CROP PROTECTION PROGRAMME

Public-private partnerships for development and implementation of entomopathogenic viruses as bioinsecticides for key lepidopteran pests in Ghana and Benin, West Africa

R 7960 (ZA 0462)

FINAL TECHNICAL REPORT

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Executive Summary

The project sought to alleviate poverty, to enhance incomes of smallholder farmers, and to increase food safety through the promotion of viral biopesticides as alternatives to broad spectrum synthetic insecticides for control of lepidopteran pests of vegetables through public-private sector partnerships in Ghana and Benin. The project focused on the diamondback moth, *Plutella xylostella* as a pest of cabbages. Brassicas, and particularly cabbage, are key cash crops for peri-urban vegetable farmers, but larvae of the diamondback moth place a severe constraint on production in the region. Research activities were grouped into two themes: (1), an evaluation of *Plutella xylostella* granulovirus (PlxyGV) for control of diamondback moth larvae on brassica crops and (2), an examination of constraints, opportunities and implementation routes for biopesticides in Ghana and Benin.

PlxyGV imported from Kenya proved highly virulent towards DBM larvae from Benin. Despite limited field persistence, during on-station field trials PlxyGV was as effective in reducing larval numbers as commonly used insecticides and *Bt*. Daily application of a $1/10^{th}$ dose of PlxyGV-Nya01 improved yields as did use of a proprietary UV protectant. In participatory trials, growers in Benin found that crop yield and value in virus-treated plots exceeded those in control and farmer-practice plots, but they were disappointed that the product was not commercially available. In Ghana, where Bt is widely used and appreciated, PlxyGV efficacy was acknowledged but it was more critically received.

Differences between Ghanaian and Beninese vegetable growers in their perception and awareness of biopesticides suggest that adoption of biopesticides may have greater success in Ghana. However, the regulatory authorities in Benin appear more favourable inclined towards PlxyGV and that may have an influence on the ease of registration.

Opportunities for the promotion of biopesticides stem from rising concern over the safety of chemical pesticides and include the national vegetable IPM strategy in Ghana, new export markets and implementation of MRLs on agricultural produce destined for export, resistance management, organic agriculture, public health campaigns and the Stockholm Convention to replace persistent organic pollutants.

Stakeholders agree that commercialization is the most appropriate implementation route for PlxyGV but see a need to establish specific registration guidelines and local production facilities, to undertake a comprehensive market study and a training programme.

Significant constraints hinder biopesticide implementation in Ghana and Benin: Commercial biopesticide production capacity does not exist currently; Uncertainty over the regulatory status of biopesticides and absence of specific registration guidelines requires an enabling legislative framework; Consumer, retailer and government pressure on growers supplying local markets is inadequate to drive a biopesticide industry despite trends toward IPM in Benin and Ghana. Thus, while farmer demand for PlxyGV was evident, this is largely a response to ineffective synthetic insecticides, and there is currently no economic imperative that would sustain longer term adoption of PlxyGV in Benin or Ghana.

Wide-scale adoption of PlxyGV in Ghana and Benin is unlikely in the short term to medium without economic incentives or donor support, and unless constraints can be overcome. Small-scale informal production of local isolates by farmer cooperatives or individual entrepreneurs is feasible in the short term. Commercial PlxyGV production in Kenya offers the possibility of importing PlxyGV to meet local demand while a West African biopesticide industry establishes. However, export crops may be more promising markets upon which to

establish biopesticides as market forces such as MRLs applied to export produce represent an significant catalyser for biopesticide uptake.

Peri-urban vegetables, Cotonou, Benin.

Good quality cabbages treated with *Plutella xylostella* granulovirus, Lokossa, Benin.

Background

Abuse and misuse of insecticides is common in Africa (Youm *et al*, 1990), and although the consumption of pesticides in Africa represents less than 5% of the global total, misuse is disproportionately high. Consumption of insecticides is determined largely by export crops, such as cotton and cocoa, and trends are therefore dependant on world prices. However, the increasingly important peri-urban sector accounts for ever-larger volumes of insecticide consumption in which products, dose and application frequency are not well regulated. Furthermore, insecticides imported for use in export crops spill over into the peri-urban sector for which products are not approved. Without specific measures, a continued increase in insecticide consumption is likely to be matched by continued misuse.

In W. Africa, the principal lepidopteran pests attacking a wide range of peri-urban crops are the targets of frequent applications of chemical insecticides. Such chemicals are associated with the familiar problems of environmental pollution and human contamination, rising costs and resistance development. With the high value of brassica crops, the larva of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is among the most serious pests in peri-urban agriculture. The appearance of widespread resistance in DBM to the commonly used insecticides in W. Africa is driving farmers to treat their crops more and more frequently, while others abandon their fields or give up brassica cultivation altogether. Farmers all claim additional pesticides are required for DBM and many have expressed interest in testing biopesticides.

Many of these same lepidopteran pests have known homologous entomopathogenic viruses. Insect pathogenic viruses offer an alternative control option because of their high virulence, minimal impact on non-target organisms and natural enemies, and because of their safety for use on human food crops. The baculoviruses of *Helicoverpa armigera, Plutella xylostella* and *Spodoptera* spp. for example are all amenable to mass production and field application as biopesticides, and have proven capacity to reduce field populations of their host insect. The literature on the use of baculoviruses for control of Lepidoptera is extensive and Moscardi (1999) provides a valuable review.

At the same time, there is a widespread recognition that, while there has been substantial technical progress with biopesticides, and while there is increasing public awareness of the need to reduce dependency on chemical pesticides, the uptake of biopesticides to date has been limited. In Africa there have been many notable and pioneering studies on the use of viruses for control of insect pests (Kunjeku *et al,* 1998), but there are few, if any, sustainable examples of their long-term commercial use. Several recent publications, as well as workshops, confidential reports and case studies, have attempted to address or explain the constraints to biopesticide implementation (Dent, 1999, Gaugler, 1997; Gelertner and Lomer, 1999; Harris and Dent, 1999, Lisansky, 1997; Waage, 1997; Warburton, 1995). Explanations have often concluded a lack of expertise in the crucial later stages of development and/or that an inappropriate model of biopesticide development is being pursued. The early stages of biopesticide R&D are often undertaken in the public sector using public finances, but many potential biopesticide products fail to go beyond the laboratory or field-trial stage. Inappropriate biopesticide development models with inadequate attention to multidisciplinarity, particularly in developing countries, have been blamed for the failure of biopesticides to reach the market or, attain a greater market (Dent, 1997; Waage 1997, 2000). Despite the acknowledged constraints, a review of microbial pest control in W. Africa (Langewald and Cherry, 2000) considers that the prospects are good, and that many opportunities remain to be fully explored. Nevertheless, successfully identifying and overcoming case-specific constraints are essential steps along the implementation pathway.

Commercialisation is an important implementation pathway for biopesticides yet failure to engage the private sector and a lack of market data are factors contributing to poor biopesticide uptake. Public-private partnerships are viewed as appropriate routes to successful implementation of development objectives (James, 1999) but early engagement of the private sector is essential. The project proposed partnerships with the private sector, to motivate and encourage commercial enterprises to establish production of the viral insecticides by first demonstrating that these products have the potential to be manufactured and sold for a profit. Without this "plausible promise", experience shows it is unrealistic to expect entrepreneurs, with many demands on their time, to become equal partners in the development of a new innovation.

Project Purpose
Promotion of pro-poor strategies to reduce impact of key pests, improve yield and quality of crops, and reduce pesticide hazards in peri-urban systems.

Research Activities
(This section is taken from section 19 of the PMF. The text following each activity explains any activities that were actually undertaken, plus any modifications).

Activity 1 Isolation, identification and molecular characterisation of baculoviral diversity in spatial and temporal terms for one key vegetable pest in Ghana and Benin.

1.1 Spatial and temporal collections of larvae of *P. xylostella* and *Helicoverpa armigera* were conducted by IITA and collaborators during 2001 according to protocols given in Annex 1. Larvae were collected in the main peri-urban vegetable growing regions of Ghana (Greater Accra, Eastern, Ashanti, Central and Brong Ahafo regions), and Benin (Atlantique, Mono and Ouème departments) at three times throughout the year with the aim of isolating pathogen-infected individuals. The primary target vegetables for the surveys were cabbage and cauliflower, for *P. xylostella*, which as an irrigated crop is grown on a year-round basis; and tomatoes for *H. armigera* (although this species has a very wide host range) that are grown only in the cool season.

1.2 Larvae sampled under 1.1 were returned under licence to IITA, Benin. Aqueous suspensions prepared from the groups of live larvae from each site were fed to conspecific larvae in replicated laboratory bioassays (Lacey, 1997) to screen for pathogenic activity. Dead larvae from the field were examined for the presence of pathogens and then similarly fed to conspecific larvae in a test of Koch's postulates.

1.3 Putative baculoviruses were semi purified according to standardised procedures (e.g. Hunter-Fujita *et al*, 1998) and forwarded to NRI, UK, for REN analysis.

1.4 Identified pathogens are housed in IITA's long-term pathogen storage facility on behalf of respective national governments. Terms for the acquisition and storage of germplasm abide by article 15 of the Convention on Biological Biodiversity. Transfer of germplasm is accompanied by Material Transfer Agreements (MTA) with the nation of origin.

1.5 Selection of pest-pathogen system for further studies.

1.6 Studies, in the form of undergraduate and post graduate theses, were conducted in collaboration with the Université National du Bénin and the University of Ghana, to provide supplementary information on the epidemiology and ecology of *P. xylostella* granulovirus. Studies included the following: 1) the importance of horizontal virus transmission to inter- and intra-generational spread, 2) transmission of PlxyGV from the soil to the plant surface, 3) persistence of PlxyGV on the leaf surface during exposure to ultra-violet solar radiation and rainfall, 4) comparative persistence of PlxyGV, Bt and acephate.

Activity 2. Socio-economic survey of the biopesticide market, of the social, institutional, political and technical constraints to biopesticide development in Ghana and Benin, and identification of potential private sector collaborators.

2.1 A socio-economic study of the biopesticide sector, including the social, institutional, political and technical constraints and opportunities for biopesticide development was conducted in Benin and Ghana by social scientists from the Institut National des Recherches Agricoles du Bénin (INRAB) and the Department of Agricultural Economics at the University of Ghana. Terms of reference (Annex 2) were drawn up with collaborators by the IITA-Benin senior socio-economist, Dr O. Coulibaly.

2.2 In response to a project activity prioritisation exercise held during a workshop at IITA in 2002, additional funds were requested from CPP to conduct a biopesticide market study before being able to proceed significantly with private sector partnerships. Proposed activities were as follows:

- Assess the willingness to use and pay for biopesticides on vegetables
    - Surveys to identify factors most likely to influence the willingness of vegetable farmers to pay for a biopesticide product, using the price of an existing biopesticide (*Dipel*)
    - Probit or Hedonic pricing model used to identify the determinants of willingness to pay for a quality or low cost product
    - Sensitivity analysis used to assess changes in prices on the likelihood of demand for biopesticides
- Assess the competitiveness and potential market niches for biopesticides
    - A policy analysis matrix used to assess the competitiveness of biopesticides compared to traditional pesticides; potential markets will be identified nationally and regionally based on past key informant and formal surveys.
    - Complementary interviews carried-out with pesticides merchants
- Determine the unit cost of production and market size for brassicas
    - A detailed cost and benefit analysis of alternative production techniques or systems of biopesticides.
    - Sensitivity analysis to determine break-even levels to match with the various levels of willingness to pay determined earlier.

Activity 3. At least one entrepreneur selected in each country interested in commercialising viral insecticides.

3.1 Enterprises / entrepreneurs interested in developing biopesticides, or already possessing the technical capacity to enter into partnership for biopesticide production were identified by socio-economic surveys in 2.1. The project contacted potential partners with preliminary information on biopesticides, the objectives of this project, and solicited their views on partnerships.

3.2 Following activity 3.1, the project proposed a series of information exchange workshops for potential private partners in Benin and Ghana. The objective of these workshops was to build awareness and understanding of biopesticides, study biopesticide development models and biopesticide company models. The project aimed to select one candidate entrepreneur / private enterprise in each country with which to pursue product implementation.

Activity 4. Pesticide registration committee members in Ghana and Benin sensitised to biopesticide data requirements and regional initiatives for establishing a biopesticide regulatory frameworks.

4.1 The permanent secretaries of the pesticide registration committees in Ghana and Benin were invited to nominate 3 to 4 candidates each to attend a biopesticide regulation workshop hosted by IITA from January 29 to February 7, 2001.

