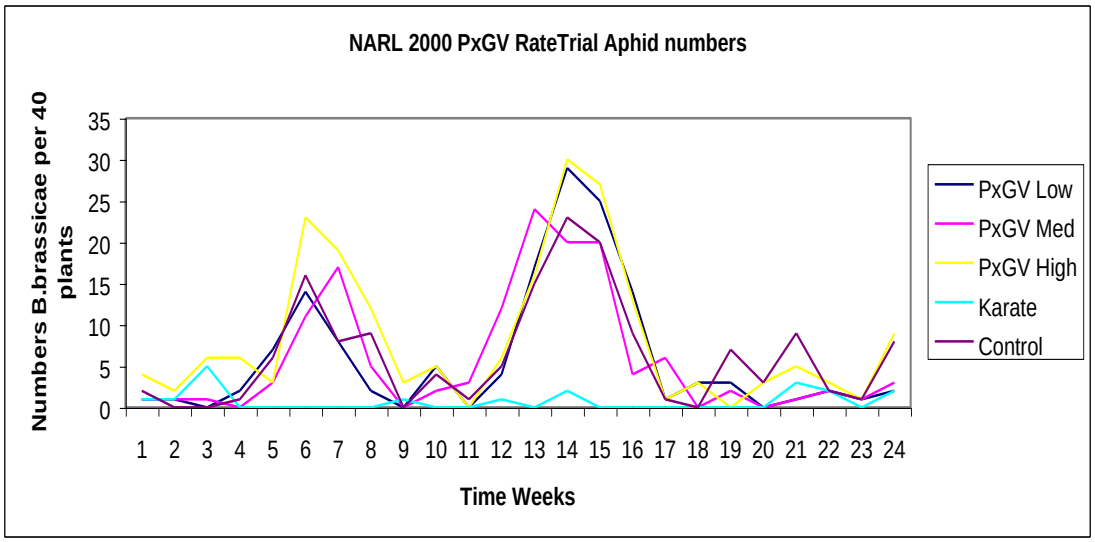
Effect on Aphids and non-target fauna

As can be seen from Fig 8 the use of Karate appeared to delay the onset of the plant virus diseases. The figures here are a score for obvious symptoms of virus diseases not differentiated into separate diseases. In Kenya Brassicas the two predominant plant virus diseases are turnip mosaic virus (TMV) and cauliflower mosaic virus (CMV). The prevalence and impact on yields of these diseases is currently being studied under project R7571 led by Dr N Spence. They are both vector transmitted

by aphids and the highly effective aphid control shown by Karate (Fig 8) would probably explain the low incidence of these diseases in the Karate treatments.

Fig 8 Graphs of virus disease scores and aphid numbers in virus application rate trials at NARL 2000





However it can also be seen that the incidence of aphid vectored disease does eventually rise in the karate plots after week 18. This may represent a gradual spread from adjoining non-karate treated plots via aphid populations that have built up in the adjoining plots. However it is more probably a result of mechanical vectoring, both CMV and TMV can be mechanically vectored on the hands of the kale harvesters as they moved from plot to plot. Harvesting in the trial in Fig 8 NARL LR 2000 started 9 weeks after the start of the trial and continued at approximately fortnightly intervals there after. Virus disease in the karate plot occurred rapidly after week 18 and this followed major aphid population peak in non-karate plots during week 14. This would be consistent with an over spill of aphids around week 14 spreading to karate plots initiating TMV and CMV infections whose symptoms become progressively observable in the plants of the Karate plots from week 18 onwards (N Spence pers comms).

As can be seen from the sample set of data shown in Table 6 *Plxy*GV has no adverse effect on non-target fauna as exemplified by syrphids in two of the trials.

The natural enemy data from all the trials was consistent with the syrphid prevalence data presented here in showing no significant negative impact of *Plxy*GV on natural enemies. This finding agrees with what is known about the high specificity of baculoviruses such as GV. A recent paper on the non-target impact of baculoviruses reviewing nearly 50 years of working studying the use of baculoviruses as crop protection agents concluded they have no impact on non-target species (Cory *et.al.,* 2003). Thus baculoviruses such as BV are among the most environmentally safe crop protection agents. This is in sharp contrast to broad-spectrum insecticides such as Karate that are toxic to syrphids, spiders and a wide range of arthropod predators and parasitoids (Cooper 2001).

Table 6 Total numbers of syrphids observed per plot in application rate trials during 2000 by treatment

Treatment	PlxyGV Low rate 3 $x10^{12}$ OB ha	PlxyGV medium rate3 $x10^{13}$ OB ha	PlxyGV High rate3 $x10^{14}$ OB ha	Karate	Control
Site/trial					
NARL LR	26	28	39	2	16
Thika LR	16	27	31	1	19

Overall from these application rate trials given the very low numbers of DBM in all trials it is not possible to be conclusive about the rate of *Plxy*GV that gives acceptable DBM control. However if all the data is considered the two lower rates would seem to be producing lower than optimal control of later instars (as indicated by pupal survival) and infection rates in the field. Thus it would appear that an application rate of *Plxy*GV equivalent to 3 $x10^{14}$ OB ha^{-1} is the minimum that would give maximal rate of infection and a standard application rate for further trials.

Persistence of *Plxy*GV in the field

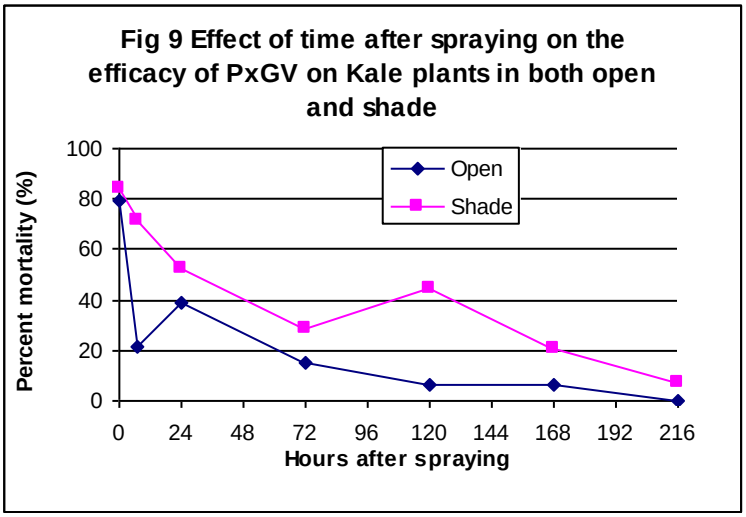
An important property determining the application intervals for a biopesticide is the persistence of the agent on the target crop. baculoviruses such as GVs or their close relatives NPVs generally have relatively short persistence times of 1-5 days on exposed leaf surfaces. This combined with their greater effectiveness against early instars often determines the application interval. On crops viruses are known to lose activity in three ways, DNA inactivation by ultra violet (UV) light, physical loss of infective particles and chemical inactivation of the particles by plant exudates.

Determining the persistence is therefore a useful exercise in helping to decide application guidelines on maximum interval between applications. Knowing the mechanisms operating to reduce persistence in specific crops can be useful as it helps the formulator to decide which type of additives might appropriately be added to the formulation or tank mix to improve performance e.g. UV blockers, stickers, specific chemicals to neutralise injurious plant exudates

A trial to investigate *Plxy*GV persistence was carried out on potted Kale plants spayed with a medium rate equal to 3 $x10^{13}$ OB ha^{-1}. After spraying plants were put either in the shade or open sunlight. Sample leaves were collected after 0, 7 hours, I day, 3 days, 5 days and 7 days. These were immediately bioassayed to determine the residual

*Plxy*GV activity remaining on the leaves (for detailed methods see Ogutu et al 2002 appendix 8).

The results of the persistence study are presented in figure 9. The activity of the *Plxy*GV on unshaded plants declines rapidly by two thirds within the first 7 hours



corresponding to daylight time on the first day of exposure. Plants in the shade also show a fall off but this is less rapid and the virus retains significant activity even after 5 days (120 hours). The slight rise in activity seen between 7 hours and 24 hours is curious and may be experimental error in this single replicate experiment. Although other researchers have reported that after exposure of other insect viruses to full daylight some recovery of activity can be seen after overnight darkness (Jones 1988). It was hypothesised that there is some repair mechanism that can partly repair the UV induced damage to the viral DNA. The other slight rise at 5 days may be an example of *Plxy*GV recycling from insects on the plant that infected on day 0. This would fit in with what we know about the dynamics of *Plxy*GV production, as the first progeny virus would appear 4-5 days after initial infection.

The main result of interest was to confirm that GV persistence is short and that most activity disappears between 8-72 hours after spraying. It is known that DBM larvae are most sensitive to *Plxy*GV in the L1-L2 stages that last for 5-6 days after hatching. Thus the seven day spray interval chosen in these trials for the *Plxy*GV applications would appear to be an appropriate application interval. These persistence results are similar to those for NPVs, closely related to GVs, used as bioinsecticides in the tropics (Cherry *et.al.,* 1997, Jones *et.al.,* 1993). Longer spray intervals would not be advisable unless a way can be found to very significantly increase the persistence of the *Plxy*GV (>10 days) by formulation.

One interesting finding was that UV might not to be the only important factor in loss of activity as considerable activity is lost on shaded plants in the first 24 hours. Previous work reported by Richards and Payne (1982) on *Mamestra brassicae* GV on cabbage found that physical loss of GV OB was relatively unimportant on cabbage. It may therefore be hypothesised that UV inactivation and chemical inactivation of the virus by plant surface exudates are major factors in reducing *Plxy*GV persistence.

Formulation of PxGV

From the field application rate trails discussed above it was concluded that a high rate of 3 x10^{14} OB ha would be required to get maximal infection in DBM. This was with simple unformulated suspensions of *Plxy*GV. This rate would be equivalent to using the virus production of 3,500 infected larvae per ha (see activity 2.3 on production). For comparison existing commercial baculovirus products are applied at rates of between 50-1000 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} (Copping 1998). This may suggest that an application rate of 3.0 x10^{14} OB ha^{-1} would be much higher than is economically viable.

It has been shown experimentally that the addition of molasses to a GV formulation can increase the virus's efficacy by a factor of ten and allow for a consequent reduction in the application rate of *Plxy*GV. (Abdul Kadir 1992, Ballard *et.al.,* 2000). Thus it was decided to carry out some trials with formulation additives to see if the virus application rate could be reduced. There was also some data from work with baculoviruses in India that the addition of neem at sublethal levels could also potentiate the action of baculoviruses (Rabindra *et*.al., 1997). A range of additives of low cost locally available in Kenya including neem and molasses were evaluated in a series of laboratory bioassay trials using the standard leaf dip assay (Ogutu 2002).