4.2 A biopesticide regulation workshop led by a USAID-funded project out of Virginia Tech (Blacksburg, VA, USA) was held for members of the Comité Sahélien des Pesticides (CSP) of the Comité Permanent Inter Etats de Lutte Contre la Sécheresse (CILSS) and East Africa regulatory authorities. The project offered to support attendance of members from Ghana and Benin to gain value from the investment by the VPI project whose mandate did not cover the coastal W. African countries. The workshop dealt with the criteria for registration; biological data requirements, physicochemical properties, residue data, toxicological and environmental fate studies, and aimed to encourage greater regional dialogue in the interest of harmonisation.

Activity 5. A tested and validated virus production system, evolved around a principle of locally sustainable practice and based on field or greenhouse production

5.1 Sustainable, low cost baculovirus production systems were investigated (as alternatives to those used in developed country production units and in industry that depend on expensive material inputs). The initial focus of this activity was *H. armigera* NPV as studies took place prior to the decision under activity 1.5 to work on PlxyGV. Undergraduate studies investigated the following: 1) The effect of host plant on the mass production and persistence of *Helicoverpa armigera* nucleopolyhedrovirus, and 2) Optimisation of parameters in the production of *Helicoverpa armigera* nucleopolyhedrovirus in larvae of *Helicoverpa armigera* fed on leaves of host plants. A subsequent study considered optimisation of PlxyGV production on plant material. The protocol (Annex 3, in French) loosely followed optimisation procedures for *Spodoptera exempta* NPV in Cherry *et al* (1997). Cost of PlxyGV production was estimated during a period of intensive mass production of the PlxyGV Togo isolate (IITA 00503).

5.2 The project proposed that private sector partners identified under section 3 would receive training on sustainable virus production techniques for their own enterprises.

Activity 6. At least one formulated biopesticide based on the homologous baculovirus of a key vegetable pest to be demonstrated in multiple participatory trials to farmers, government departments, NGOs and potential private sector entrepreneurs in both Ghana and Benin**.**

6.1 Close liaison was proposed during PM development between the current project and the CPP-funded NRI project ZA0319/R7449 (“Biorational Brassica IPM”) in Kenya. Additional funds were approved to permit IITA research assistant Mr Denis Djegui to attend the NRI-KARI field trial of PlxyGV in Kenya as part of the Biorational Brassica

IPM project to familiarise himself with methods of PlxyGV formulation, application and data collection.

6.2 PlxyGV isolate Nya01, under evaluation in Kenya as part of the "Biorational Brassica IPM" project, was imported to Benin from Kenya under the terms of a MTA with the Kenya Plant Health Inspectorate Service (KEPHIS), the Kenyan authority with responsibility for phytosanitary affaires, for testing against the diamondback moth in Benin. PlxyGV-Nya01 was evaluated in field trials at IITA, Benin and at PPRDS, Accra in Ghana to validate dose, application strategy and formulations drawing on outputs from DFID-CPP funded project "Biorational pest control" (ZA0079/R6615). The trial protocol is presented in Annex 4.

6.3 Visit by Mr. Douglas Miano from the Kenyan Agricultural Research Institute (KARI), to IITA, Benin: Under the terms of the transfer of PlxyGV Nya01, IITA agreed to furnish KEPHIS, the Kenyan authority with responsibility for phytosanitary affaires, with performance data collected during evaluation trials under 6.2 above. In the interest of promoting greater exchange of information between the Kenyan authorities and IITA, IITA invited a nominated member of the Kenyan authorities to visit the PlxyGV Nya-01 trials in Benin. Following contact with the Kenyan authorities via Dr. George Oduor of CABI Kenya, Mr. Douglas Miano, from KARI's National Agricultural Research Laboratory, was nominated by KEPHIS and KARI to visit IITA. The visit was funded by additional funds from CPP. Terms of reference for the visit are given in Annex 5.

6.4 Farmer participatory field trials were used as the principal mechanism to promote PlxyGV for DBM control. Trials were conducted in cabbage growing regions of Parakou, Lokossa and Cotonou in Benin, and in Jowulu, Mampong and Kumasi in Ghana. Fifty farmers were involved in trials per country. Site selection and trial execution was the responsibility of PPRSD (Mampong and Jowulu) and the Crop Research Institute (CRI) (Kumasi) in Ghana, and the responsibility of IITA and SPV in Benin. NRI provided advice on formulation, application and evaluation procedures based on their DFID-funded work in E. Africa, India and Thailand. No written protocol is available as trials were designed and conducted by farmers themselves. Teams of facilitators operated at each site to offer training in cabbage pest IPM and use of PlxyGV. Data on pesticide use and perceptions of PlxyGV were collected by questionnaire before and after the trials. Yield data and pest and invertebrate population data were collected at harvest.

6.5 In an addition to proposed activities, a postgraduate study investigated the impact of PlxyGV application frequency on efficacy of DBM control in the field. As part of the same field trials, an undergraduate study investigated the effect of Coax®, a proprietary adjuvant with phagostimulatory and UV protectant properties, on the field efficacy of PlxyGV.

Activity 7. Recommendation of a strategy/strategies for commercialisation of a viral biopesticide in Ghana and Benin. All activities under 7 were conducted as part of a final project meeting for Beninese and Ghanaian stakeholders.

7.1 In collaboration with a CABI consultant, the project leader reviewed progress with the private sector in target countries and considered whether uptake pathways identified in 2.2. for commercialisation of a viral product remain valid, or whether alternative promotion and implementation pathways need to be researched.

7.2 With reference to, and contingent upon the outcome of Activity 6.1, a strategy for commercialisation of a viral biopesticide was proposed.

7.3 With the assistance of the IITA socio-economist, a review in the form of a brief questionnaire of awareness and perceptions of biopesticides among project stakeholders was undertaken during the final project meeting. This data, together with the data gathered in 2.2 will form the basis of a project impact-evaluation exercise.

Outputs

Output 1 Isolation, identification and molecular characterisation of baculoviral diversity in spatial and temporal terms for one key vegetable pest in Ghana and Benin.

1.1 & 1.2 Approximately 9000 *P. xylostella* larvae were collected from 93 farms in Benin and 85 farms in Ghana (Annex 6), none of which were symptomatic for baculoviruses infection, and none led to isolation of PlxyGV. By contrast, Parnell *et al* (2002) collected 127 larvae infected with PlxyGV from 8 of 27 farms visited during a three week survey in 1999 in and around the Nairobi region of Kenya. Absence of PlxyGV can not be concluded but evidence points to a lower incidence in the survey areas relative to the Nairobi region. The areas surveyed in Ghana and Benin were geographically and climatically different to the Nairobi region of Kenya. Given this difference, in 2002 two surveys were mounted in the cooler highland area of Togo where conditions are closer to those around Nairobi. In all, 28 sites were surveyed and one isolate of PlxyGV was recovered from Apeheheme, Danyi Kpalime, which is now stored in the IITA pathogen collection (IITA 00503).

Since PlxyGV was isolated neither from Ghana nor Benin during 2001, while the need to progress with objectives remained pressing, PlxyGV isolate Nya01 (Parnell, 1999) was imported from Kenya under MTA between IITA and KEPHIS. The original terms of the MTA allowing experimental work in Benin was later expanded on request to include Ghana. The Togolese PlxyGV isolate was not used during the current project.

Several isolates of *H. armigera* virus were discovered during surveys but as *H. armigera* was dropped from the project programme at the end of 2001, these isolates are not discussed further. Isolates are available from IITA's pathogen collection (IITA 00601 – 00605).

1.3 & 1.4 Samples of Togolese PlxyGV were multiplied and sent to NRI for identification by REN analysis. REN profiles were identical to those for PlxyGV-Nya01 suggesting contamination had occurred during multiplication. Since larvae were already symptomatic for PlxyGV infection in the field in Togo, there is no suggestion that larvae were contaminated with PlxyGV Nya01 on arrival in the in the laboratory at IITA. No further action has been taken.

1.5 Based on pathogens identified from surveys, on the socio-economic report (Activity 2.2) and feedback from initial meetings *P. xylostella* granulovirus was selected as the focus of the remainder of the project.

1.6 Brief summaries of the results of student theses are given here. Theses are reproduced in full in Annexes 6 to 9.

*Osae, M. (2002). Virulence and transmission of an East African Plutella xylostella granulosis virus (*PlxyGV*-Kenya) in a West African diamondback moth, Plutella xylostella (L.) (Lepidoptera: Plutellidae) population. MSc. University of Ghana, Legon, Zoology (Faculty of Science) & Crop Science (Faculty of Agriculture). (Annex 9).*

Abstract: The virulence and transmission of PlxyGV-Nya01 were assessed in the laboratory in a population of *P. xylostella* from southern Benin. LC_{50}, LT_{50} and virus yield per larva were used as virulence determining factors. PlxyGV-Nya01 was >44 fold more virulent in 2nd instar *P. xylostella* larvae from the southern Benin population than the Kenyan population based on LC_{50} values. Virus yield per larva was approximately two fold lower in southern Benin *P. xylostella* populations. PlxyGV was transmitted in a density dependant manner from infected cadavers to healthy larvae and these were observed to feed on infected cadavers. Highest levels of transmission (>94%) were observed for 4,10 and 20 cadavers per 7.5cm leaf disc and one and two cadavers per same size leaf disc gave significantly lower levels (61% and 67%, respectively). Transmission from infected live to healthy larvae increased with time post-infection. Transmission from infected to healthy larvae was significantly higher (> 96%) at the 95% confidence level on day 6. There was moderate transmission on days one, three and four (> 28%< 36%) with a highly significant (P< .0001) increase in transmission on the fifth and sixth day. This kind of transmission might be through the faeces or regurgitate from infected larvae. Seedlings emerging from PlxyGV-loaded soil acquired adequate virus to kill 2nd instar *P. xylostella* larvae. The amount of virus acquired and mortality were dose dependent. The virus acquired was not inactivated by day 8 and leaves sampled on day 8 appeared to be preferred by *P. xylostella* larvae. Hence, higher mortality was recorded for larvae fed with leaves sampled on day 8 than on day 3 across all doses. The high virulence and transmission of PlxyGV in the southern Benin *P. xylostella* are indications that the virus holds great potential for DBM control in the West African region and must be developed and made available to farmers.

Ahoyo, C. (2003). [Effect of ultraviolet solar radiation on the activity of (Plutella xylostella) granulovirus and determination of the median lethal dose]. Dipome d'Ingénieur Agronome, Université d'Abomey-Calavi (Faculté des Sciences Agronomiques), Protection des Végétaux. (Annex 7).

The effect of solar UV radiation on PlxyGV was evaluated by exposing virus-treated potted cabbage to varying periods of sunlight between 10:00 and 16:00 daily when UV intensity was maximal (measured in the 294-320 nm range by UVX radiometer). Leaves were returned to the laboratory where they were fed to *P. xylostella* larvae. Following this protocol, the half life of PlxyGV was approximately 4 hours. When the experiment was repeated in the greenhouse, with treated cabbages held in screened cages protected from direct solar UV radiation (maximum intensity = 102 μWcm^{-2}), PlxyGV half life was approximately 7 days. Growth dilution accounted for only a small proportion of this loss of activity. LD_{50} values for 2nd and 3rd instar larvae using a leaf disc dip protocol (see Annex 7) were 8,227 et 21,988 OB respectively.

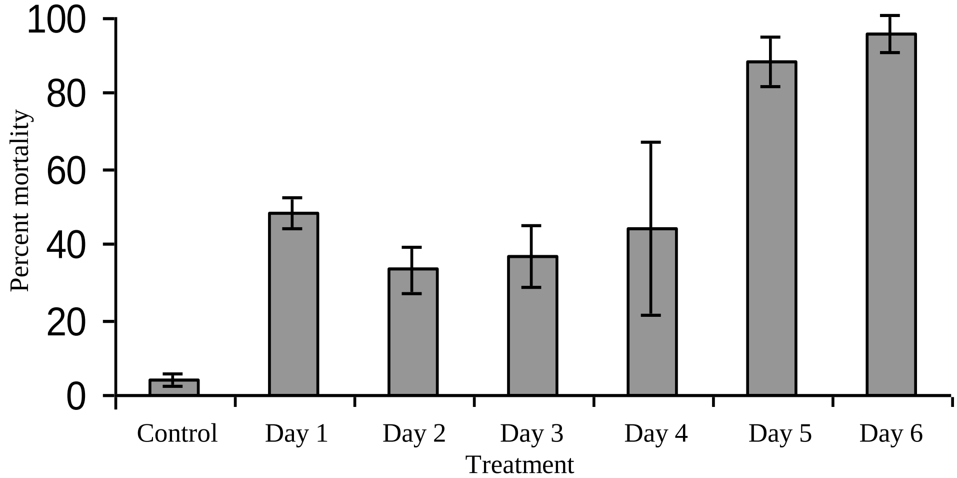
Zinzidohoué, E. (2003). [A contribution to the study of horizontal transmission of Plutella xylostella granulovirus. (Lepidoptera: Yponomeutidae)]. Dipome d'Ingénieur Agronome, Université d'Abomey-Calavi (Faculté des Sciences Agronomiques), Protection des Végétaux. (Annex 8).