Formulation with Neem/Molasses

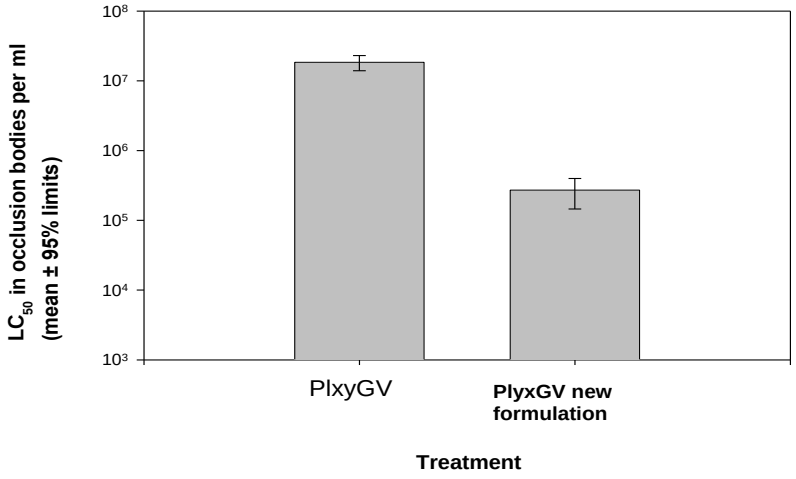
A series of bioassays were conducted to test candidate formulation reagents using the standard DBM laboratory LC50 leaf dip trial protocol (Parnell 1999), These bioassays followed recommended protocols for baculovirus bioassays (Jones 2000). Formulation additives tested included a number that had been shown to work with baculoviruses (Abdul Kadir 1992, Burges and Jones 1998) concentrating on those readily available in Kenya. The results of these assays were to show that molasses used at one percent consistently increased *Plxy*GV potency. Neem oil added at a sublethal concentration of 0.1% to the molasses formulation also had some synergistic effect on PlxyGV, although this was a much less consistent finding than with the neem. The latter result was somewhat surprising as neem was widely reported to have a strong antifeedant effect on insects (Copping 1997) and might therefore be expected to reduce ingestion of virus particles and reduce the effectiveness of a formulation. However there were a number of reports from Indian scientists that suggested neem could at non toxic concentrations enhance baculovirus pathogenicity by reducing the infective dose in lager insects (Rabindra 1997).

The laboratory results with neem were somewhat inconsistent so its value was not definitely proven in the laboratory before the start of the field trial. It was decided to include molasses and neem in the field trial of formulation where the PlxyGV was formulated at 3 x10^{13} OB ha^{-1} with 1% molasses and 0.1% neem oil. In these field trials the neem used for the field studies was a local Kenyan product Neemroc EC 0.3% WW neem oil; equivalent to 0.03% WW azadirachtin. The neem application rate was 0.1% rather than the 0.25-0.5% required for direct insecticidal action.

The laboratory bioassays of simple molasses/neem formulations of *Plxy*GV showed the LC_{50} for the PlxyGV formulated with 0.1% molasses and 0.01% neem to be 3.62 x10^{5} OBs/ml compared to 3.65 x10^{7} OBs/ml with the unformulated control a reduction of x100 (see Fig 10).

This result indicated that formulation could indeed very significantly increase the

Fig 10 Laboratory LC_{50} value of the standard PxGV suspension compared to the LC_{50} of the neem/molasses formulation



activity of *Plxy*GV and perhaps enable the active ingredient level to be reduced to more economical level. The molasses itself may have a dual function being both a gustatory stimulant encouraging DBM larvae to feed on virus containing droplets thus increasing the likelihood of larvae ingesting a lethal dose in the first few hours when the virus retains maximum activity. It may also mask the antifeedant effect of the neem in the formulation so that the insect will ingest the neem. In addition molasses may also have some function as a UV protectant (Burges and Jones 1998).

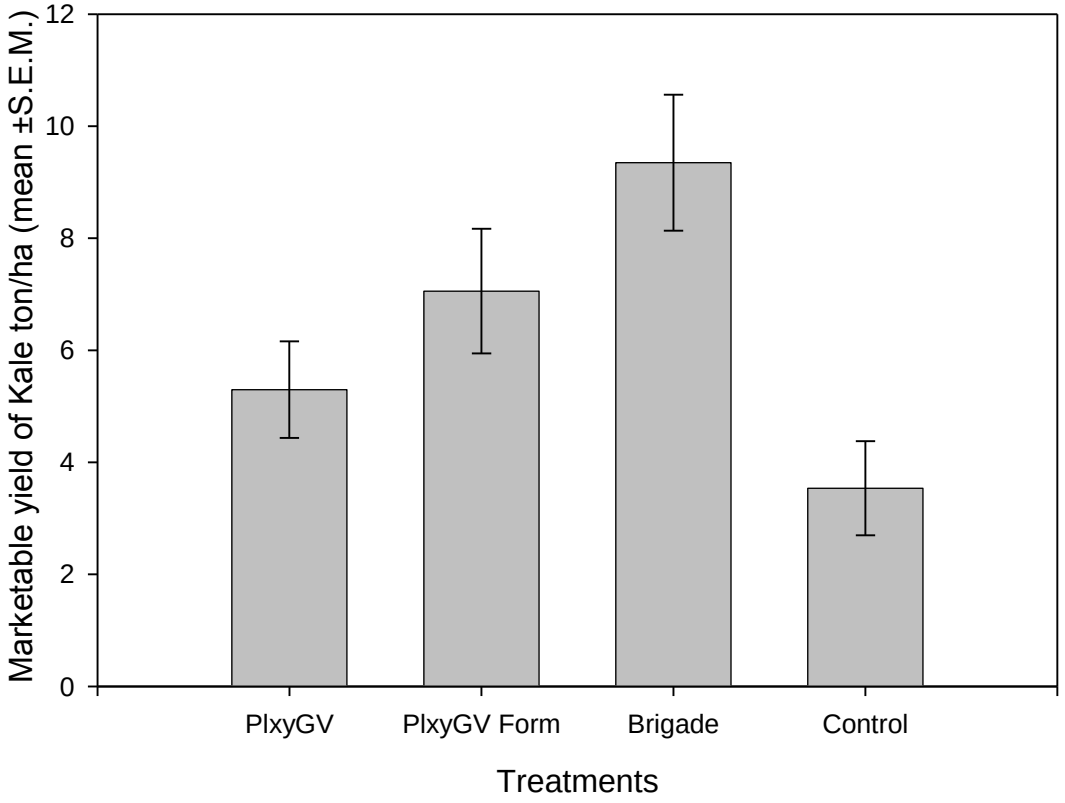
It was decided therefore to conduct next trials of the *Plxy*GV formulation in the field using reduced application rates to see if the same positive effect of formulation was seen in the field.

Testing improved formulation of *Plxy*GV in the field

Two sets of field trial were conducted to test the new formulation out at the two field stations used before. The methods are detailed in Appendix 8 (Ogutu *et*.al.,. 2003). Although it was recognised that the Thika site in particular appeared to have a low incidence of DBM it was not possible to use an alternative site due to the regulatory restrictions on the use of *Plxy*GV at that time.

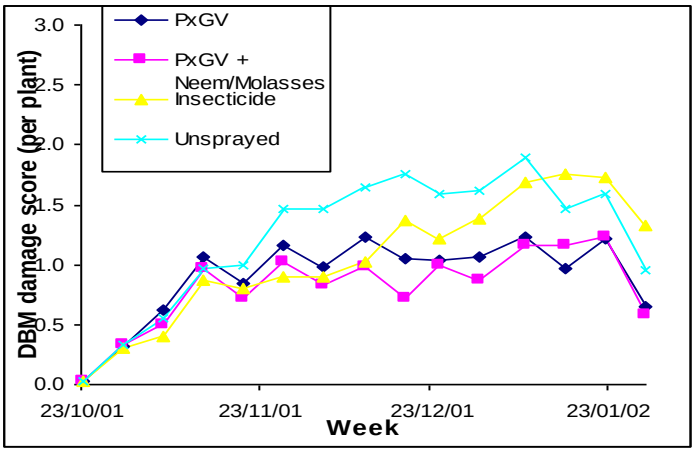
In this series of trials the conventional insecticide comparison used was a newly introduced insecticide Brigade® (Bifenthrin) at the recommended rate. This had replaced Karate as the KARI recommended insecticide for Brassica IPM as the increasing resistance of DBM to Karate had been clearly documented by work carried out under the earlier CPP projects R6616 & R7413. Bifenthrin is a newly introduced pyrethroid to which there was as yet no evidence of resistance in Kenya. A major drawback to Bifenthrin though was its high cost which prevent its widespread adoption by farmers many of whom who continue to use the less effective Karate.

Fig 10 Effect of formulation of PlxyGV on marketable yield: Results of field Trial comparing effect of PlxyGV at 3 x10^{13}, PlxgGV at 3 x10^{13} formulated, Insecticide(Brigade) and unsprayed control NARL short rains 2002 on marketable yield of Kale.



The results (Fig 11) when analysed showed significant treatment effect (p = <0.001 F = 5.02, df = 3 & 23). The only significant individual treatment differences was between Bifenthrin and the control (t = 3.96 p = 0.03) and *Plxy*GV formulated and the control (t = 2,52 df 10 p = 0.03). DBM damage scores in this trial (Fig 12) were lowest in the two PxGV treatments in the latter half of the season but higher in the control and Bifenthrin treatments. The results could be interpreted as suggesting that the formulation is having a positive effect on *Plxy*GV efficacy but that this is still not producing yields equal to that obtained with the insecticide Bifenthrin.