The study considered two aspects of horizontal transmission: transmission via the faeces of infected larvae, and transmission via infected cadavers.

In an experiment on PlxyGV transmission via the faeces, 3rd instar larvae were infected with a viral suspension containing 1.89×10^7 OB ml^{-1} and their faeces collected separately on each of six days post-infection. Suspensions were prepared with collected faeces in aqueous Tween 80 at 0.1% with a ratio of 1 g faeces per 1 ml of Tween 80. Suspensions were used to treat leaves and infect 2nd instar larvae whose survival was monitored for 9 days. Faeces collected on the 1st to 6th day gave 48.3%, 33.3%, 36.7%, 44.2%, 88.3% and

95.8% mortality (Figure 1) respectively demonstrating that PlxyGV infection can be transmitted via this pathway. Faeces became more infectious as disease progressed.

Figure 1. Nine-day mortality among *P. xylostella* larvae treated with suspensions of faeces taken from PlxyGV-infected larvae on days 1 – 6 post inoculation. (After Zinzidohoué, 2003).



In an experiment to measure PlxyGV transmission from cadavers to healthy larvae, 2nd instar larvae were infected with a viral suspension containing 3.77×10^6 OB ml^{-1}. On the 6th day after infection, morbid larvae were placed on 7.5 cm cabbage leaf discs at densities of 1, 2 and 4 per disc. Healthy 2nd instar larvae were also introduced at densities of 5, 10, 15 and 30 per disc and monitored for 9 days. Mortality among previously healthy 2nd instar larvae ranged from 52.3% to 90.3% demonstrating PlxyGV transmission from cadavers to healthy larvae. Analyses showed correlation between the cadaver density and mortality among introduced larvae, with higher cadaver density leading to higher mortality among introduced larvae. The density of introduced larvae had little effect on their mortality.

Kassa, A. (2003). Evaluation of the field persistence of PxGV, Bt and Orthene (acephate). Diplôme d'Ingénieur des Travaux, Université d'Abomey-Calavi (Collège Polytechnique Universitaire), Aménagment et Protection de l'Environnement. (Annex 10).

Experiments were conducted to compare the persistence of PlxyGV Nya-01 with that of acephate (a commonly used organophosphate insecticide) and *Bacillus thuringiensis*. A series of three assays were carried out in which cabbages were sprayed in the field with the above treatments. Cabbage leaves were sampled at intervals of 0.5, 16, 24, 48, 96, 168 hours after treatment. Treated leaves were fed to 2nd instars *P. xylostella* larvae in the laboratory and larval mortality checked daily. Persistence was taken as a function of the rate of larval mortality obtained with different periods of exposure. Results obtained showed that PlxyGV Nya-01 has short persistence in the environment. With leaves taken at 0.5 hours after application, larvae mortality due to PlxyGV Nya-01 was 98% while with leaves taken after 168 hours it was only 3%, and 2% in the control. For Bt and acephate after 0.5 hours there is 75% and 15% mortality respectively, while after 168 hours the figures are 25% and 22% respectively against 2% in the control. PlxyGV Nya-01 is almost completely inactivated after 168 hours in the environment.

Output 2. Socio-economic surveys of the biopesticide market, of the social, institutional, political and technical constraints to biopesticide development in Ghana and Benin, and identification of potential private sector collaborators.

2.1 *In-country surveys of biopesticide sectors.* Final reports of socio-economic surveys conducted by project collaborators can be found in Annexes 11 (Benin) and 12 (Ghana). Summaries only are reproduced here.

2.1.1. Benin (translated from French):
In the third phase in this study we noted a real need for biologically-based products for cabbage and tomato protection. In fact, pest attacks constitute the principal constraints for these crops. Urban area are the main zones of cabbage production while tomatoes are produced in large quantities in rural zones.

The cabbage crop occupies much of the market gardeners time in urban centres. Areas cultivated per farmer vary from 126 m² to 446 m². The periods of intensive production are during the months April to June, and July to September. The length of the crop varies from 72 to 118 days. Cabbage is a profitable crop for market gardeners but profits decrease with increasing pest attack through the use of pesticides or the loss of harvestable yield. The use of pesticides account for about 30% of the costs of production.

The average area under cultivation per farmer for tomatoes is 2.5 ha in the regions of intense production. Peak production occurs during the months from January to March and from July to September. The length of the crop is from 96 to 107 days. Like cabbage, the profitability of tomato is satisfactory. The use of pesticides account for approximately 40% of production costs although their use is restricted in certain rural zones.

The probable rate of adoption [of biopesticides] is around 75%. There are five main factors determining market adoption of these biopesticides: these indicators are relative to the application method, the style of packaging, the formulation, the price and the distribution mechanism. In this respect, growers [in Benin] would like to see a water soluble liquid formulation, stocked in plastic bottles, with a price that is lower than the most commonly used pesticides. In order of importance, the methods of distribution proposed by growers are via CARDER (the state extension service), via private distributors, and via growers' cooperatives.

Distributors sell various pesticides for cabbage and tomato protection. About 71% of the products distributed for cabbage protection are for "caterpillar" control . Among the synthetic products distributed for tomato protection, 33% are for control of larvae. The annual quantities of pesticides distributed are of the order of 10,602 litres for liquid products and 8,000 kg for powdered products. Profit margins vary from 167 fcfa lt^{-1} to 6,000 fcfa lt^{-1} for liquid products against 850 fcfa kg^{-1} to 5,000 fcfa kg^{-1} for powdered products (£1.00 ≈ 960 fcfa).

In discussions about the organisation of a biopesticide sector / industry for cabbage and tomato crops, distributors would prefer to help with marketing products. However, the large agricultural input import companies would prefer to be sole distribution agents for the manufacturer. In this respect, among the types of contract proposed the contract with deposit on sales commission is one of the most important. Provision of products with credit from a manufacturing plant is the type of contract suggested by the individual distributors. They would like to see training and sensitisation of growers in order to increase usage of these products and guarantee demand. Furthermore, liquid formulations in small containers are key propositions of the distributors relating to biopesticide characteristics.

2.1.2. Ghana:
This study was the socio-economic component of a project aimed at developing and commercialising a biopesticide in Ghana through public-private partnerships. The purpose of

the study was to assess the constraints and opportunities for developing a biopesticide in Ghana.

The study included consultations with officials of relevant government departments and NGOs, researchers, pesticide distributors, and farmers. A structured survey of farmers and consumers was also conducted to assess their attitudes and perceptions about produce quality. A probit model was used to identify the determinants of farmers willingness to pay for a new biopesticide.

The major findings of the study are summarised below:

Cash crops are the main target for application of pesticides. Pesticides are mostly imported as ready-to-use products on a commercial basis, and the import bill has been rising since 1983. Although there are 33 licensed registered pesticide importers, about seven of these handle 80% of the market. Pesticide distribution is through a network of wholesalers and retailers, located mostly in cities and towns. There are no subsidies on pesticides. However pesticides imported through Japanese aid may enjoy some level of subsidy.

The Environmental Protection Agency is the regulatory body responsible for the pesticide sub-sector and it operates under the Pesticide Control and Management Act (Act 582). The effectiveness of the EPA is however limited by inadequate staff and logistics.

Sellers of fresh vegetables do not perceive consumers to be aware of dangers of chemical residues in vegetables; therefore most consumers would not be willing to pay higher prices for vegetables produced without chemical pesticides. Consumer preference for vegetables with little visible sign of insect damage also puts pressure on farmers to use chemical pesticides. Consumers are more aware of the problem of bacterial contamination of fresh vegetables through use of sewage water than they are about chemical residues on produce. Consumers are willing to pay for non-contaminated produce. Farmers perceive intensity of pests to be on the increase. The large number of pests with varying characteristics poses a challenge to efforts at reducing use of synthetic pesticides, although farmers also attribute the increasing incidence of pests to resistance of pests to synthetic pesticides.

The biopesticide Bt, was introduced to contain pest resistance in cabbage production. Pest specificity of the biopesticide has been a limitation to its wider use as farmers prefer broad-spectrum pesticides. Although the biopesticide, Dipel is widely used for the control of caterpillar on cabbage, it is often used in combination with synthetic pesticides.

On average, farmers also expressed willingness to pay for a biopesticide, which would increase their yields or reduce pest control costs. The main determinants of willingness to pay among farmers were education, access to extension services, and previous experience in the use of Dipel.

The main issues in pesticide use in Ghana are: Effectiveness due to poor handling; ensuring safety to farmers; consumers and the environment; necessary policy to support initiatives aimed at reducing excessive use and misapplication of chemical pesticides.

Opportunities for the promotion of biopesticides in Ghana are: The government's ongoing IPM strategy; rising concern over safety aspects of chemical pesticides; and implementation of EC's Maximum Residue Limits (MRL) on imported produce.

Some of the constraints to be overcome are: Farmers' preference for fast acting broad spectrum pesticides; the wide range of pests that have to be controlled; inadequate distribution networks and inappropriate storage facilities especially at the retail level; and lack of regulatory framework for registration, importation, production and use of biopesticides.

2.2 *Market study:* For each of the three parts to this activity, a draft report has been prepared by the IITA socio-economist who conducted the work. Abstracts or conclusions are presented here, while the full draft reports can be found in Annexes 13, 14 and 15. The first report has been prepared as a draft paper and has been approved by CPP for submission to an international journal.

2.2.1 Coulibaly, O., Cherry, A.J., Al-Hassan, R. and Adegbola, P.Y. (in prep) Vegetable Farmer's Perceptions and Willingness to Pay for Biopesticides in Benin and Ghana. (Annex 13).
Abstract: High value vegetable crops in coastal West African countries are subject to intensive pesticide applications that raise concerns about health and environmental risks. Increasingly strict MRL in export markets are likely to place restrictions on their use. Surveys in Benin and Ghana assessed factors influencing vegetable farmers' willingness to pay for biological pesticides as alternatives to pesticides. In Ghana key factors are access to non-formal education, access to extension, and experience with the use of Dipel. In Benin, ineffectiveness of current chemical pesticides, access to extension and perceived capacity to control new pests are the key factors. Adoption of a novel biopesticide, such as *Plutella xylostella* granulovirus for control of the diamondback moth on cabbage, would be more likely in Ghana given the greater emphasis on IPM in that country. The lack of distinction in Benin between biological and chemical pesticides presents unrealistic expectations for biopesticide introductions at this time.

2.2.2 Coulibaly, O., Cherry, A.J., Nouhoheflin, T. and Aitchedji, C. (in prep) Assessment of the competitiveness of vegetable production systems in Benin and Ghana: Case of cabbage and Tomato. (Annex 14).
Conclusions: The results of the study of cabbage and tomato production systems have shown that cabbage is basically grown under three major production systems and tomato under two systems in Ghana, while in Benin cabbage is grown under four systems and tomato under only one production system. Vegetable production is profitable. Even if farmers do not produce with the best production system, the actual production systems are financially profitable and generate for farmers a significant income as indicated by the private benefit-cost ratio and the net private profitability. This income generation is higher in Ghana than in Benin for both vegetables (cabbage and tomato). The result shows that cabbage and tomato production has become a highly profitable activity. The ratios B/C and DRC indicated that Ghana has a better comparative advantage in cabbage and tomato production. The best system that gives the highest profit is the one where farmers combined biopesticides and chemical pesticide methods to control pests and diseases. It's followed by the system where cabbage or tomato is controlled by biological control. Finally, all the production systems of vegetable are profitable for farmers. But, they must take into account environmental risks and human health hazards in their decision making linked in chemical use.