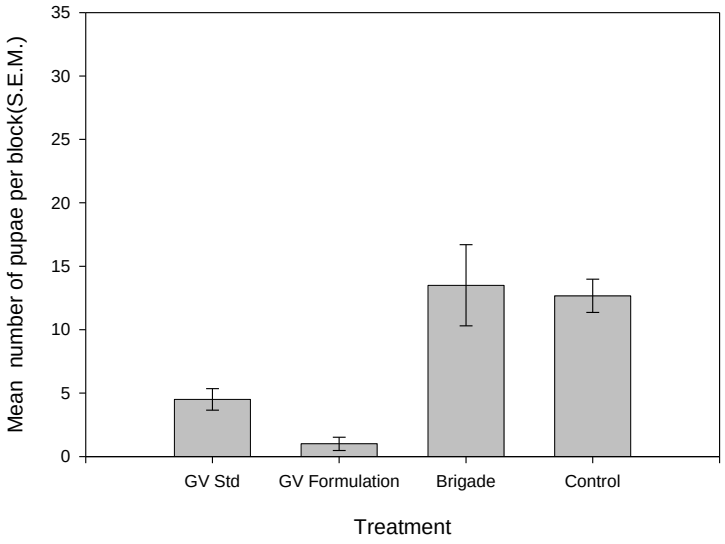
Fig 12 Record of DBM damage in NARL 2002 SR trial



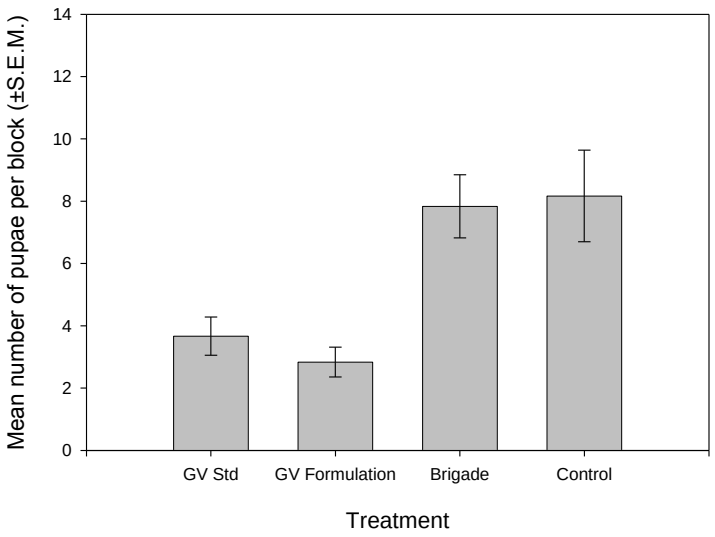
What cannot be determined precisely from this trial is how much of the greater yield with Bifenthrin was due to DBM control and how much due to control of other pests. *Plxy*GV only controls DBM but Bifenthrin is active against a wide range of pests.

Fig 13 Graphs of pupal numbers in field trials of PlxyGV formulation mean pupal numbers per block (±S.E.M.) for different formulation of PlxyGV, insecticide and Control.

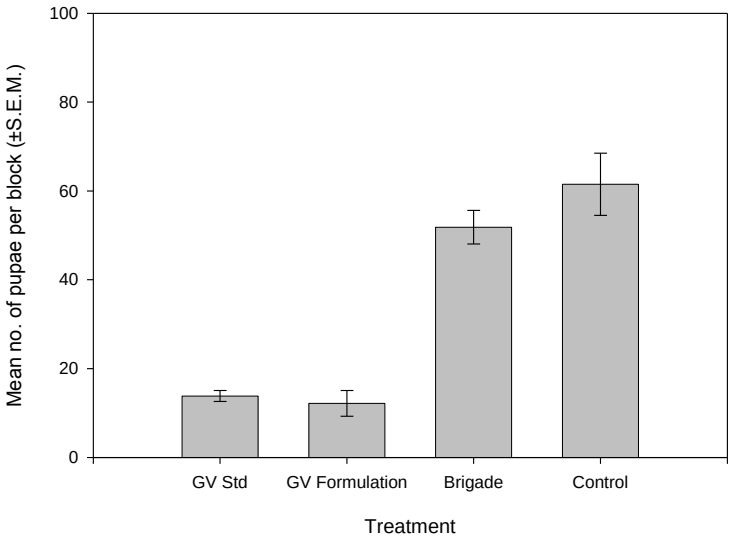
Effect of treatment on pupae. Formulaton trial, Thika short rains.



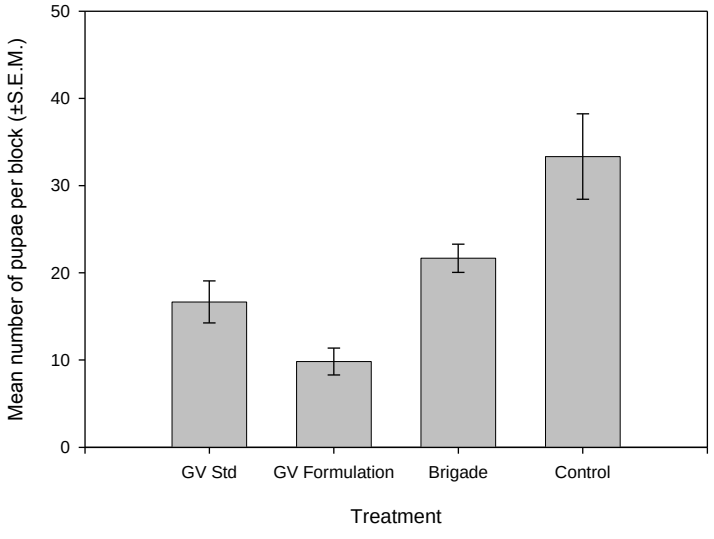
Effect of treatment on pupae. Formulation trial, Thika Long Rains.



Effect of treatment on pupae. Formulation trial, NARL Short Rains



Effect of treatment on pupae. Formulation trial, NARL Long Rains



The data on the DBM damage certainly suggests that Bifenthrin did not seem to give as effective DBM damage control as either of the *Plxy*GV treatments in the later part of the season (Fig 12). Bifenthrin is still one of the pesticides recommended to growers in Kenya for DBM control. These results indicate that it is not effective in

this role and thus resistance may already have appeared or cross resistance to some other previously used pesticide may be operating.

The pupal results again show very effective suppression of DBM pupae by PlxyGV though not with Bifenthrin (Fig 13).

Bifenthrin did give however total control of aphids and a significantly reduced plant virus disease score ($p = 0.012$, $F = 2{,}51$, $df = 3$ & 23) Table 7. That aphid occurrence and plant virus disease incidence are correlated is no surprise as in many brassicae plant viral diseases vector transmission by aphids is an important route of infection.

Table 7 Average Aphid numbers and plant virus scores for plants in NARL 2002 SR trial.

Treatment	*Plxy*GV	*Plxy*GV formulated	Bifenthrin	Control
Aphid numbers per plant	0.233	0.133	0.00	0.117
Plant Virus scores	1.36	1.35	1.067	1.350

In the long rains trial only the insecticide gave a significantly higher yield (Fig 14) and there was no significant difference in yield between the improved formulation of *Plxy*GV and unformulated *Plxy*GV or the control. This absence of any effect of either *Plxy*GV treatment could be a reflection of low overall losses due to DBM pest pressure in this trial. The is backed up by the data on the marketable yield as percentage of total were much higher than the earlier formulation trial ranging from 62-76% while in the SR trial above marketable yield was only 24-50% of total yield. DBM damage scores were also consistently low and showed no differences between treatments.(Fig 15)

Fig 14 Effect of *Plxy*GV formulation on marketable yield
NARL 2002 Long Rains trial

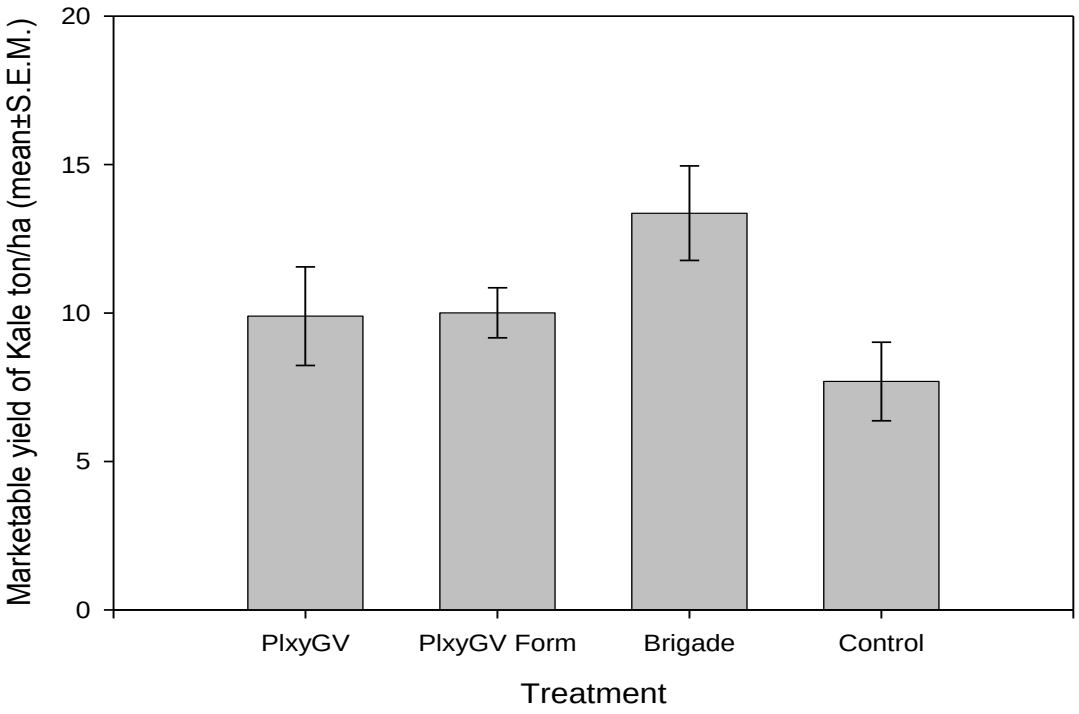
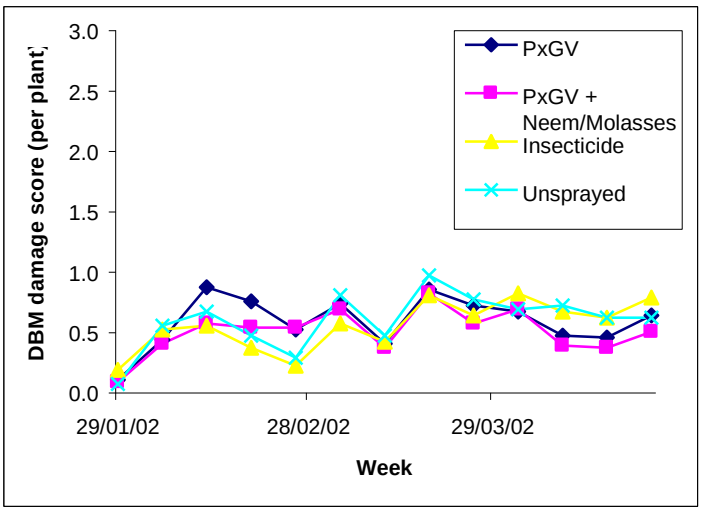


Fig 15 DBM damage scores NARL 2002 LR



In this trial (table 8) there were very significant differences (p = <0.001 F = 2.93 df = 3 & 23) in aphid numbers between Bifenthrin and the other treatments and aphid scores were much higher than in the earlier short rains trial. This could be an indication that much of the yield difference seen was negatively correlated with the size of the aphid population. It could also be related to the effects of plant viruses, which are aphid transmitted, as plant virus scores in this trial showed significant differences between treatments (p=<0.001, F = 2.37, df = 3 & 15) with the Bifenthrin having the lowest virus score.

Table 8 Aphid and plant virus scores in NARL long rains trial 2002

Treatment	PlxyGV	PlxyGV formulated	Bifenthrin	Control
Aphid numbers per plant	0.467	0.35	0.0	0.45
Plant Virus scores	1.317	1.267	0.850	1.250

Two sets of trials with improved formulation were conducted at Thika as duplicates of the NARL trials. In the short rains trial (Table 9) the results showed no significant differences in yields between treatments and the unsprayed control yields. One could conclude therefore that there was no evidence of significant pest pressure (DBM or aphid) during this trial.

Table 3 Yield results for Thika trials short rains

	*Plxy*GV	*Plxy*GV formulated	Bifenthrin	Control
Mean yield ton/ha (±SEM)	20.77(1.55)	19.15 (2.03)	18.86 (0.71)	20.67 (1.38)

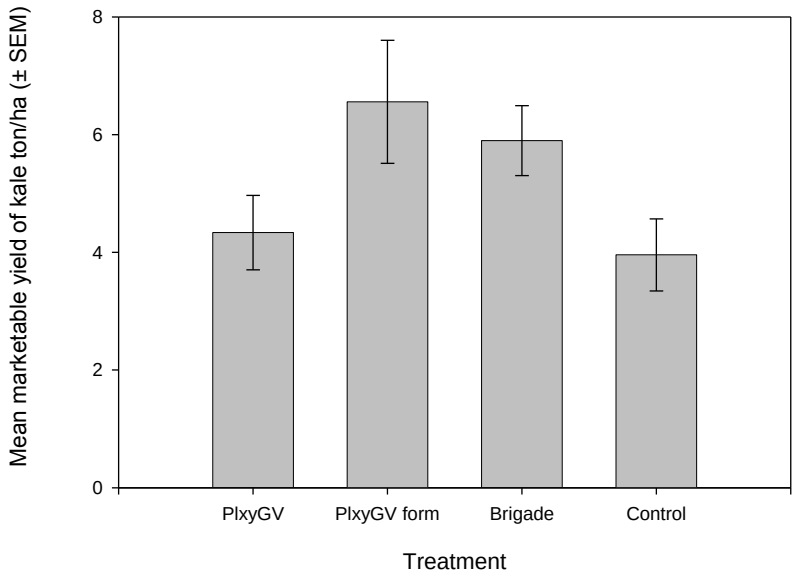
In the Thika trial carried out during the long rains the yields (fig 16) did show significant treatment differences (P = 0.001, F = 11.06, DF = 3 and 23). The highest yield was in the PlxyGV formulation treatment, though this was not significantly different to the Bifenthrin.

Once again the data showed that Bifenthrin had a marked effect on aphid occurrence and plant virus scores (table 10).

Table 10 Average Aphid numbers and plant virus disease scores for trials at Thika during long rains 2002

Treatment	*Plxy*GV	*Plxy*GV formulated	Bifenthrin	Control
Aphid numbers per plant	0.467	0.333	0.00	0.450
Plant Virus scores	1.617	1.60	0.933	1.55

Fig 16 Thika long rains 2002 effect of formulation of PlxyGV on marketable yield of Kale



To draw definitive conclusions from these formulation trials is not easy as few significant yield differences between treatments were obtained in these trials.

The improved formulation appeared to improve the efficacy of *Plxy*GV in reducing pupal numbers in two of the four trials. From the data here it is not possible to determine how close the *Plxy*GV application rate of the formulation used here, 3 $x10^{13}$ OB $^{ha-1}$, is to the optimal for DBM control. In order to isolate the effect of other pests in order to determine the optimal application level for *Plxy*GV it should be compared to an effective but lepidopteran specific insecticide of proven efficacy against DBM (such as a *Bacillus thuringiensis* product). Alternatively in order to compare *Plxy*GV to a broad spectrum insecticide it would be necessary in a trial to use a *Plxy*GV formulation that includes Pirimor to control aphids that *Plxy*GV does not effect.

There is therefore still a need to carryout further trials to determine a minimum effective application rate for the improved *Plxy*GV formulation. Without such a trial it is not possible to compare the likely costs of *Plxy*GV to the alternative chemical controls. Such trials are now due to be undertaken by the follow on project to commercialise the *Plxy*GV being undertaken by the Kenyan company Dudutech.

Subsequently further laboratory work (Ogutu 2002b) has not shown a consistent additive value to including neem. Without further detailed validation of its efficacy its incorporation into PlxyGV formulations therefore cannot be recommended. This decision is also influenced by the well-recognised difficulties in chemically standardising neem extracts and the highly variable nature of the chemical constituents in simple neem extracts from different sources/locations/cultivars. Given these uncertainties it would be impossible to make firm recommendations concentrations of neem that should be used in a tank mix with PlxyGV.

Trials with *Plxy*GV applied through V lance applicator

Project R7413, is promoting the adoption of the more efficient V lance applicator for conventional hydraulic sprayers as this improves the targeting of insecticide especially on the leaf under surfaces where young DBM larvae tend to feed. Its use with *Plxy*GV seemed worth investigating to similarly improve *Plxy*GV targeting. In addition it was hoped that greater under leaf application of the GV might reduce the UV inactivation and increase GV persistence. The use of the new V lance was tested in four season long field trials small plot trials at Thika and NARL. In this trial the virus application rate used in the previous virus rate trials of 3 $x10^{13}$ OB ha^{-1} used with the addition of molasses and neem formulation additives. The check insecticide used was Fipronil as Regent (50 SC) at the recommended rate of 500 ml . per hectare. Fipronil is one of the newer insecticides introduced into Kenya and is highly effective against pyrethroid resistant DBM (J. Cooper per. comms.). The trial format was the same as that for the previously described formulation trials with 3.6 x4.2 metre plots with each treatment replicated six times. Applications were weekly as was monitoring again as per earlier trials.

The overall yield results showed no significant increase in yields or lower DBM populations was experienced when using the V lance in comparison to the standard conventional lance (Table 11).

Table11 Effect of applying *Plxy*GV through V lance and conventional spray lance on mean yield (ton ta^{-1}) of Kale, average of 6 replicate plots per site

Trial site / season	Yield of Unsprayed Control	Conventional Lance	V Lance
Thika 2001 long rains	9.93	8.92	9.06
NARL 2001long rains	41.74	44.20	40.36
Thika 2001 short rains	34.06	31.49	34.60
Thika 2001 short rains	21.77	23.19	25.45

However as can be seen by comparing to unsprayed controls their was no significant difference in yield between plots sprayed with *Plxy*GV or the unsprayed controls suggesting DBM damaged was not a significant factor affecting yield in these trials. This makes an assessment of the value of the V lance as a delivery system for *Plxy*GV very difficult from this yield data.

Examination of the larval numbers in one trial at NARL during the 2001 short rains reveals that their was no significant difference in the numbers of DBM between *Plxy*GV applied by conventional lance and V lance (Table 12). Fipronil was effective at reducing DBM numbers but again there was no significant difference between the V lance and conventional lance. The same result was obtained in the other four trials

Table 12 NARL Short rains V lance trial 2001 Total DBM larvae per plot

	*Plxy*GV CL	Fipronil CL	*Plxy*GV VL	Fipronil VL	Control
	650	187	504	160	423
	437	169	360	133	467
	386	104	363	123	279
	417	95	334	140	408
Mean	472.5a	138.0b	390.3a	111.0b	394.3a
SD					

Treatments significantly different F= 16.2 P= <.0.0001 Df = 4 &19

If the pupal numbers data are examined for the same trial (Table 13) again there is no difference in the efficacy of the conventional lance or the V lance when used to apply *Plxy*GV or Fipronil. However the DBM pupal numbers for PlxyGV are not significantly different to those in the Fipronil treatments. This indicates that PlxyGV as effective as Fipronil in killing DBM before they can become pupae and the same result was seen in all the V lance trials (See Fig 17)

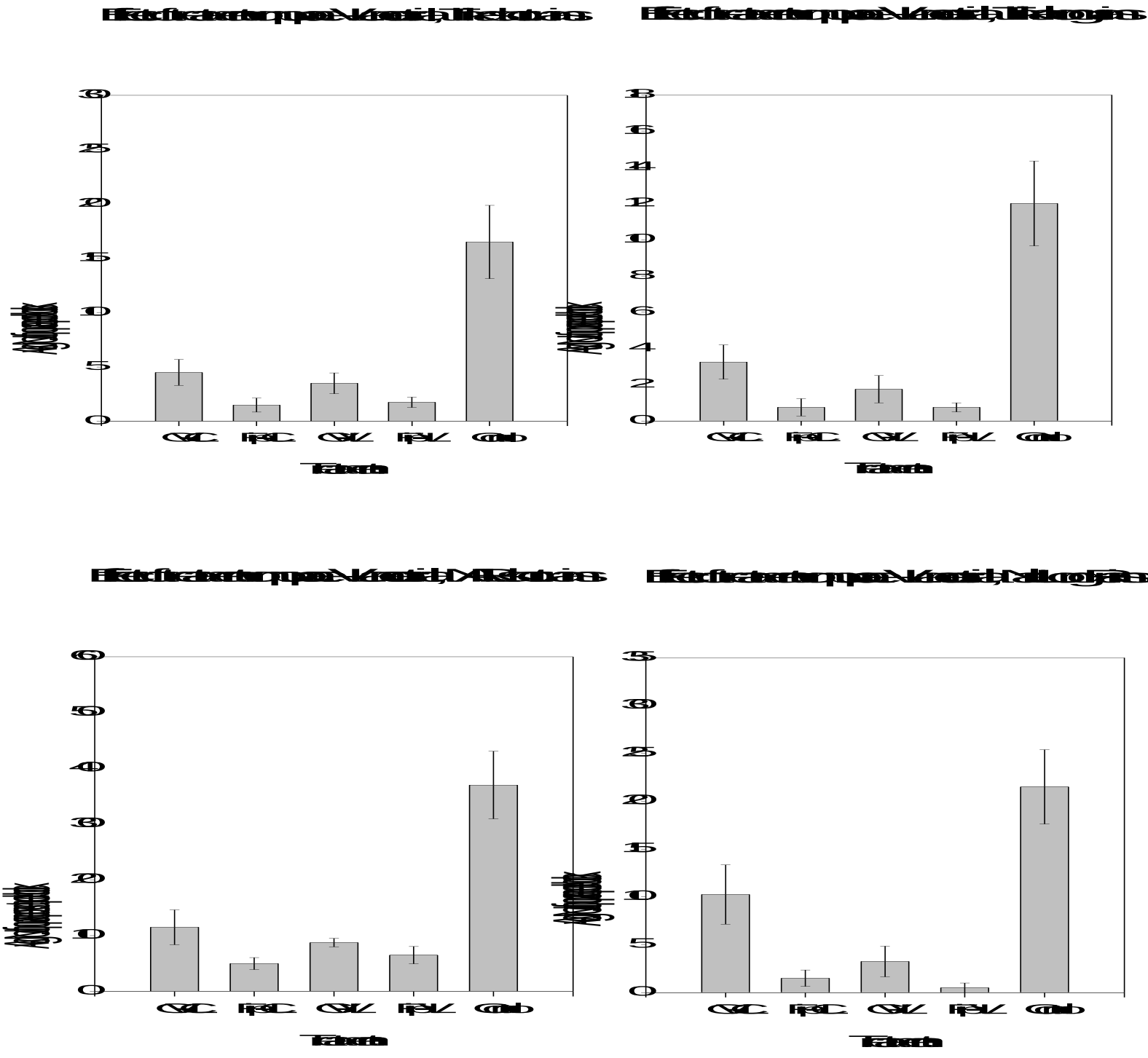
Table 13 NARL short rains 2001 Total DBM pupae per plot

	*Plxy*GV CL	Fipronil CL	*Plxy*GV VL	Fipronil VL	Control
	18	4	7	5	30
	12	3	10	4	27
	13	3	10	5	37
	3	5	8	11	54
Mean*	11.5a	5.09a	8.75a	6.5a	37.0b
SD	6.25	2.66	1.5	3.11	12.08
**Log Mean	0.981a	0.670a	0.937a	0.780a	1.552a

*Non Normal variance Kruskal Wallace P = 0.016 H = 12.2 4 Df.
** Sig. F = 11.1 p = < 0.0002 DF 4 &19

No observable effect of PlxyGV or Fipronil on aphid numbers or occurrence of plant virus was found indicating neither has any effect on aphids..