2.2.3 Coulibaly, O., Cherry, A.J., Aitchedji, C. and Nouhoheflin, T. (in prep). Consumers' perceptions and willingness to Pay for organic vegetable in Benin and Ghana, (Annex 15).
Abstract: The study assesses the market potential of organic vegetables in Ghana and Benin. The specific objectives are to analyze consumer awareness and perceptions of chemical pesticide residues in vegetables and assess the premium levels that consumers are willing to pay for chemical free vegetables. The results indicated that the characteristics that consumers are looking for in assessing the quality of cabbage and tomato are: damage free, freshness, big size, bright colour and hardness. Consumers are aware of the heavy use of chemical pesticide on vegetables. The level of awareness of the health hazards

linked to chemical residues among consumers is more widespread in Ghana than Benin. Consumers are willing to pay more than 50% as a price premium for chemical free vegetable. Factors likely to affect consumers' willingness to pay for chemical free vegetable produce are the socio-professional category acting as a proxy for income level, the awareness of chemical residue in vegetables, the availability, the label and the taste.

Output 3. At least one entrepreneur selected in each country interested in commercialising viral insecticides.

3.1 Socio-economic surveys conducted under activity 2.1 were to identify enterprises and entrepreneurs interested in developing biopesticides, or already possessing the technical capacity to enter into partnership with IITA for biopesticide production. However, one major observation of the surveys was the absence of biopesticide manufacturing capacity in either country. All pesticides are imported to Ghana and Benin, and while there may be some re-formulation and re-packaging, there is no manufacture. This was the first indication that our objective of establishing partnerships for biopesticide production might have been over optimistic. Nevertheless several groups or individuals from the private sector did express interest in working with IITA to learn more about biopesticides and these were invited to participate in a series of three information exchange workshops, one for Ghanaian partners held in Accra (February 2002) and two at IITA, Cotonou, the first for Beninese partners (January 2002) and the second for all project partners (April 2002).

3.2 Reports for the three workshops are presented in Annexes 16, 17 and 18. Summaries are presented here.

3.2.1 *Information exchange workshop on biopesticides for private and public sectors in Benin, 29 to 30 January 2002 (Annex 16, in French).*

Following a day of presentations to reach a common level of understanding among participants, the focus of the workshop was a round table discussion to address a series of prepared question posed by the organisers:

In the creation of a biopesticide sector the following should be included as key players: Biopesticide manufacturers (not yet established in Benin), pesticide distributors, the technical and extension services of the Ministry of Agriculture, farmer and village unions, consumer associations, technical training centres, the health service and the division of Food and Nutrition (to operate quality control (QC) of treated vegetables), NGOs and mass media to sensitize, the pharmaceutical agency, and the Benin Environment Agency.

Commercialisation was tacitly accepted as the most appropriate implementation route but a major concern to emerge was whether the capacity of the Beninese and regional market was adequate to return a profit on investment made in establishing the product.

Principal constraints to the adoption of PlxyGV were felt to be linked to existing legislation, and the institutional and political environment. Additional constraints were related to the particular characteristics of baculoviruses. Removing pesticide subsidies (under the Japanese KR2 round) and a reorientation of the policy on input supply were considered important to opening opportunities for biopesticides. Participants considered that raising awareness of the advantages of organically produced vegetables might permit value to be added to the product, and promotion of an export market could be considered.

Participants considered a market study to be essential to convince donors and distributors of existing outlets. To establish product price participants proposed that the State should provide a decreasing subsidy over a five year period in the interest of environmental protection. The subsidy could be in the form of tax relief on sales or on import of raw materials in its manufacture. The cost price of pesticides should be compared with that of biopesticides after integrating environmental costs.

Participants proposed a series of steps to be taken, science apart, once a virus had been isolated (see Annex 16) (note that at the time of the meeting no virus had been recovered from surveys and PlxyGV-Nya01 had not been imported from Kenya).

3.2.2. *Public-private partnerships for biopesticides in West Africa: stakeholder information exchange workshop, 26-27 February 2002 (Annex 17).*

This initial meeting brought together members of the public and private sectors in Ghana with interest in the promotion, implementation and commercialisation of microbial biopesticides for control of pests of peri-urban insect pests. In a series of 5 presentations, participants learned about the global biopesticide market, the mode of action and mass production of baculoviruses, constraints to biopesticides in Ghana, the perception and potential use of biopesticides in Ghana, and the pesticide registration procedure in Ghana. Armed with this information participants discussed a series of questions addressing biopesticide constraints and opportunities.

The meeting discussed routes to implementing a new biopesticide. Commercialisation was accepted as a valid route to implementation although other routes such as village level production were also discussed. The latter, although receiving some support, was generally felt to be less sustainable than commercialisation.

Accepting that commercialisation was the preferred implementation option, the meeting was asked to discuss means of promoting a biopesticide towards commercialisation. Here discussion was polarised with two fairly distinct points of view arising. One point of view held that the market should be supplied with significant quantities of the new product, either free or at a reduced price, for farmers to test. Such is the case with newly introduced chemical pesticides which leads to market demand. The second point of view held that a new biopesticide could be introduced to the market through a farmer field school IPM approach. FFS are the adopted mechanism for IPM implementation in Ghana and several projects as well as the Ministry of Agriculture itself are using this approach. It would be a relatively simple matter to insert a new biopesticide into this system.

Technology transfer to the private sector would be addressed by a partnership between those developing the technology, currently the international agricultural research institutes, and those private sector companies interested in adopting and implementing the technology. The nature of such a partnership was not elaborated, nor was the involvement of the various public sector departments in Ghana clarified.

When participants were asked to propose potential partners, both individuals and pesticide distributors expressed interest in learning more about biopesticides with a view to going into partnership towards biopesticide commercialisation. This was not the case in Benin. Like Benin however, it is clear that there are no existing companies or individuals who currently have the capacity to produce and sell microbial insecticides based on baculoviruses.

From the information that was available it seems that credit facilities for start-up companies are difficult to access. This will need further investigation.

Although the current project has specific objectives and milestones, the meeting was asked to propose crucial steps that should be addressed in the near future to facilitate progress.

Although there were several suggestions, perhaps the most urgent was the need for a market study (as in Benin) and economic study of the cost of production. Other suggested priorities were promotion, demonstration and education.

The floor was invited to offer other constraints and opportunities that had not been considered during the meeting. One of the most frequently cited opportunities concerned the development of an organic vegetable sector whose products could eventually attract a green premium. Mechanisms for certification of an organic sector gave rise to concern.

Potential constraints were seen from both technical and non-technical perspectives. Technical constraints included resistance development, shelf life and specificity. Non-technical constraints included acceptance [of viruses], sustainability when project funding ends, scientists' long-term commitment, technical backstopping and political stability.

Although products based on baculoviruses for control of some pests are already available on the market outside Africa, participants felt that it was preferable, although not essential, for Africa to develop its own options, rather than to import products from overseas.

3.2.3 *Public-private partnerships for biopesticides in West Africa, biopesticide implementation meeting, IITA Cotonou, Benin, 24 - 25 April, 2002 (Annex 18).*

The purpose of this third meeting was, like the first and second, to exchange information on the development and commercialisation of biopesticides in Ghana and Benin. The meeting was more focussed and the specific objective was to develop a strategy for the commercialisation of a biopesticide based either on the baculovirus of the tomato budworm, *Helicoverpa armigera*, or the diamondback moth, *Plutella xylostella*. The strategy should identify the pathway to commercialisation, the anticipated constraints and mechanisms to overcome those constraints. Participants worked in two groups, Francophone and Anglophone, to address a series of issues related to commercialisation of a biopesticide. The country reports are presented below:

1. Benin

1. Research Base:
The need for an inventory of the existing research base in Benin was seen as an indispensable first step. Among the institutes mentioned were INRAB, Service Protection des Végétaux et Contrôle Phytosanitaire (SPVCP), the University of Abomey-Calavi, and the Direction de l'Alimentation et de la Nutrition Appliquée (DANA). A comparison with international information was proposed in order to demonstrate differences and needs in Benin, but the utility of such information was not clarified. In a second stage, meetings should be organised between the project and the above mentioned sectors to improve relations, reinforce the research capacity and sustainability of the project. It was recommended that the project organise training and offer scholarships.

2. Commercial route to implementation:
The private sector was felt to be the best route but the following data would be needed to encourage their participation:

- Efficacy data.
- Socio-economic study on feasibility
- Market analysis of crop protection in brassicas and tomatoes.
- Commercialization report
- Technical dossier on storage, transport and application of biopesticides
- Information on regulation & registration issues
- Storage and transport conditions

- Availability of the virus

3. Manufacturing plant
It was agreed that a small-scale pilot plant should be established at IITA-Cotonou. When questioned on the rationale, participants felt strongly that IITA was the most appropriate place. Techniques could be mastered at IITA then pilot units could be set up around the country.

4. Production quality issues
The need for an independent QC body was recognised that should be different from the production unit. It was suggested that QC could come under CNAC (pesticide registration committee) with assistance from DANA. There was no disagreement on this issue.

5. Routes to market
This question caused much discussion, possibly because the group lacked the technical knowledge on which to make a judgement. There are existing routes to market: market gardeners' groups and existing distribution networks such as COMAKO; SAMAC; CAGIA; SDI. An analysis of distribution networks and their suitability was proposed.

The issues of labelling, storage, packaging and product identity came up under this question although they were not discussed in depth. The need for information was identified. There was discussion of liquid formulation in an un-refrigerated and refrigerated distribution system and dry powder formulation. Participants were clear about the benefits of a powder but seemed less sure as to what sort of refrigeration would be required. This is where a discussion about markets arose and there was a proposal that the *Hear*NPV product should be produced in a powder formulation for distribution to northern Benin. Bringing in a second product would widen a company's portfolio.

6. Registration
Here, the starting point of the group was that the registration requirements for a virus biopesticide should be greater than for a chemical. Dr David Dent explained that for most of the toxicological issues, biopesticides were much safer than chemicals. Participants were however concerned about the introduction of an exotic isolate from Kenya and wanted special ecotoxicity testing in Benin. Participants considered this an issue that was separate from registration and wanted a special study. The issue was raised as an additional point to the questions asked. Participants were interested in a generic study but perhaps because this had been previously suggested. There was a clear need to ensure no safety hazard. The provision of an Environmental Impact Assessment, using generic data is very important.

7. Market development
Participants were in favour of a knowledge first approach and the establishment of farmer field schools. The establishment of a local production unit was seen as important to help develop the market, production, promotion and progressively teaching technology to farmers. The idea of supplying market with free or subsidised sample product did not get much discussion.

2. Ghana

1. Research Base
Training and upgrading skills to extend research capacity in Ghana was the main need identified here. The primary institution was the University of Ghana as a focus for building the research base but the CRI and PPRSD could also provide supporting resources. The suggested mode of implementation was through training at IITA.

2. Commercial implementation

Established private sector companies were seen as the most appropriate and effective mechanism to achieve commercial implementation. DBM was seen as the most appropriate pest target as brassicas have few other significant pests. *Helicoverpa* on tomato was a candidate secondary target. However the private sector would need the following additional data before they would consider firm involvement:

- Host range and susceptibility data on PlxyGV (& *Hear*NPV)
- Safety dossier
- Market analysis of crop protection in brassicas and tomato
- Analysis of likely resistance risk with PlxyGV & *Hear*NPV
- Economic analysis of costs of biopesticide production
- Report on market growth and potential of biopesticides
- Technical dossier on storage, transport and application of biopesticides
- Information on regulation & registration issues
- Identify government policy on biopesticides, production and use

[Note the close agreement with the list from Benin above.]

3. Manufacturing plant

The suggested way forward was to establish a pilot plant in IITA followed by a commercial plant in Ghana. The commercial sector is keen to start production but feel the need to see a working pilot to evaluate potential and determine costs.

4. Production and quality issues

The provision of an independent QC agency was considered essential. This should fall under EPA of Ghana but all felt they were too busy to run a QC laboratory and that they should contract another organization (for example University of Ghana). This body could then exercise product stewardship to monitor post-registration quality and prevent substandard products.

5. Routes to Market

The group considered that these do exist in Ghana and that existing networks are adequate to bring biopesticides to market. However, except in a few intense peri-urban locations, handling a refrigerated product would not feasible. Farmers preferred liquid formulations, as these are easier to measure. But powder formulations would be acceptable if sold in pre-measured single-use packages standardized to match existing sprayers.

6. Registration

This should be the job of the manufacturers who should provide data to the appropriate body. This would require outside technical expertise to aid it to evaluate the registration dossier. The following information would be needed for registration:

- Efficacy data from Ghana or a similar ecological environment,
- Environmental and safety dossier (largely generic with a few in country non-target organism assays e.g. bees etc.)

7. Market development

The commercial sector has its existing routes using identified organisations to test and evaluate new candidate products before any decision is made to launch in Ghana. But it was felt that IITA through FFS and on farm demonstrations also had a role. Organisations such as CRI, PPRSD and UoG also have a clear role.