Fig 17 Graph of pupal numbers in plots treated using PlxyGV and Fipronil with different application systems comparing conventional lance and new V lance



Analysis of the observed infectivity rate in all these four trials showed that there was a small but significant increase in the overall *PlxyGV* infection rate observed with the V lance (38.9%) as opposed to that seen with the conventional lance (33.2%) (P= 0.028, F= 5,32, df = 31 &1). Both were much higher than the GV infection rate seen

in the unsprayed controls (3%).. However the pupal and larval numbers data would appear to indicate that this slightly higher rate has little overall impact on PlxyGV effectiveness. A conclusion would be that one could not recommend farmers to invest in V lance solely to improve PlxyGV effectiveness but if the equipment was available it should be used for *PlxyGV* application.

The most interesting result from this trial would be the result that formulated *PlxyGV* at could be as effective as Fipronil in controlling the numbers of DBM pupae.

2.3 Develop local mass production technology

PlxyGV is at the moment produced by infecting the larvae of DBM moths and multiplying the virus in these larvae in an i*n vivo* technique. All viruses currently are reared in host cells either in whole animals or in cell culture. Currently all commercial biopesticides are produced in whole insects as tissue culture of GV and other baculoviruses has yet to reach the industrial scale (circa 20,000 litres) or costs to be competitive. Although large-scale tissue culture of baculoviruses is a very active field of research it is likely the *in vivo* approach will remain for at least another decade. Of course *in vivo* mass rearing of insects is widespread in some areas of the tropics for silk culture. *In vivo* rearing is easy to establish as a small-scale operation and has been the entry technology for a number of producers in India, Thailand, China Brazil, Bolivia, and Peru, who now produce baculovirus insecticides. These are mainly NPVs mainly but some production the GV of potato tuber moth and the sugar cane borer.

Low technology production on natural diet

The *PlxyGV* used in the project trials was grown in DBM larvae reared on fresh brassica plants. This is a cheap and effective method of small-scale production and would be a viable input method for local small to medium scale production (Lisansky 1997). Similar on plant systems are used in Brazil to mass produce another baculovirus insecticide for controlling the velvet bean caterpillar and here production reaches 2 million hectares per annum at a cost of 1.24 US$ ha making it much cheaper than most insecticides (Moscardi 1999).

Work on refining this system continued as part of the project. Simple low cost means of extracting the *PlxyGV* from large numbers of larvae were developed. Productivity here was a maximum of 3.7 $x10^{10}$ OB per larva. At this rate the highest rate of field use of 3 $x10^{14}$ would require the virus contained in 8000 larvae to treat one hectare. Using the formulated lower rate 3 $x10^{13}$ would require the equivalent of 800 larva. In comparison current commercial virus insecticides require between 250-1000 larval equivalents per hectare. The lower rate improved formulation of *PlxyGV* looks potentially economically viable. The low input on plant system could produce an acceptable product using simple locally available domestic and industrial utensils and equipment without the need for expensive and imported high-speed centrifuges. One aspect of the system that still could be improved is to develop a large-scale method of inoculation.

The DBM larva is rather small (<100mg) and as costs tend to be related to the number of larvae produced rearing in a larger larva (>500mg) would have considerable cost saving implications. It had been thought that *Plxy*GV infected and replicated only in *P.xylostella*. However there were suggestions that Asian strains of *Plxy*GV could be propagated in non-homologous host (Abdul Kadir pers. comms.) specifically *Artogeia rapae*. It was felt that producing *Plxy*GV in this large easily

reared insect might lower production costs so a study was undertaken at HRI to explore this option further

Alternative Host production

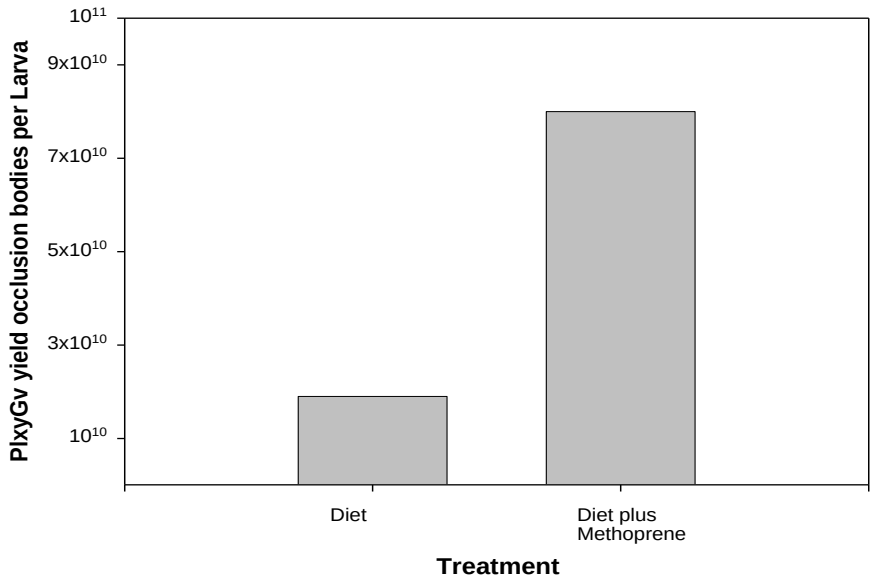
A series of experiments to were carried out to infect *A. rapae* with *Plxy*GV (Taiwan). A small percentage of larvae died of a GV infection. However when the progeny virus was characterised it was found not to be *Plxy*GV but *A.rapae* GV. It was later found that this same genotype of *A. rapae* GV could be stressed out of the insect stock using other methods. This suggested strongly that early work had not in fact obtained *Plxy*GV replication in the *A. rapae* but had stressed out an *A.rapae* latent GV. Thus in conclusion it seems that producing *Plxy*GV in another more easily reared species is not as yet a viable option.

Artificial diet based production

Most commercial viral insecticides are now produced on insects reared on artificial diets as opposed to natural plant material. These allow all the diet to be sterilised and prevent diseases entering and destroying the insect culture that supplies the virus production. Also production with artificial diet can continue as a year round activity whereas plant supplies and growing seasons often limit the plant based production to certain times of the year. It was felt that developing an artificial diet based production system would give any potential commercial producers an alternative system to the plant system that could make production more commercially attractive. Kenyan strains of *P. xylostella* have been successfully adapted to artificial diet. An adaptation of the Pieris diet was used initially. It is possible to rear thousands of *P.xylostella* in a relatively small space without cabbage plants. It is possible to produce a simpler and more cost-effective diet based on soy bean flour but this requires more development and will now be undertaken as part of the Dudutech follow on project.

Experiments at HRI showed that it was possible to inhibit pupation of *P. xylostella* by incorporating a juvenile hormone analogue into the diet. Concentrations as low as 0.0004 active parts/litre prevented pupation. As a result it was possible to inoculate larger third instar larvae with *PlxyGV* and for the infected insects to die as larger fourth instar larvae without pupating. This has increased the yield of virus by almost 4 fold compared with the yield of virus from larvae reared on plants (Fig 18). A significant step in increasing productivity. At this rate of *PlxyGV* the formulated product with an active ingredient of $3x10^{13}$ OB hectare would require the equivalent of 375 infected larvae per hectare well within the range of economic feasibility.

Fig 18 Effect of including Methoprene in artificial diet on yield of PlxyGV



Thus two options for *Plxy*GV production now exist and are available for potential producers. The artificial diet based system and the natural plant system. The artificial production system could meet international quality standards (Jenkins & Grzywacz 2000) but would require a specially modified production plant. The product would be appropriate for local sale or export. This is the system currently being adopted by the Kenyan producer Dudutech.

There is an alternative low input on plant system that would be appropriate for artisanal production of *Plxy*GV for local use by small-medium enterprises, NGOs, or farmer own production. This technique if taken up by local small enterprises could ensure a supply of product at price probably lower than that of the factory produced product but might have difficulty meeting international quality standards appropriate for the export market. While on farm home or community based production would be theoretically feasible, and has been utilised in Asia on a small scale, maintaining sustained quality would be a serious challenge and without further evaluation this approach cannot be recommended at present.

2.4 Establish registration requirements for viral pesticides

At the start of the project their was no system for registering viral biopesticides A draft registration protocol for biopesticides, such as *Plxy*GV, in Kenya has been developed and adopted by the Kenyan authorities based upon FAO guidelines. Kenya is currently harmonising this package with Tanzania, Uganda and Ethiopia so that the same product registration dossier can be used in all countries. Draft application form and data dossier are attached as appendices. Kenya has sent representatives to the international conference on harmonising African biopesticide registration in Cotonou in January 2001 and the proceedings are due to be published in 2003 (L Vaughan pers comms). However while the system is in place the actual registration of the first viral biopesticide, due to be *Plxy*GV by Dudutech will be a considerable challenge to the appropriate authorities. To support this training and a dossier of information and papers on granuloviruses and their registration and safety has been prepared as part of this

project to assist eventual registration. Also key staff in Kenya have been involved in meetings with project staff to help them acquire the necessary background to handle registration.

As part of the field trials on *Plxy*GV a considerable dossier of field data on the impact of *Plxy*GV on non-target insects, predators and parasitoids had been collected. In summary it showed no adverse impact of *Plxy*GV on any species. There is little point in presenting this extensive data set in this report but it is expected to be very important data that will be useful for the environmental Impact assessment part of the registration dossier of *Plxy*GV. A copy of the Kenya regulations for registering a biopesticide with specifications for the data needed for a registration dossier is attached (appendix 10).