Following this third workshop, the project solicited expressions of interest from individuals or companies, who, having participated in the workshops, were willing to work closely with IIITA and its project partners during the remainder of the project, and possibly in a next phase, towards product commercialisation. The objective was the continued exchange of scientific, commercial and market information through close collaboration, but on a more detailed level than previously. The higher goal was to reach consensus on the viability of a viral biopesticide for the West African brassica market and to develop a clear strategy for its implementation. Four positive responses were received (Table 1)

Table 1. Potential partners in a second phase.

Company	Contact name	Address	Email / Tel / Fax	Comment
Agro-Conseil	Mr. Raymond Afouda	02 BP 8196 Cotonou, Rep. of Benin.	lafouda@yahoo.com Tél.: 92 25 29	Individual entrepreneur with no manufacturing capacity but interested in PlxyGV production in Benin.
Nature's Way Company limited	Mr. Newton Amaglo	Ghana.	amaglonewton@yahoo.com	Was with Jeloise Ltd., contact lost, but renewed Apr 04. Potential partner interested in PlxyGV production in Ghana.
Reiss & Co. Ltd.	Mr Henry Korboe	P.O. Box ct 5064, cantonments, Accra, Ghana.	agric@reissco.com.gh Fax: +233 21 772942	Distributor company interested in marketing, but no manufacturing capacity
Vegetable Producers and Exporters' Association of Ghana	Mr. Emmanuel Annan	P.O. Box SD 239, Accra, Ghana.	vepag@hotmail.com Tel: (233) 21 660740 ; Fax (233) 21 675580	NGO with no manufacturing capacity.

Output 4. Pesticide registration committee members in Ghana and Benin sensitised to biopesticide data requirements and regional initiatives for establishing a biopesticide regulatory frameworks.

4.1 The board of Ghana's Environmental Protection Agency, the agency responsible for pesticide registration, was unable to make a timely decision on participation in the project and were, as a consequence excluded as a collaborator. Regrettably they also declined participation in the workshop on biopesticide registration citing a conflict with their ongoing internal reorganisation programme.

4.2 The following short article on the "Pan-African Biopesticide Registration Workshop " was produced for the local press and IPM web-server. A draft meeting report prepared by Virginia Tech is presented in Annex 19.

The "Pan-African Workshop on Biopesticide Registration" was held in West Africa, from January 29 - February 2, 2001 at the Plant Health Management Division of the International Institute of Tropical Agriculture (IITA) in Cotonou, Benin. The workshop was sponsored by Virginia Polytechnic Institute and State University (Virginia Tech) and IITA [with DFID-CPP funds]. The event was part of Virginia Tech's USAID-funded project to develop biopesticides for locust and grasshopper control in Sub-Saharan African using indigenous insects. USAID support came from the Africa Emergency Locust and Grasshopper Assistance (AELGA) project in the Africa Bureau of USAID.

The workshop was attended by 40 representatives of plant protection services, pesticide registration authorities, and other stakeholder organizations from fifteen countries across Africa. FAO/Rome, the FAO Emergency Prevention Service (EMPRES), the Inter-African Phytosanitary Council of the OAU, and the Pesticide Action Network were represented. An expert on biopesticide registration from the US Environmental Protection Agency also participated.

The group spent five days reviewing how different microbe-based biological control products work, understanding how they are currently used in Africa and other parts of the world, and examining the current national and regional regulatory frameworks for registering biopesticides in Africa. Of particular interest to participants was the contribution from the South African representative who explained the procedures by which Green Muscle^{TM} was registered in South Africa. The participants developed recommendations regarding how existing regulations and guidelines for the registration of synthetic chemical pesticides can be better adapted to the unique properties of biocontrol agents.

Following the workshop, working groups for West Africa and Eastern Africa spent three days drafting relevant documents for their regions based on the recommendations. The West African working group revised its draft biopesticide registration guidelines and initiated the design for a decision document for use by the Comité Sahelien des Pesticides (Sahelian Pesticide Committee, CSP) of the Permanent Interstate Committee for Drought Control in the Sahel (CILSS). This document will be used to consistently evaluate biopesticide registration dossiers in the regional CSP system, which comprises nine countries. Through the USAID/Virginia Tech biopesticide project, two components of the guidelines had been previously prepared with the leadership of Senegal's Direction de la Protection des Végétaux (DPV). These documents have been drafted at the request of the CSP.

In Eastern Africa there is no regional system comparable to the CSP, although the South and East African Regional Committee on Harmonization (SEARCH) is working to harmonize data requirements for synthetic pesticides. The objective of the Eastern Africa work group was to develop a framing document that can be used by countries in Eastern Africa to harmonize national guidelines and regulations on pesticide registration with respect to microbial biopesticides. The work group represented pesticide registration authorities from five countries (Eritrea, Ethiopia, Kenya, Tanzania, Uganda). During the workshop and work group sessions, the individual team members made plans for how these recommendations can be put to use to facilitate biopesticide registration, including their presentation to national regulatory bodies, SEARCH, and the OAU Inter-African Phytosanitary Council.

Output 5. A tested and validated virus production system, evolved around a principle of locally sustainable practice and based on field or greenhouse production.

5.1 During year one, this activity was addressed by two students working on aspects of the sustainable mass production of *H. armigera* NPV. These studies were initiated prior to the decision of April 2002 to focus on *P. xylostella*.

5.1.1:

Yadouléton, A. (2002). [Effect of host plant on the mass production and persistence of Helicoverpa armigera nucleopolyhedrovirus]. Diplôme d'Ingénieur des Travaux, Université d'Abomey-Calavi (Collège Polytechnique Universitaire), Aménagment et Protection de l'Environnement. (Annex 20).

Nine host plants of *H. armigera* (cotton, tomato, castor bean, chick pea, *Cleome* sp., aubergine, sweet potato, tobacco and sunflower) were evaluated as potential alternative substrates to expensive artificial diet, based on imported components, for sustainable local NPV production. In small scale laboratory host plant preference trials and NPV production trials, tobacco and cotton were the most preferred host plants. Those fed on tobacco showed the greatest weight gains and also produced the greatest viral titres. Similar results were obtained from *H. armigera* larvae fed on NPV-treated whole potted plants held in cages. Felton and Duffey (1992) obtained similar results in India. Nevertheless, a locally adapted artificial diet is likely to provide greater economy because of the cost of growing potted cabbage, of keeping them pest free, and the space required to house adequate cages and rear potted plants.

5.1.2:
Kossou, I. (2002). [Study of optimisation parameters in the production on Helicoverpa armigera nucleopolyhedrovirus in larvae of Helicoverpa armigera (Hübner) fed on leaves of host plants] Diplôme d'Ingénieur des Travaux, Université d'Abomey-Calavi (Collège Polytechnique Universitaire), Aménagment et Protection de l'Environnement. (Annex 21).

The optimum host plant, viral inoculum concentration and incubation period for *Hear*NPB mass production were determined to assist development of a low cost, sustainable *Hear*NPB production system that did not depend upon expensive imported ingredients for artificial diet. Tobacco leaves treated with aqueous *Hear*NPB suspension containing $1x10^6$ OB ml^{-1} gave the heaviest larvae although higher vial yields were obtained from larvae fed with treated sunflower leaves (see graphs in Annex 21).

5.1.3 Artificial diets for *P. xylostella* larvae are available by importation but colony adaptation is required. In an alternative system using locally available materials, PlxyGV was produced in larvae fed on potted cabbage plants held in cages in a green house. Production was first optimised on excised cabbage leaves in the laboratory then the system transferred to whole potted plants for larger scale production. Optimum infection rates and PlxyGV yields were obtained by spraying cabbage leaves to run-off with aqueous suspensions of PlxyGV in 0.05% Tween 80 containing $3.8x10^5$, $3.8x10^6$ and $1.9x10^7$ OB ml^{-1}. Treated and dried leaves were used to feed groups of 2nd and 3rd instar larvae in sandwich boxes. Given the difficulty of enumerating *P. xylostella* granulovirus, optimisation of yield was based on maximising the infection rate among test larvae. Individual larvae were assumed to yield similar quantities of virus if they reached full size before death. Peak infection rates under the experimental conditions at IITA occurred on day 6 post-infection at $1.9x10^7$ OB ml^{-1} for both 2nd and 3rd instars, while at the lower dose of $3.8x10^5$ OB ml^{-1} peak infection occurred on day 7 post-infection for both instars.

The cost of producing PlxyGV at IITA Benin was estimated using a simple linear model which includes the estimated costs of all consumables and utilities, transport, staff time, depreciated capital items and construction of all buildings. A 22% overhead on all costs is included. Excluded from the model are the costs of formulation, packaging, storage, marketing & sales and profits. At a field application rate of $3x10^{12}$ OB ha^{-1} the estimated production cost per hectare would be 6053 fcfa (≈ £6.31 ha^{-1}). The retail price of common insecticides to treat 1 ha ranges from £10 to £80 depending on the product.

5.2. Given the lack of production capacity among the potential partners listed in Table 1 in a second phase, and the emphasis placed on registration and legislation, specific training in PlxyGV mass production was not given.

Output 6. At least one formulated biopesticide based on the homologous baculovirus of a key vegetable pest demonstrated in multiple participatory trials to farmers, government departments, NGOs and potential private sector entrepreneurs in both Ghana and Benin.

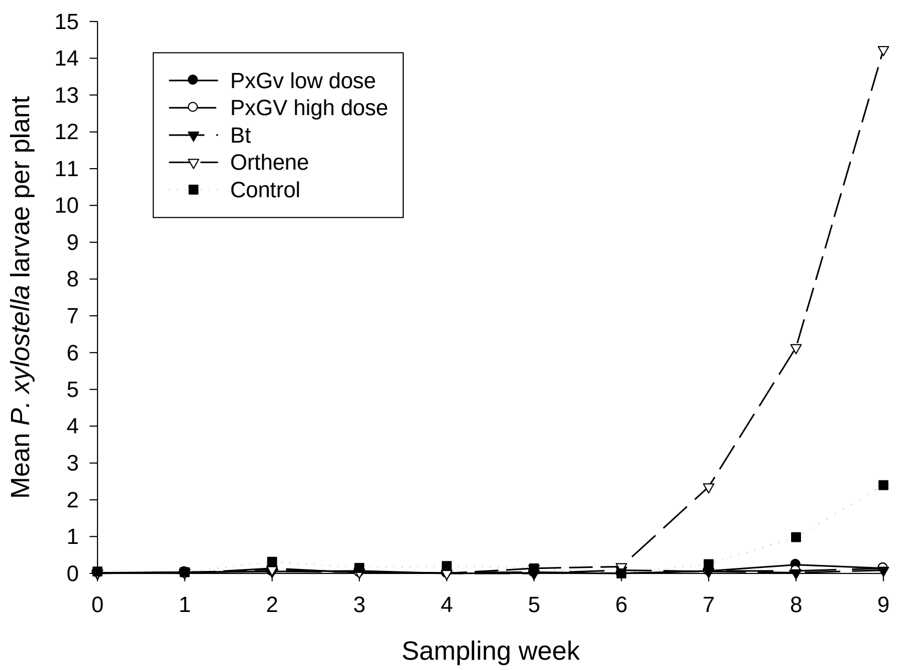
6.1 The visit by Mr Denis Djegui to Kenya established collaboration between projects ZA0462 at IITA and ZA0319/R6615 in Kenya, and also promoted good relations with KEPHIS, who were later to approve release of isolate PlxyGV-Nya01 to IITA. Protocols were adopted for PlxyGV formulation, application and data collection. Mr Djegui's report of that visit, along with protocols, is presented in Annex 22.

6.2 A brief summary of initial trials at IITA and PPRSD is presented below. A more extensive draft report in presented in Annex 23 and the data set can be found as Excel spreadsheets in Annexes 24 (Benin) and 25 (Ghana).

The locations of initial trials in Benin and Ghana were constrained by regulations relating to the release of exotic organisms. Sites were distant from peri-urban and urban vegetable production areas and consequently distant from sources of *P. xylostella* adults to invade trial plots. Low *P. xylostella* population pressure during both trials prevented thorough evaluation of treatment effect. The effect of low population density was compounded by poor soil quality and poor irrigation in both sites leading to low yields. Early attack by larvae of *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae) led to significant plant loss in PlxyGV and control plots. Nevertheless, useful data emerged relating to the impact of acephate (a common organophosphate insecticide used by farmers in the region) on natural enemies.