2.5 On farm validation and demonstration trials of novel DBM control technology

It had been hoped to conduct on farm trials to validate the use of *Plxy*GV during the project. However delays in determining the optimum field rate meant that it was not possible to get permission from the Kenyan authorities to do research trials on farm before the end of the project. These trials are due to be carried out in the first stage of the follow on commercialisation project lead by the Dudutech company by mid 2002.

Output 3 A system of DBM control based upon pheromone mating disruption developed and evaluated for use on Kenyan farms.

The previous project R6615 had obtained promising results with a pheromone mating disruption technique for DBM during 1998. This system used a commercially produced PVC formulation "Selebate®". However the trials used a very high application rate of active ingredient (120g ha^{-1}) and also a high density of individual dispensers so that the costs of both materials and labour for application were high. Th first objective of this work was therefore to validate the earlier results and determine if the dispenser application rate and active ingredient rate could be reduced to improve the economics of the system. Trials of pheromone mating disruption of diamondback moth (DBM), *Plutella xylostella*, L. were carried out near Nairobi, Kenya from March - August 2000 and October 2001 - January 2002.

The first trial was carried out at three different sites. 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. In the treatment plots, individual dispensers were set out at a density of approximately 625 ha^{-1}. 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 (*e.g.* Fig 16.).

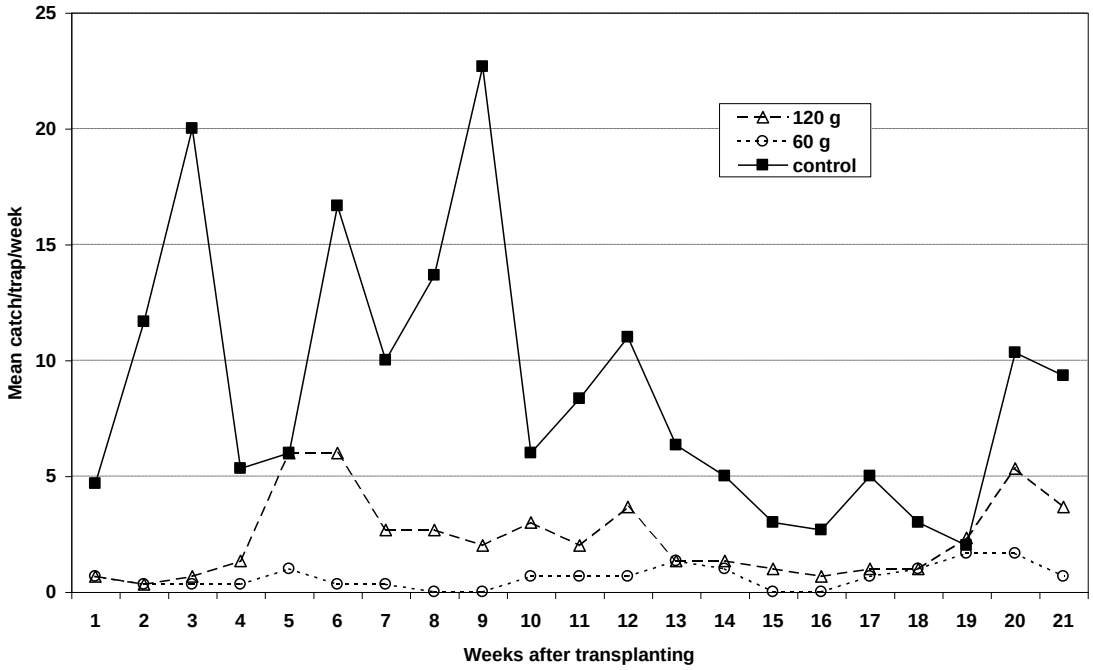


Figure 19. Mean weekly trap-catch results for trial at Jomo Kenyatta University of Agriculture and Technology (means of 3 traps for each plot).

From the results it was concluded that the pheromone treatments tested did not disrupt mating of DBM. Previous, apparently successful, results in Kenya using 60 g ha^{-1} of pheromone 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 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 (through the use of 'buffer zones'). Accordingly a second trial incorporating these changes was conducted.

The second trial was carried out at the Jomo Kenyatta University of Agriculture and Technology farm (JKUAT), one of the sites of the trial in 2000. This trial incorporated recommendations from the first trial that were intended to improve the effectiveness of mating-disruption treatment as well as the evaluation of results. Dispenser density was more than doubled to 1425 ha^{-1} in treated plots and the area application rate was 110 g a.i. ha^{-1}. 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 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. 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 fewer larvae in treatment plots (Table 6) and leaf damage scores were consistently slightly lower; total marketable yield over six harvests was 50% higher (Fig 20.). However these differences were not statistically significant.

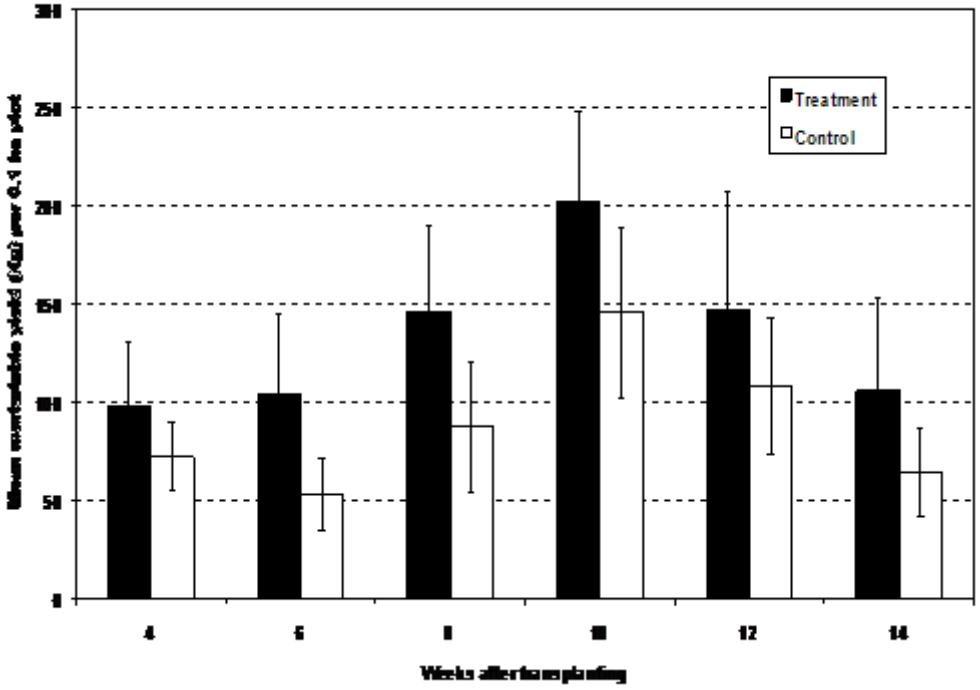
Table 14**.** Mean numbers of DBM larvae and pupae per plant sampled over first eight and all weeks of the second trial at JKUAT.

	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.

Figure 20. Mean weight of marketable yield per 0.1 ha plot (in Kg) in pheromone



treated and control plots on successive harvest dates of the second trial, JKUAT (error bars indicate standard errors of the respective means).

Data from three of four separate sets of observations to determine rates of female mating were limited for a variety of practical reasons, but overall results indicated that the pheromone treatment did not suppress mating as envisaged (Table 7). Pheromone trap monitoring again showed incomplete suppression of catches in the pheromone-treated plots, even in the early stages of the trial, and thus 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. So that the dispensers initially deployed would have been weak or even have lost effect just over half way through the Kale crop cycle. 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 the small beneficial effects were due to non-treatment related effects.

Table 15. 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.

The overall conclusions of the two trials indicate that pheromone mating-disruption of DBM in the context of small-holder farmers in Kenya is not feasible. Trials could only possibly succeed if it proves feasible to increase plot sizes, unit-area dose rates and physical isolation of plots. This is only likely to be possible on the larger commercial farms. Other successful trials reported from Japan and the USA have generally used much larger plots (> 1 ha) and higher application rates (≥ 250 g a.i. ha^{-1}). The economic feasibility of such high application rates given the current costs of DBM pheromone mix – even if technically successful – appear doubtful.

Output 4 A system for aphid control compatible with both pheromone and virus technologies identified.

It had been recognised from the outset of this project that in developing a new brassicae IPM system based upon specific non chemical insecticide technologies, both pheromone and *Plxy*GV, there would be a need to develop compatible solution(s) to aphid problems. Aphids had been identified prior to the project, as the second most important pest perceived by farmers. Their yield impact was both through direct feeding damage and as vectors of virus diseases. The chemical insecticides used to control DBM were broad-spectrum insecticides that could control both DBM and aphids.

Both pheromone and *Plxy*GV are specific DBM controls with no activity against aphids so that identifying a compatible solution to controlling aphids was an important part of the project and essential if an IPM system based upon the new technologies was to be viable. In the previous project the option of combining an aphid specific fungus with the *Plxy*GV was researched and found to be viable in principal but progress was not judged sufficient to pursue this work further as part of this project. The alternative approach adopted was to try and identify a specific chemical aphicide product that could be combined as a tank mix with the *Plxy*GV when needed. The linked biorational project R6614 was charged with identifying such an aphicide as part of its outputs and identified Pirimor® (Pirimicarb) as the most promising candidate as this was a specific effective aphicide.

Activity 4.1 Evaluate biorational compatible technologies for aphid control

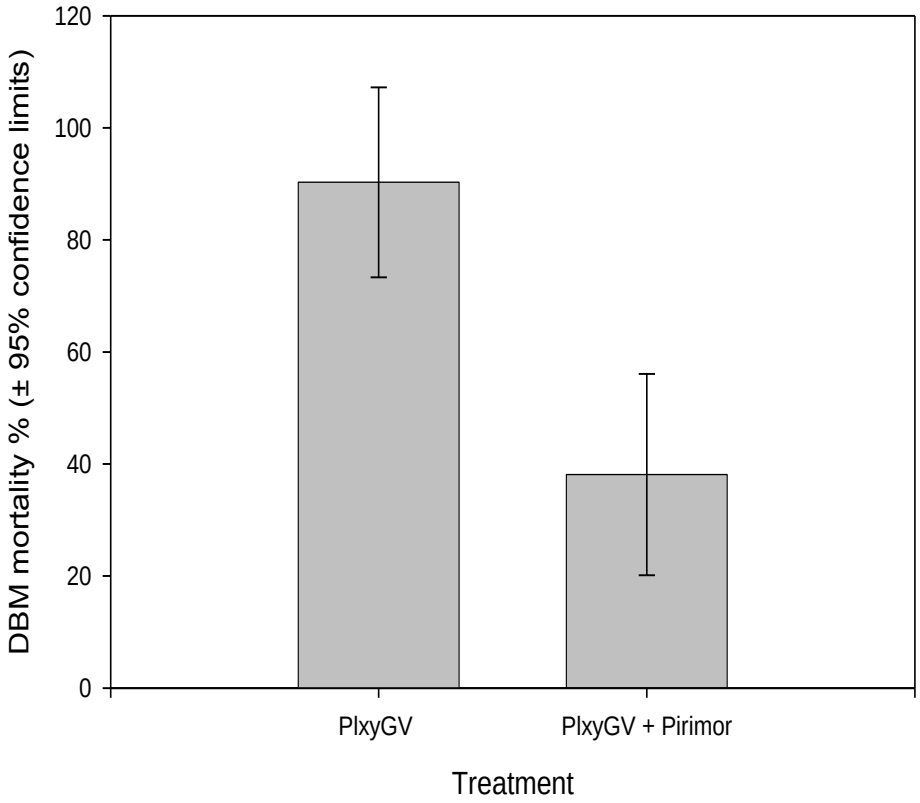
The first stage was to conduct assays in the laboratory to evaluate the compatibility of the candidate aphicide

Laboratory Results

The bioassays to evaluate the effects of aphicide on virus infectivity were carried using a neonate larvae leaf paint assay developed previously (Parnell 1999). This involved dipping a leaf in either a suspension of virus alone, virus plus Pirimor and a control. At each treatment three separate suspensions were made up and a single leaf used for each replicate suspension. The treated leaves were allowed to dry then the leaves mounted in water agar to retain viability and avoid desiccation. On to each treated leaf ten neonate DBM larvae were then introduced and the leaf incubated for seven days with mortality and infection being assessed daily for seven days. The whole experiment repeated three times.

A result of bioassays carried out in the laboratory show that mixing Pirimor in with *Plxy*GV appeared to reduce the efficacy of *Plxy*GV (Fig 21). There are no previous reports of insecticides of this group (carbamates) interfering with baculovirus pathology and observations suggested that some antifeedant effect was operating to reduce larval feeding and therefore ingestion of *Plxy*GV. Whatever the mechanism though these laboratory bioassays did suggest tank mixing of Pirimor and *Plxy*GV might not be a viable option.

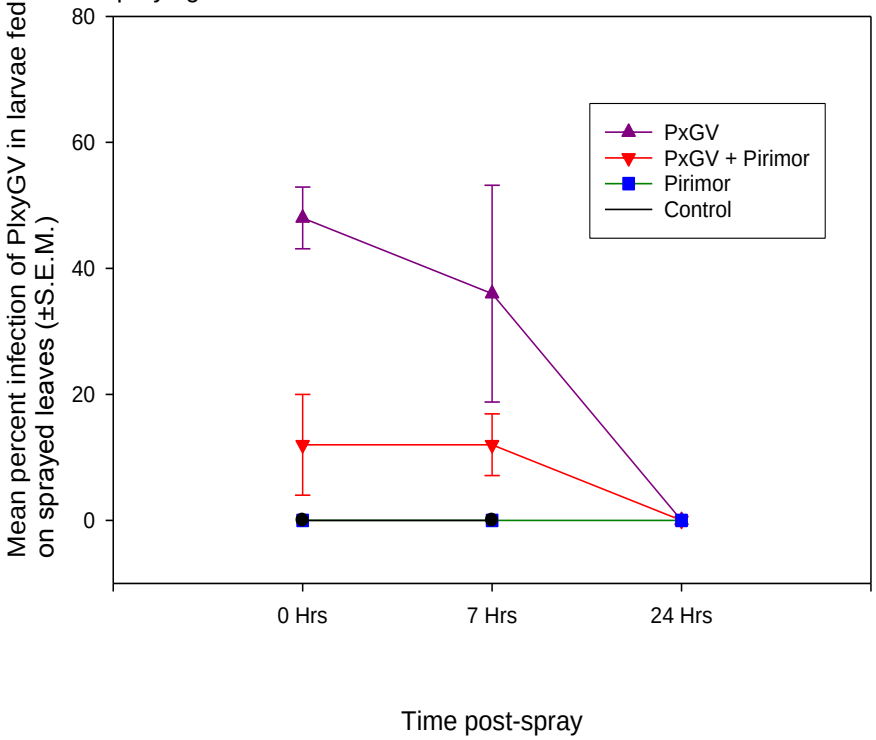
Fig 21 Effect of Pirimor on PlxyGV: laboratory bioassays of PlyxGV as aqueous suspension and mixed with Pirimor when used to treat leaves



To study further the interaction of Pirimor and *Plxy*GV the laboratory bioassay was repeated on whole plants which were sprayed with *Plxy*GV and *Plxy*GV plus Pirimor used at a rate equivalent to recommended 125 g active ingredient (a.i.) per hectare. The sprayed leaves collected and brought into the laboratory for bioassay, the protocol is described in more detail in appendix 8 (Ogutu *et*.al., 2002).

The results presented in Fig 22 clearly again show that there seemed to be clear incompatibility between Pirimor and *Plxy*GV as the two applied together resulted in a large reduction in *Plxy*GV infectivity. While no quantified data to elucidate the possible mechanism was collected observations seemed to indicate that the incompatibility might be based upon an antifeedant effect of Pirimor. An antifeedant could reduce the intake of *Plxy*GV and need not be prolonged as even a 24 hour cessation of feeding combined with the loss of *Plxy*GV activity over this period could prevent insects acquiring an infection.

Fig 22 The effect of Pirimor insecticide on the efficacy of PlxyGV
Infection rate in DBM larvae fed on leaves from plants sprayed with PlxyGV or PlxyGV plus Pirimor collected at different times post spraying



Given the findings from the socio-economic study (see output 1.5) of the importance of aphid control to brassica farmers it was decided not to dismiss the combination of *Plxy*GV and Pirimor solely on the basis of these laboratory and pot plant trials but to conduct more comprehensive field trials. Four small plot field trials with Pirimor used in combination with *Plxy*GV were conducted during 2001-2002. Again small plot trials with treatments were carried out at NARL and Thika. Treatments were *Plxy*GV, *Plxy*GV plus Pirimor, Pirimor alone (at recommended rate 125 a.i. ha^{-1}) and unsprayed control using techniques detailed in Appendix 8 (Ogutu *et*.al., 2002).

The combined results of all the four field trials showed that aphid control in plots sprayed with the *Plxy*GV combined with Pirimor was significantly better than with *Plxy*GV alone (Table 15) . The presence of the *PlxyGV* in a formulation with Pirimor did not interfere with the action of the aphicide.

Table 15 Mean numbers of aphids found per plant on plots treated with *Plxy*GV, Pirimor and combination in four trials in Kenya in 2002

	*Plxy*GV mean aphid numbers	*Plxy*GV + Pirimor Mean aphid numbers	Pirimor mean aphid numbers	Control mean aphid numbers
NARL 2002 SR	0.15a	0.01b	0.02b	0.18a
NARL 2002 LR	0.45a	0.03b	0.05b	0.52a
Thika 2002 SR	0.06a	0.00b	0.01b	0.09a
Thika 2002 LR	0.51a	0.02b	0.04b	0.46a

Treatments significantly different Kruskal Wallis H= 11.8, 3df P= 0,0082
(F= 4.8 DF= 3&15 p = 0.02)
Treatment contrasts followed by same letter not significantly different at 5% level Student- Neumann-Keuls

In field trials both Thika and NARL the combined *PlxyGV* plus Pirimor treatment did not have significantly lower yields (table 16) compared to *Plxy*GV on its own.

Table 16 Mean (n=6) yields of Kale from plots treated with virus and virus plus Pirimor

Trial site	Yield with *Plxy*GV (ton/ha)	Yield with *Plxy*GV + Pirimor	
Thika SR 2001	14.8	12.95	NS
Thika LR 2001	4.30	5.40	NS
NARL LR 2001	14.8	12.95	NS
NARL SR 2001	10.35	9.59	NS
Total yields	44.25	40.89	

NS differences not significant

In the field DBM both larval numbers and pupal numbers were not significantly different in any treatment (see Table). Analysis of pupal numbers (Table 17 and 18) showed significant treatment differences (F = 5.86 df = 3 &15, p = 0.0106). The treatments *Plx*yGV and *Plx*yGV plus Pirimor were not different showing that Pirimor did not significantly impair the reduction in pupal numbers by *PlxyGV*. There was no difference in either larval or pupal numbers between the Pirimor treatment and the control confirming Pirimor had no affect on DBM numbers.

Table 17 Mean numbers of DBM per plant in Pirimor PlxyGV combination trials

	*Plxy*GV mean aphid numbers	*Plxy*GV + Pirimor Mean aphid numbers	Pirimor mean aphid numbers	Control mean aphid numbers
NARL 2002 SR	2.88	3.54	3.73	3.00
NARL 2002 LR	1.24	1.09	0.96	1.14
Thika 2002 SR	2.22	2.77	2.11	2.08
Thika 2002 LR	1.97	2.14	1.96	1.77

Table 18 Mean numbers of DBM pupae per plant in Pirimor PlxyGV combination trials

	*Plxy*GV mean aphid numbers	*Plxy*GV + Pirimor Mean aphid numbers	Pirimor mean aphid numbers	Control mean aphid numbers
NARL 2002 SR	0.06a	0.09a	0.21b	0.18b
NARL 2002 LR	0.09a	0.09a	0.25b	0.19b
Thika 2002 SR	0.02a	0.04a	0.14b	0.13b
Thika 2002 LR	0.03a	0.03a	0.10b	0.06b

Means within column followed by same letter not significantly different at 5% level

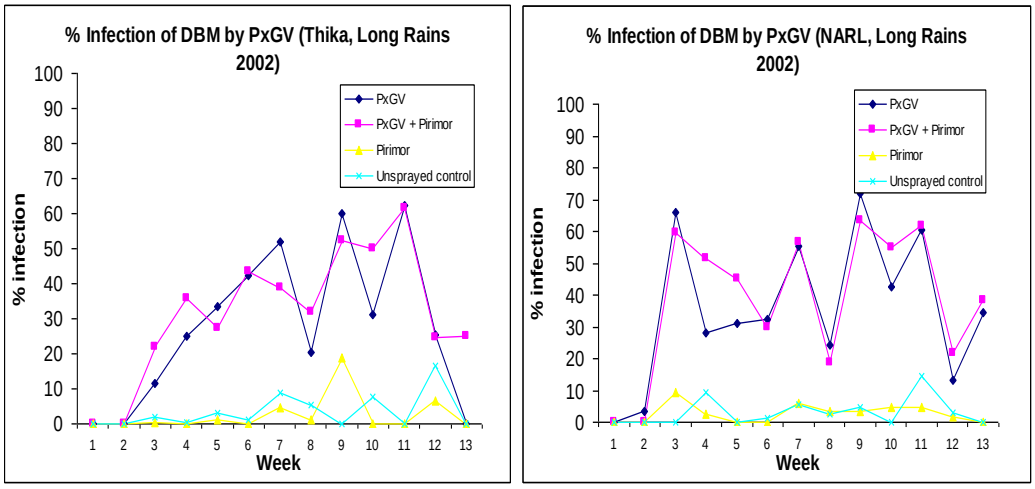
Analysis of the mean *Plxy*GV infection rate among DBM larvae in treatments with *Plxy*GV plus Pirimor showed this was slightly, though not significantly higher than for *Plxy*GV on its own (Table 19)

Table 19 Effect of combining *Plxy*GV with Pirimor on the % infection rate of DBM at NARL and Thika field trial sites

Treatment	Percent infection with PlxyGV (95% confidence intervals)	
	NARL (mean of two seasons)	Thika (mean of two seasons)
PlxyGV	32 (29-35)	33 (30-36)
PlxyGV + Pirimor	36 (33-38)	34 (32-36)
Pirimor	5 (4-7)	2 (1-3)
Unsprayed control	6 (4-7)	2 (1-3)

Graphs of the mean infection rates throughout the season in two representative trials at both Thika and NARL both show very similar patterns of infection in the PlxyGV alone and PlxyGV plus Pirimor during the season long trials (Fig?? & ??).