In the Benin trial, the density of *P. xylostella* larvae remained below 0.3 larvae per plant in PlxyGV- and Bt-treated plots throughout the trial period. In control plots there was evidence of a slight increase in population pressure from the sixth week of sampling with mean density rising to a maximum of 2.4 (± 0.65) larvae per plant. In plots treated with acephate larval density rose dramatically from the sixth week of sampling to a maximum of 14.2 (± 2.03 s.e.) larvae per plant (Figure 2). Similar trends were observed in Ghana.

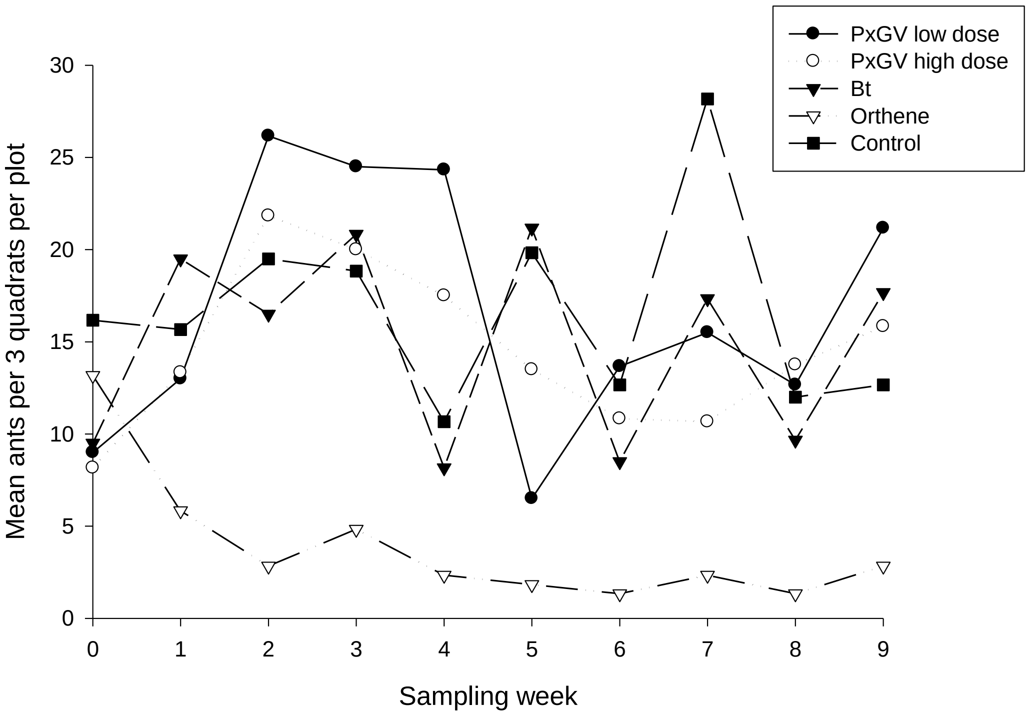
Figure 2. Density of *P. xylostella* larvae per plant by treatment throughout the Benin trial.



Despite low larval densities, PlxyGV infection among *P. xylostella* larvae could be observed and measured in virus-treated plots, although differences between high and low doses were not detected. PlxyGV was as effective as Bt at reducing larval density. Mean in-field rates of instantaneous infection observed in Benin were 55% and 28% in Ghana. This compares with 40% observed during field trials on kale in Kenya at the higher application rate of $3x10^{14}$ OB ha^{-1} (Grzywacz, 2002). The actual rate of infection is likely to be higher. Kenyan larvae sampled in the field and returned to the laboratory demonstrated mortality rates varying between 60 and 90% according to instar. Because of low larval numbers, similar measurements could not be taken in the present trials.

Natural enemies were abundant in all but acephate-treated plots. Use of acephate led to significant reductions among all natural enemy groups recorded. Ants were the most numerous predators during the present trials and in Benin the most common species were in the genus *Pheidole.* Other common species during the trial included *Camponotus serieus, C. acvapimensis* (?) and *Dorylus* sp. In the Benin trial, the ant population density fluctuated in virus-, *Bt*-treated and control plots but remained within similar limits throughout the trial period. In plots treated with acephate mean ant density was five to six fold lower (Figure 3). The trends were similar but less exaggerated in Ghana. Despite the apparent causal relationship, a correlation could not be established between ant and larval density in any of the treatments, suggesting absence of any numerical response.

Figure 3. Relative density of ants on the ground by treatment in the Benin trial, November 2002.



The results of two manipulative experiments strongly suggested that the presence of ants, particularly those in the genus *Pheidole*, was associated with the removal of larvae from cabbage plants, and may have contributed to *P. xylostella* suppression during trials despite the apparent absence of a numerical response. In the first experiment, predation of *P. xylostella* larvae in cabbage plots treated with acephate, where predator numbers were low, was less than half that in control, virus and *Bt* plots. In caged experiments, predation of larvae was significantly greater in the proximity of an ants' nest entrance.

Other natural enemy groups were also negatively impacted by acephate treatments. The mean number of spiders per plant was less than half the number in control and PlxyGV treated plots, while syrphid larvae were totally absent from acephate treated plots. Spiders increased in abundance during the trial in all treatments except acephate. They were however much less numerous than ants, reaching a maximum density of 1 per cabbage between the seventh and eighth sampling. The spider density in acephate plots declined after the first application and remained close to zero through the season. There was no correlation between the densities of spiders and *P. xylostella* larvae.

Of the three species of parasitoids attacking *P. xylostella* in Benin *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) is dominant in southern Benin. Mean annual parasitism in the Cotonou peri-urban zone was about 55% for the period 1995-1998 (Goudegnon *et al*., 2002) but overall parasitism by *C. plutellae* in the Benin trial was approximately 2% so did not play an important role in biological control of larvae.

Because of the very short persistence of PlxyGV (see Ahoyo, 2003, Annex 7), and assuming continuous recruitment to the DBM larval population, a significant proportion of DBM larvae would be expected to avoid lethal infection. As a consequence, one might have expected to see a rise in density in PlxyGV plots when population pressure increased later in the trial. That this did not occur might be attributable to natural enemies. By contrast, in acephate-treated plots, the sharp rise in *P. xylostella* density above that observed in control plots suggests not only the absence of any control by acephate, but that the absence of natural enemy groups in acephate-treated plots was a contributory factor. Despite the absence of a numerical response by ants to larval density, there is strong circumstantial evidence to suggest that natural enemies played an important role in suppressing the *P. xylostella* larvae. Any control strategies should therefore be built around conserving or encouraging their populations.

Farmers frequently reported that acephate, along with many other products, is no longer effective against *P. xylostella* larvae. The extent of acephate use among vegetable growers in Benin is likely to have led to significant resistance in *P xylostella*. At the time of writing (Apr. 04) reports from Benin suggest acephate is now less available.

Farmers' pesticide spraying practices can negatively affect natural enemy populations, particularly when applied early season. Natural enemies that might otherwise have built up and suppressed the pest are killed and more insecticides are required. Reductions in the use of hazardous insecticides and early season substitution of broad spectrum insecticides with softer biological alternatives such as Bt, NPV and botanical insecticides may permit early establishment of natural enemies and contribute to pest suppression.

6.3 *Visit by Douglas Miano to Benin*: Mr Miano's report on the visit is provided in Annex 26. The lessons learned and conclusions are reproduced below directly from that report.

Lessons learned from the visit

It was extremely useful to meet and work alongside scientists with similar interests, especially in biological control, which allowed exchange of ideas. New contacts were made, and I was exposed to a different farming system, which broadened my perspective. In PlxyGV field trials, data collection has only been done for some few weeks and thus I was able to assist with the experience gained from the Kenyan trials, in the identification of some insects especially the aphids. I also had the opportunity to see the diversity of other pests attacking different crops in Benin and possibly West African region.

It was also important to experience the fact that the virus can be used outside the area of origin. Though this sounds obvious, its practicality has been demonstrated. PlxyGV Nya-01 is endemic in Kenya and has now been shown to be effective in DBM control in West Africa, far from its place of origin. This is significant in that the virus can be used in any other part of the world where brassica is grown, as an introduction without depending on epizootic. The benefits accrued away from the place of origin in terms of better control may be far more. The best and easiest way to make use of the virus is to develop it into a commercial product for ease of access and marketing.

The trip highlighted the dire need to support the resource-poor smallholder vegetable farmers living in the urban and peri-urban regions both in Benin and Kenya. The introduction of the virus will go far in meeting the demands of the farmers in DBM control, thus giving them an alternative approach to pest control, increase their income, and help reduce pesticide hazards in peri-urban systems. The Crop Protection Programme (CPP) has been in the forefront of pushing for such technologies to alleviate poverty and improve the livelihood of farmers. This strongly supports the CPP's purpose of generating benefits to poor people through application of new knowledge on crop protection in peri-urban production systems. It will also be an important output towards achieving CPP's objective of developing sustainable rural livelihoods and in particular to combat vegetable pests. Extending the use of the virus to West Africa will also disseminate further outputs developed by CPP peri-urban Vegetable Projects Cluster in Eastern Africa.

The visit also highlights the importance of having strong linkages between researchers / projects in different regions. In so doing knowledge is shared freely and research outputs reach a wider group of beneficiaries. Such linkages will help promote strategies developed from one region to another thus reducing the impact of key pests common in the regions. The success of PlxyGV Nya01 in Benin will open doors for further collaborations, creating the need for exchange of such materials, like is already the case with Ghana and West African region. The demand for the use of the virus will be expected to spread to other regions of the world, especially with the reports from UK that the Kenyan strains of the granulovirus are more virulent than strains from other regions. With such demand expected, it will be necessary for Kenyan authorities to make it easy for researchers to access the virus, and have rules and protocols governing such transfers in place while still protecting the rights of Kenyan researchers. This will broaden the horizon for using and marketing biopesticides.

Conclusions
It seems clear that the Kenyan isolate of PlxyGV is highly virulent to Benin DBM in the laboratory, and thus has a great potential for DBM control in the region. The potential is enhanced by the absence of PlxyGV in the region, making any introduction more or less a new association. Establishing a new association between the isolate and West African DBM population holds great promise for DBM control in the region, and may be other regions in Africa and elsewhere where DBM is a threat. This creates a real need to develop the virus into a product that is readily accessible to farmers. This is in line with DFID objectives of developing environmentally friendly acceptable alternatives to chemical control.

The success story of the use of PlxyGV in Kenya, and now in West Africa and the demand by farmers, researchers and private companies such as Dudutech to take the virus to the farmers calls for an urgent need to move on to the next step of formulating the product and making it accessible to farmers. Registration for such a product still follows the normal protocols for chemical pesticides. The question still remains unresolved as to whether biopesticides should be subjected to the rigours of chemical pesticides registration process.

6.4 *Farmer participatory field trials.* The following is a brief summary of results. A more comprehensive report is in preparation. A draft is attached in Annex 23 and harvest data is available in Annexes 28 (Benin) and 29 (Ghana).

In Benin, PlxyGV performed well at all three sites in comparison to farmers' own practices with average yields from PlxyGV treated plots exceeding those in control and farmer practice plots (Table 2). Acephate (Orthene), an organophosphate insecticide was the most commonly cited insecticide used by farmers (cited by 67% of farmers) for cabbage pest control, followed by deltamethrin (Decis) cited by 39% of farmers, and Bt products cited by 32% of farmers. Earlier trials at IITA had already demonstrated significant *P. xylostella* resistance to acephate. Farmers' impressions of PlxyGV were universally positive, noting that PlxyGV was either very effective, effective, effective for now, better than other products, or that it gave good control. All wanted to see the virus registered and / or made available as soon as possible.

Table 2. Mean net weights ± s.e. (grams) per saleable harvested cabbage head after removal of outer leaves for the three treatments at the three sites in Benin; Cotonou, Lokossa and Parakou.

	Cotonou		Lokossa		Parakou		Total	
Treatment	Mean*	s.e.	Mean*	s.e.	Mean*	s.e.	Mean*	s.e.
Control	405.31[a]	33.30	589.38[a]	46.33	513.75[a]	51.27	502.81[a]	25.93
PlxyGV	895.07[c]	38.59	1,056.88[b]	52.08	1,417.50[b]	54.81	1,133.07[c]	32.09
Farmer	544.69[b]	35.87	682.50[a]	58.60	1,130.00[b]	186.08	785.73[b]	67.80
Total	602.85	24.53	776.25	32.93	1,020.42	70.92	802.61	28.30

**Within sites, means followed by a different letter are significantly different at the 5% level according to the SNK means separation test.*

At harvest, the density of *P. xylostella* larvae was significantly lower on PlxyGV-treated cabbages than in other plots at both Cotonou and Parakou. At Lokossa farmers had removed the outer leaves from plants prior to harvest for fear of introducing *P. xylostella* into their adjacent plots of cabbage. Natural enemy populations at harvest were highest in control plots and lowest in farmer practice plots.

In Ghana, PlxyGV performed less well in comparison to farmer practice and was perceived less favourably than in Benin. Data on pesticide use by farmers in the survey were collected only in Kumasi where all farmers used Dipel 2X and report very good results. In a previous survey, Bt-based products were in more common use in Ghana than in Benin. Yield data is less consistent than in Benin: Yields were greater in farmer practice plots at Kumasi and Jowulu than in PlxyGV or control plots (Table 3) while in Mampong yields were greatest in control plots. According to collaborators running the Ghana FFS trial, in Mampong the control plot performed well because it was located in a marshy area and needed no irrigation and pest pressure was very low, while the farmer responsible for the PlxyGV plots failed to properly irrigate and weed. In Jowulu, and curiously also in Mampong, PlxyGV farmers nevertheless reported good efficacy of PlxyGV although there were reservations about its specificity, while in Kumasi, when asked about PlxyGV efficacy the overall impression of PlxyGV was more negative with farmers reporting poor efficacy, narrow activity and poorer yields. On the other hand, when asked about expectations for the product, farmers in Kumasi acknowledged PlxyGV to be a good product but that its limited activity spectrum should be improved. In both Jowulu and Mampong farmers reported that use of PlxyGV gave good cabbage head quality while in Kumasi, the majority of farmers responded that cabbage quality with PlxyGV was inferior to cabbage treated according to standard farmer practices. It is likely that the more widespread use of effective Bt products in Ghana, and in Kumasi particularly, places greater expectations on new products such as PlxyGV.

Table 3. Mean gross weights ± s.e. (grams) of harvested cabbage heads before removal of outer leaves for the three treatments at the three sites in Ghana; Jowulu, Mampong and Kumasi.

	Jowulu		Mampong		Kumasi		Total	
Treatment	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Control	33.11[a]	7.47	655.00[a]	44.57	494.63	31.79	272.85	25.12
PlxyGV	631.75[b]	29.95	36.25[b]	10.41	857.63	35.97	744.68	24.98
Farmer	852.50[c]	33.95	336.25[c]	36.07	1,075.50	57.28	964.00	34.35
Total	523.33	27.48	342.50	25.32	809.25	29.25	665.42	21.09

**Within sites, means followed by a different letter are significantly different at the 5% level according to the SNK means separation test.*

6.5 Student theses

6.5.1 *Yadouleton, A. (2004). A study of the frequency of application of the granulovirus of* Plutella xylostella (L.) *(Lepidoptera: Plutellidae) and its economic impact on productivity in a cabbage field (Annex 30).*

The initial PlxyGV release trials and FFS trials in Ghana and Benin left a number of questions unanswered, and following recommendations from project ZA0319 (Biorational brassica IPM), Yadouleton (2004) conducted a large replicated on-station trial at IITA Benin to investigate and compare the effects of applying PlxyGV on a weekly basis at $3x10^{12}$ OB ha^{-1}, on a daily basis at 10% dose, and at 10% dose but according to an action threshold of 0.2 larvae per plant. Sixteen plots each measuring 12x12 m were arranged in a Latin square such that each of the 4 treatments was represented by 4 plots, with each plot containing approximately 625 cabbage plants. The third replicate served as a demonstration plot for participants at the final project meeting discussed below under output 7. At the time of writing, the thesis which will be submitted for an MSc to the University of Abomey Calavi, Benin, is still in preparation and a draft version is attached in Annex 30. In previous trials at IITA *H. undalis* and aphids caused serious losses. To avoid similar problems in this trial, Dipel (*Bacillus thuringiensis*) was applied uniformly across all plots when the action threshold density of 3 *H. undalis* larvae per 10 plants was reached.

Table 4. Mean head weight and economic value of cabbages under various PlxyGV treatment regimes.

Parameter*	Treatment			
	Weekly $3x10^{12}$	Daily $3x10^{11}$	Threshold $3x10^{11}$	Control
Mean head weight (g)	995	1595	988	632
Max. economic value per plot (cfa)[1]	45650	67500	39850	22500
Min. economic value per plot (cfa)	38500	57500	30000	15000

**At the time of writing data have not been analysed and standard errors are not yet available*
[1]There are approximately 960 cfa to the UK pound at the time of writing (Apr. 04)

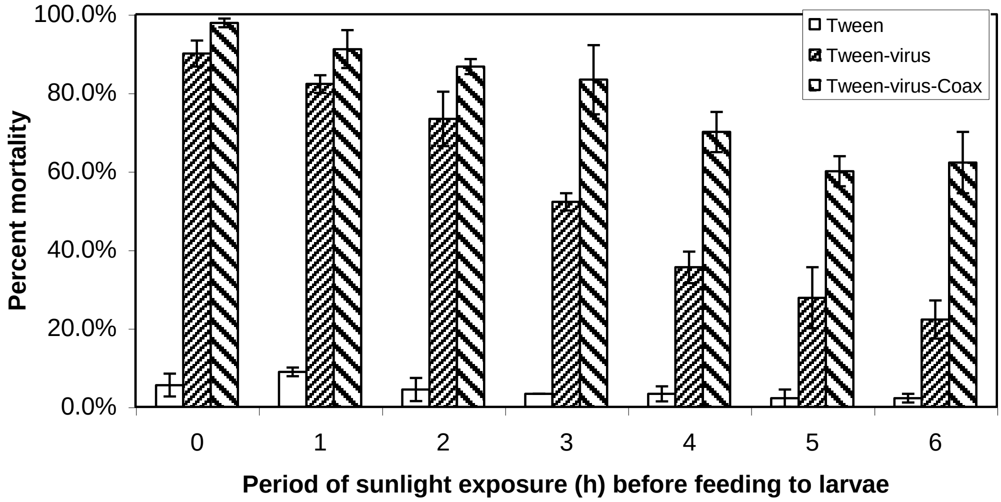
In all plots treated with PlxyGV, there was a reduction in mean larval density relative to the density in control plots and as a consequence, an increase in the mean cabbage head weight and commercial value. The greatest reduction in larval density and hence highest yield and economic return occurred in plots treated on a daily basis at $3x10^{11}$ OB ha^{-1} (Table 4). As in field trials under 6.4, economic value was attributed by market retailers with no prior knowledge of the differences in treatments between plots.

6.5.2 *Tchougourou, A. (2004) Comparative study of the efficacy and persistence of the granulovirus of Plutella xylostella (PlxyGV) in two different formulations (Annex 31).*

As a addition to the field trial reported under 6.5.1, Tchougourou (2004) investigated the ability of the proprietary adjuvant "Coax®" to provide greater persistence and hence greater efficacy of PlxyGV. A draft version of the thesis (in French) is reproduced in Annex 31. At the time of writing the thesis had not been submitted to the University of Abomey Calavi, Benin. A brief summary of the major results is presented here.

In the laboratory, Coax alone applied to cabbage leaves and fed to second instar larvae had no appreciable effect on larval development compared to a control treatment. In combination with PlxyGV, Coax led to a slight but non-significant rise in larval infection and mortality over the 9-day bioassay period. However, when cabbage leaves treated with Coax + PlxyGV were exposed for periods of 0 to 6 hours to peak solar radiation between 10:00 and 14:00 hr, the protective effect of Coax became evident (Figure 4).

Figure 4. Mortality of *P. xylostella* larvae fed on cabbage leaves previously treated with PlxyGV and exposed to varying periods of solar radiation. (After Tchougourou, 2004).



While larval mortality due to PlxyGV alone fell off rapidly following increasing periods of exposure to sunlight from 0 to 6 hours, when combined with Coax, the decline was significantly reduced. Formulated alone, PlxyGV that had been exposed to 6 h sunlight caused only 22% larval mortality, while after the same period, virus formulated with Coax still caused 62% mortality. The difference in mortality due to the two formulations was significant (P=0.05) from 2 h sunlight exposure onwards.

The protective effect of Coax demonstrated in laboratory assays on potted plants was translated to the field, leading to greater absolute numbers of infected *P. xylostella* larvae, reduced damage and increased yields in plots treated with Coax + PlxyGV compared to plots treated with PlxyGV alone. Mean gross weights of cabbage head in grams (± s.e.) were 716.87 (± 36.74), 927.50 (± 35.04), and 1385.70 (± 50.96) for control, virus alone and virus + Coax treatments respectively.

Output 7. Recommendation of a strategy/strategies for commercialisation of a baculoviral biopesticide in Ghana and Benin. (All activities under 7 were conducted as part of a final project meeting for Beninese and Ghanaian stakeholders.)

7.1, 7.2 & 7.3. As part of the process of bringing this project phase to a close, a final two-day conference was held in November 2003 at IITA-Benin to discuss strategies for development of PlxyGV and / or other biopesticides for the region. The meeting, attended by 40 stakeholders from Benin and Ghana, including several growers who had participated in farmer field schools, was facilitated by biopesticide experts from NRI and CAB International. Project staff summarised achievements and additional presentations were given by representatives of the public and private sectors, as well as by growers themselves.

Questionnaire

Participants at the meeting were requested to complete a questionnaire (Annex 32) addressing output 7.3 that sought to assess participants' perception of biopesticides and of their markets in Africa, participants' involvement in the project, and participants' proposals for a second phase. The questionnaire was designed prior to the start of the workshop in collaboration with the IITA socio-economist, Dr Coulibaly.

A spreadsheet containing responses from the 30 participants who completed the questionnaire is included in Annex 33. Among the listed benefits of biopesticides, protection of human health, protection of the environment, and reducing the use of chemical pesticides were each cited by over 80% of respondents. Ghanaian participants were more concerned about reducing the use of chemical pesticides, while Beninese participants were more concerned with protecting human health and the environment. When asked about the major disadvantages of biopesticides, specificity was cited by 63%, problems of storage, handling and persistence by 53%, speed of action by 26% of respondents, availability was cited by 20% and cost by 16%.

All Ghanaian respondents believe there is a market for biopesticides in their country but only 26% of Beninese (although a subtle difference in the question due to translation may have influenced this result). Participants were asked which markets they felt were most amenable to biopesticides. Responses were mixed but dominated by vegetable crops / market garden crops and the peri-urban and urban markets. The crops most frequently cited as potential targets for biopesticide use were unsurprisingly cabbage (60%) followed by tomatoes (33%), cowpea (26%) and cotton (23%). To encourage biopesticide use in the proposed markets over 90% of respondents agreed that they should be made available and accessible to farmers in towns and villages.

Most respondents were able to name at least one biopesticide available in their country but the frequency was greater in Ghana than in Benin. Dipel and Biobit were the most commonly named biopesticides, with neem products mentioned occasionally. Few respondents were aware of any biopesticide producers in the region although several Beninese participants cited IITA. Only 60% of respondents believed their countries should import biopesticides while fully 100% believed they should establish local production of biopesticides. The principal reasons for establishing local production were based on the belief that locally produced biopesticides would be of lower cost and be more available, and that it would create employment.

Among the major constraints to biopesticide adoption proposed in the questionnaire, problems with registration and lack of information were considered the most important. Registration was more of an issue in Benin than in Ghana.

Questions about participation in the project where almost entirely positive. Over 93% of respondents agreed that their involvement in the project had had a positive influence on their perception of biopesticides and 70% believed their opinion of biopesticides had changed (favourably). Up to 90% believed that their knowledge of biopesticides had increased, and 93% agreed that as a result of involvement in the project opportunities for biopesticides in their respective countries would increase.

In the third part of the questionnaire, participants were asked about a second phase of the project. Among the subjects that participants felt should have been included in the first phase, the most frequently cited was biopesticide registration. Although the project did address registration, participants felt that the project should have gone further towards registration of PlxyGV in either Benin of Ghana. It is interested to recall that an early version of the PMF did include biopesticide registration as an objective, but in response to a comment made during PMF reviews that implied registration of a biopesticide would be an (over-) ambitious objective, the objective was dropped. It should also be recalled that although the Ghanaian Environmental Protection Agency (the agency responsible for pesticide registration in Ghana) was invited to collaborate in the project, they were unable to reach a decision by the time the PMF was submitted and were consequently excluded. Similarly, an invitation to EPA to participate in the biopesticide registration workshop of Jan 2001 was declined.

Priorities and important activities for a second phase according to respondents were, in order of importance (frequency of citation), registration / legislation issues (cited by 73% of respondents), training and education (for general public and stakeholders) (70%), establishing (local) mass production capacity (43%), and market and economic feasibility studies (27%).