Fig. 23 Infection rates of PlxyGV in different treatments for aphicide trials during 2002 long rains at Thika



There appears therefore no evidence that when used in the field Pirimor has any adverse effect on the infectivity of PlxyGV. Thus the incompatibility between

*Plxy*GV and Pirimor identified in the laboratory and leaf bioassay experiments seems not to be a significant factor in the field. Thus it may be concluded that in practice Pirimor could be used in combination with *Plxy*GV as a dual aphid/DBM treatment.

Output 5 Demonstration and dissemination of new technologies

Activity 5.1 Workshop and demonstration field trials

The plan to start active dissemination to farmers of the *Plxy*GV technology was delayed and curtailed because development field trials to validate the application rates and formulation continued up until April 2002. This was because lack of DBM pressure in earlier years had slowed down the trials to determine the effect rate and develop a practical formulation. However a field day was held at Nyathuna for 30 farmers to demonstrate the use of *Plxy*GV on the farm where it was originally found as part of a farmer demonstration trial. A video of the field day was produced (Kimani *et.al.,.,* 2002) in Kikuyu with English captions for use as a training aid in future promotional activities run by KARI-Ministry of Agriculture.

Activity 5,2 Scientific dissemination.

An active programme for scientific dissemination of project findings was undertaken as follows.

- A project collaborator attended the DBM International workshop (held every four years) at Melbourne in December 2001 where a paper was read on the project work and findings. A full draft has been submitted for publication in the conference proceedings later this year (Grzywacz *et.al.,.,* 2002).
- A paper has been accepted in the Journal of Invertebrate Pathology on the Kenyan strains of *Plxy*GV and their pathogenicity (Parnell *et.al.,.,* 2002).
- Two papers have been accepted for the forthcoming International symposium on improving biocontrol of Plutella xylostella to be held in Montpellier October 2002 (Ogutu *et.al.,.,* 2002 and Grzywacz *et.al.,.,* 2002) and will be published in the proceedings.
- A paper will be presented at the forthcoming International conference on IPM in sub-Saharan Africa in Uganda in September 2002 (Ogutu *et.al.,.,* 2002).
- A paper will be read at the Society of Invertebrate Pathology's International Colloquium on Invertebrate Pathology in Brazil in August 2002. (Parnell *et.al.,.,* 2002)
- An article on biopesticides and improved spraying techniques is in preparation (Ogutu and Oduor 2002).

It is intended that at least two papers on *Plxy*GV field trials will be submitted to appropriate international scientific journals for publication by the end of 2003.

Contribution of Outputs to developmental impact

Include how the outputs will contribute towards DFID's developmental goals. The identified promotion pathways to target institutions and beneficiaries. What follow up action/research is necessary to promote the findings of the work to achieve their development benefit? This should include a list of publications, plans for further dissemination, as appropriate. For projects aimed at developing a device, material or process specify:

a. What further market studies need to be done?

b. How the outputs will be made available to intended users?
c. What further stages will be needed to develop, test and establish manufacture of a product?
d. How and by whom, will the further stages be carried out and paid for?

The project investigated two novel technologies for DBM control and has shown that one of them the *Plxy*GV is a feasible alternative to chemical insecticides though the other the pheromone system did not prove to be appropriate for use on smallholder vegetable farms in Kenya. The outputs of the project have therefore been achieved in advancing the development of a novel, safe, natural control for this major vegetable pest. The summary table (table 24) illustrates the project's findings on the specificity of *Plxy*GV to key elements of the brassica crop system.

The *Plxy*GV work has made major progress and in the next two years is due to be developed into a commercial product that will be produced in Kenya by a local company. This will provide the farmers with a new, safe and effective product to combat the problem of insecticide resistant DBM. Controlling this pest in a safe and sustainable way would be very important in ensuring the viability of vegetable production in Kenya by smallholder farmers.

Table 24 Summary comparison of the effectiveness of Brassica pesticides against Brassica pests diseases and natural enemies

Pesticide	DBM	Aphids	Plant viruses TMV and CMV	Effect on Natural enemies
Karate	- (resistance)	++	+	Yes
Brigade	- (possible resistance)	++	+	Yes
Pirimor	-	++	+	Yes
Fipronil	++	-		Yes.
PlxyGV	+	-	-	No

++ = very effective, + partly effective, - = not effective
N.D. Not determined insufficient sample data for analysis.

In conclusion the project has shown *Plxy*GV can reduce DBM survival to pupae when applied as an insecticide. The results indicate that PlxyGV can reduce the average number of DBM pupae present to the same levels as the most effective locally available chemical insecticide (Fipronil) and much better than older widely used Brassica insecticides such as Bifenthrin and Karate. Whether such a specific insecticide taking several days to kill its host can effectively prevent unacceptable levels of farmer damage and thus establish itself as a commercially viable pesticide can only be determined in on farm trials when incorporated into an actual cropping system. While it has the disadvantage of being slower than chemical insecticides it has the important advantages of being self replicating and infectious. If it can in practice suppress the population growth of DBM in brassicas it could be a potent tool in DBM pest management.

The adoption of *Plxy*GV by using a safe natural insecticide with no toxic residues will ensure the continued acceptability of produce in both national and export markets.

This is important, as in the next five years EU legislation on maximum residue levels (MRL) for chemical pesticides will effectively ban the use of many existing chemical insecticides on produce imported to the EU. This has raised concerns about its impact on poor people who depend on export horticulture for their livelihoods. Export of vegetables to the EU is a major source of income both for Kenya and for many thousands of small outgrowers in the Kenya. The banning of many older cheaper chemical insecticides will be a major threat to small holder vegetable producers, as these do not have the resources to afford the latest more expensive chemical insecticides that alone will still be effective at acceptable MRL. The availability of a new locally produced non-chemical control technology like *Plxy*GV will be an important tool in enabling small farmers to produce MRL acceptable produce and to maintain export market share.

A plant to produce *Plxy*GV is now being set up in Kenya under CPP co-funding (R7960) with a commercial partner Dudutech Ltd. This company will commercially produce the *Plxy*GV using the technologies of production and formulation developed by the project with KARI as a collaborator. This company is in the process of registering *Plxy*GV as a product for sale in Kenya to smallholder vegetable producers in the next two years. This will then be sold alongside other agro-inputs and pest control products to be marketed by Dudutech. This will be the first viral insecticide to go into commercial production in sub-Saharan Africa.

The product will be produced and sold initially around Naivasha and Nairobi. In both areas overuse of chemical pesticide is creating a serious pollution problem. The product will be promoted specifically to smallholder farmers and outgrowers to provide a safe effective alternative to the increasingly expensive and unreliable chemical pesticides currently available. It will thus enable them to maintain production of a crop crucial to the economic survival of small family run vegetable farms.

A draft registration protocol for biopesticides in Kenya, including *Plxy*GV, has been developed and adopted by the Kenyan authorities based upon FAO guidelines. Dudutech has initiated registration for *Plxy*GV and this can be expected in the next two years. Kenya is currently harmonising the registration package with Tanzania, Uganda and Ethiopia so that the same product registration dossier can be used in all countries.

In order to complete commercialisation further development work will be required as follows

- To validate the best application protocols and active ingredient rates for the *Plxy*GV in on farm trials
- To adapt the production system to use cheap local inputs and scale up to a full commercial scale.
- To develop product packaging
- To develop and implement a farmer training package
- To complete a registration dossier including an environmental impact assessment
- To develop and implement a marketing strategy

All of these activities are currently underway or planned as part of the CPP joint promotion project with Dudutech. This will run until 2003 after which the company will fund further development from its commercial operations. This promotion project will use the outputs of the project as well as further technical inputs from project collaborators at KARI, HRI and NRI to ensure continuity and effective uptake of project outputs. These experts will work alongside commercial scientist and marketing specialists from Dudutech to build up the local expertise. It is expected that after 2003 the further work will be solely involve Kenya nationals and local employees without further need for outside scientific input.

As the source strains of *Plxy*GV were isolated in collaboration with KARI they will be in a position to license their use to Dudutech or other interested companies. This would generate income that could be used to sustain KARI and its research.

While Kenya and East Africa are the primary target areas for promoting this technology DBM is a world wide problem and *Plxy*GV could have significant export potential to West Africa, South Asia, Australasia and the Americas. As part of the West African biopesticides private partnership project (R7960) funded by the CPP the *Plxy*GV has been taken to IITA in Benin for evaluation. Initial results look very promising and a recent workshop has identified commercial producers in Ghana who are interested in starting up local production and sale of PlxyGV to reduce their reliance on expensive imported Bt insecticides that are currently the only available effective option for DBM control.

Low cost production in Africa could make it very competitive with chemical insecticides sold in developed countries. Its attraction to growers would be that it could be incorporated into resistance management strategies. Alternating the use of biological biopesticides for DBM and specific chemicals for DBM and secondary pests can provide a sustainable and cost effective pest management programme that prevents resistance building up to any particular control (Roush 1997). Many existing strategies rely crucially on the biopesticide Bt however when used over long period's total resistance to this is known to appear in DBM. However by alternating the use of PlxyGV and Bt DBM resistance to both could be prevented from building up to unmanageable levels.

In conclusion the project has made considerable progress towards establishing commercial supply of *Plxy*GV to meet the need for reliable, environmentally safe locally produced pest control for DBM in Kenya. This in turn will help ensure that local vegetable production can be to be sustained and that smallholder vegetable farms remain viable and productive. Local *Plxy*GV production will create jobs and may lead to the development of export markets for biopesticides to other African countries.

4. Publications:

[List only those published and in press i.e. accepted for publication. Please highlight in bold or with an asterisk outputs which have not been previously reported]

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* 79, 192-196

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