At the end of the two days' of presentations and discussions, visiting experts Grzywacz (NRI) and Dent (CABI) conducted two analyses to assist participants in preparing a future strategy for biopesticide development:

1. *Participants' priorities for a second phase*. Although this subject was also investigated within the questionnaire, it was addressed a second time by linking to major themes coming out of discussions during the meeting. Participants were presented with a series of seven options for a future phase that they were asked to prioritise by ranking (1 = highest priority, 7 = lowest priority) and results are given in Table 5:

1. Identify the full market potential for virus biopesticides in all relevant crops and countries in the region – West Africa.
2. Encourage importation and distribution of existing biopesticide products to stimulate market.
3. Farmer/extension education programme.
4. Facilitate introduction of biopesticide registration procedures (including training) at national and regional level (CEPHAOC).
5. Establish production capability at appropriate level for example local / national / regional.
6. Ensure appropriate mechanisms exist to assess and maintain quality standards.
7. Other ideas.

Table 5: Results of a ranking exercise to determine priority activities for a second phase.

Question	Cumulative score	Rank
1	88	2
2	150	6
3	102	3
4	61	1
5	126	4
6	148	5
(7	197	7)

Other priorities suggested by responding participants under question 7 of the priority ranking exercise included the following:

- Ensure GV product purity.
- Assistance to small producers.
- Study the financial profitability of production and commercialisation of the virus.
- Publicise organic products.
- Analyse the economic profitability of installing a national biopesticide production industry relative to importation.
- Sensitize people about the advantages of use of biopesticides for biological control.
- Training for producers on best storage practices.
- Dialogue with policy makers.

- Train national distributors in biopesticide awareness/knowledge.
- Involvement of national companies in biopesticide promotion.
- Encourage private sector involvement in production and distribution.

2 *SWOT analysis of* PlxyGV: Participants contributed to an open discussion of the strengths, weaknesses, opportunities and threats to PlxyGV. The summarised results presented below confirm broadly held beliefs about biopesticides

Table 6: Results of a SWOT analysis of PlxyGV implementation in Benin and Ghana.

Strengths	**Weaknesses**
Good efficacy	Narrow spectrum
No mammalian toxicity	Slower speed of action
Harmless to natural enemies	No registration framework
Not persistent in environment	Short crop persistence
Low risk resistance	Store ability limited
Interest from farmers	Unavailability
Good crop quality	Lack of training (industry + farmers)
Yield increase	Low public awareness
Specific to pests	Intellectual property rights
Safe to handle	Poor promotion mechanisms
Horizontal transmission	Cost of production
No residue problems	Lack of production capacity
Small scale production easy	Lack of market information
	Market limited
	Limited supply
	Lack of awareness of gender issues
Opportunities:	**Threats:**
Positive attitude by regulators	Public concern about viruses
Large potential market	Competition from chemical pesticides
Expectant farmers	Availability of promotional information
MRLS	Uncertainty about importation and regulation
Concern about pesticides	Quality enforcement
Alternative technology	Product piracy
Failure of alternative technologies	Poor handling of the product
Potential on farm production	Inappropriate distribution infrastructures
Human resources available to sustain industry	Product failure (resistance)
Possible sources of funding	Introduction of exotic species
User friendly	Maintenance of quality
Technology available	Product robustness
Good research background	Lack of interest in production by private sector
Good collaboration Benin/Ghana	Human nature
	Lack of capital investment

The results of both the analysis of priorities and of the SWOT analysis fed into the report by Dent (2004) on the strategy for commercialisation of a baculoviral biopesticide in Ghana and Benin (Annex 34). The recommendations of that report are presented below (Box 1).

Box 1: Recommendations from Dent (2004).

Phase 1 of the project has stimulated interest among the complete range of players that could be involved in future development of Biopesticides in Benin and Ghana including importers, distributors, regulatory authorities, research organisations, farmers and co-operatives. This is a significant and highly commendable achievement. Many projects may try to make such claims but this project has genuinely achieved this. There is an interest, enthusiasm and commitment for continuing the process to ensure establishment of biopesticides in the countries and the region, driven largely by the commercial companies, regulatory authorities and vegetable farmers. However, the constraints faced to successful introduction of a biopesticide manufacturing capability are still considerable. There remains however both an opportunity and a need for Biopesticides in Benin, Ghana and the rest of West Africa and a second phase project could greatly facilitate that process by adopting options 3 and 2 above. The following steps are recommended:

1 Workshops for regulatory authorities in West Africa on the introduction of biopesticide product specific regulations including provision of model regulations.
2 A market analysis for Biopesticides as a whole in West Africa (from a commercial rather than an academic perspective).
3 An analysis that completes the gaps in the phase I project and the study of Langewald and Bruntrup (2002) to identify potential commercial partners and countries for a biopesticide manufacturing plant.
4 Funding of a two person team to champion biopesticide production and uptake in West Africa.

Contribution of Outputs to Developmental Impact

Among the many achievements of this project, two stand out for their important contribution to DFID's developmental goals:

In the first place, the project has made a significant and sustainable contribution to promoting environmentally friendly biopesticides in Benin and Ghana as alternatives or adjuncts to existing synthetic pesticides that could alleviate the familiar problems of over-reliance on synthetic chemical pesticides in vegetable crops. This has been achieved through training and information exchange: Firstly, the project hosted six undergraduate students during their six-month placements, two MSc students and a further two vacation students who in their turn will go on to promote safer crop protection for future generations. For example, one former MSc student now occupies an important post at the Ghana Atomic Energy Commission with responsibility for biopesticide research. Secondly, scientists from national plant protection programmes who participated in project workshops or trials are now more aware of the benefits of biopesticides and the inherent danger of over-reliance on the older groups of synthetic pesticides so common in W. Africa. These key stakeholders have been positively influenced by participation in the project and have an improved perception of biopesticides. In the medium to long term this will have an influence on pest control strategy in these countries. Thirdly, the private sector pesticide distributors who participated in meetings have become more favourably inclined towards biopesticides and at least one major distributor in Ghana has expressed interest in including biopesticides in his company's product portfolio. Thus in these broad terms the project has done much to increase the profile of biopesticides.

Secondly, and in more specific terms the project has demonstrated the efficacy of PlxyGV against larvae of the diamondback moth and created a demand among farmers, particularly in Benin for a product based on this virus. The responses of the 100 farmers who participated in trials were encouraging and suggest that adoption would be widespread if the product were available. Adoption of PlxyGV for DBM control would dramatically reduce synthetic pesticide residues on cabbage and thereby contribute to greater food safety, as well as reducing farmers' exposure to pesticides. Farmers also noted that DBM could be controlled with fewer applications of PlxyGV thus saving time. Farmers who have abandoned cabbage production might also be encouraged to return to the crop.

Promotion pathways:

Stakeholders and project collaborators agree that the most suitable promotion pathway for PlxyGV is via commercialisation in the private sector. Private sector [microbial] biopesticide manufacturers do not exist either in Benin or Ghana. Nevertheless, a small number of individuals and distribution companies have been identified in both countries who, with technical and financial backstopping from a second phase of the project, could establish a manufacturing and marketing capacity for PlxyGV.

Follow-up action:

PlxyGV is not available to farmers in W. Africa. Implementation and adoption depends on several factors and a number of steps need to be taken to move forward. Thanks to stakeholder feedback at project meetings and assistance from expert collaborators, there is clarity and broad agreement regarding the steps that have to be tackled. These steps would form the outputs of a second phase in which the technology provider and private sector manufacturer establish an equal partnership. The role of the technology provider would be product stewardship and technical backstopping while the private partner would bring commercial and marketing experience. Donor support will be required during this phase.

- Potential investors and manufacturers require detailed information on potential sales volume and product pricing. Socio-economic and market studies conducted in this phase of the project have addressed opportunities and constraints as well as farmer and consumer attitudes. The cost of PlxyGV production at IITA has been determined but there is no information on full-scale manufacture and marketing costs, or on potential demand.

- Local production units should be established to supply the market: IITA is the favoured site for a pilot plant during the establishment phase that would be used to determine product cost and potential sales volume. Proposed mechanisms to promote PlxyGV during the establishment phase include product subsidies and the use of demonstration plots.

- Guidelines for biopesticide registration are seen as necessary; however in establishing guidelines it will be essential to proceed with caution to avoid erecting unnecessary barriers. Registration without specific biopesticide guidelines is possible, but dossiers may be delayed. It is also conceivable that using local isolates, individual entrepreneurs could operate informally without registration in the first instance to establish a market. In Benin, the permanent secretary of the pesticide registration committee who participated in the project, is in favour of biopesticides and has indicated that a provisional sales licence could be granted prior to full registration if sufficient evidence were to be provided to support the safety of PlxyGV to the satisfaction of the full committee. Evidence of baculovirus safety exists in the public domain to support such an application. The Ghanaian Environmental Protection Agency is more guarded. The registration authorities of both countries would like to receive training in evaluation of biopesticide dossiers once registration guidelines are in place.

- Development of robust formulation, packaging, distribution network and label information (i.e. user guidelines).

- Compile and submit a registration dossier. Achieving PlxyGV registration will be a key step in the implementation process. If this responsibility falls within the remit of a second phase, it will determine the scale, cost and complexity of the phase.

Excessive or unnecessary data requirements on the part of the regulatory authorities will lead to delays, increased costs and could ultimately jeopardise product feasibility.

- Use of PlxyGV-Nya01 from Kenya is currently restricted under the terms of the Material Transfer Agreement with KEPHIS. Any proposal to develop the isolate commercially would require prior approval from KEPHIS and may incur additional costs if a licensing fee is imposed. Using local isolates could avoid potential delays and licensing fees but PlxyGV has not yet been isolated from either Benin or Ghana although a Togolese isolate is available. This issue was not raised during stakeholder meetings but satisfactory resolution would have to be the first priority of even precondition of any second phase.

- Training and education. Almost all stakeholders have, at one time or another, expressed the importance of further training. Training will be a central element in any future phase but defining the level of training necessary for by each group of stakeholders in order to achieve to the goal of biopesticide implementation will be essential for efficient project resources management.

The outputs of the current project should be viewed not only on their own merit, but more importantly in the wider context of the achievements of the IITA Benin station in promoting biological pest control in agriculture in sub-Saharan Africa. In the absence of any eventual follow-up or second phase of this specific project, it is important to recognise that project outputs feed into on-going and future biological control projects at IITA. One existing project at the IITA Benin station focuses on the production of healthy peri-urban vegetables, including cabbage and the advances achieved by the current project feed into the latter both directly and indirectly.

Furthermore, outputs should also be viewed in the context of results of the cluster of CPP-funded vegetable pest projects in Kenya. The potential for commercial PlxyGV production in Kenya to supply the export crop market is viewed as a very positive development that could be exploited to meet the demand for PlxyGV in W. Africa in the absence of local production capacity, and / or in the absence of economic imperatives to sustainably adopt PlxyGV.

Publication list:

Please see Annex 35, or the Project Completion Summary Sheet.

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Biometrician's Signature

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I hereby confirm that all biometric issues have been adequately addressed in the Final Technical Report of the above Project.

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Index of Annexes

Annex	Title
1	Protocol: Field sampling and laboratory screening for pathogens of *Plutella xylostella* larvae.
2	Terms of Reference for biopesticides socio-economic studies in Ghana and Benin, elaborated during a meeting at IITA, Cotonou, 8 and 9 March, 2001.
3	Excel spread sheet: PlxyGV optimisation data set.
4	Protocol: *Plutella xylostella* granulovirus (*Plxy*GV) field trials, IITA Benin. November 2002.
5	Terms and conditions for a visit to IITA Cotonou station, Benin by Mr Douglas Miano, from the Kenya Agricultural Research Institute to observe and participate in trials of Kenyan *Plutella xylostella* granulovirus (isolate Nya-01), November / December, 2002.
6	Excel spreadsheet of data from surveys in 2001 for pathogens of *P. xylostella* and *H. armigera* in Ghana and Benin.
7	Ahoyo, C. (2003). [Effect of ultraviolet solar radiation on the activity of (*Plutella xylostella*) granulovirus and determination of the median lethal dose]. Ingénieur agronome, Université d'Abomey-Calavi (Faculté des Sciences Agronomiques), Protection des Végétaux
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16	Rapport sur la première réunion « Échange d'Information sur les Biopesticides entre les Secteurs Publics et Privés au Bénin », du 29 au 30 janvier 2002.

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28	Excel spread sheet: FFS harvest data set Benin
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