

# **Integrated management of the banana weevil in Uganda**

**R7972 (ZA0472)**



## **FINAL TECHNICAL REPORT**

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## 1. Executive Summary

The DFID-CPP funded Weevil Project R7972 “Integrated management of the banana weevil” aimed at develop an integrated pest management strategy for banana weevil with emphasis on the use of the entomopathogenic fungus *Beauveria bassiana* as a microbial pesticide.

### The project had the following outputs:

1. A technique of producing consistent good quality inoculum of *B. bassiana* in an appropriate formulation developed and optimum conditions for deployment described
2. Method of production, harvesting and packaging of *B. bassiana* refined.
3. *B. bassiana* delivery systems evaluated under farmers conditions
4. A protocol for integrating different banana weevil control options with *B. bassiana* developed and evaluated.
5. Reports, referred papers prepared and delivered
6. Workshop conducted to consider success of technologies and further promotion

To achieve the above named outputs, the project scientists in the National Agricultural Research Organisation-National Banana Research Programme Kawanda in collaboration with the International Institute of Tropical Agriculture (IITA) and CAB International – Africa Regional Centre, were to develop a technique to produce large quantities of viable *B. bassiana* conidia using a fine mass production technique. In 2001, the project initiated studies at Kawanda-Uganda and at CABI-ARC Kenya, to evaluate use of waste products and other locally available carbohydrate sources for the mass production of *B. bassiana*. The mass produced and formulated local isolate of *B. bassiana* that was found to be highly infective to *Cosmopolites sordidus* from on-station experiments was further evaluated under farmer-participatory trials to determine optimum conditions under which the fungus can be applied in combination with appropriate cultural practices. An understanding of the epidemiological potential and persistence of the fungus under different banana management conditions was investigated at Kawanda by evaluating the performance of *B. bassiana* applied in fields with different organic and inorganic amendments and also under different banana plant densities.

In November 2001, a research assistant was attached to the project, to undertake an MSc research project (enrolled at Makerere University) on the influence of soil organic amendments used in the banana farming system on efficacy and persistence of *B. bassiana*. Other research work linked to the project was the development of kairomones and pheromones in delivery systems of *B. bassiana*, was undertaken by two other students an MSc student funded by BMZ through IITA and a PhD student funded by the Rockefeller Foundation through IITA. In addition to these students’ studies, an on-station field trial to evaluate the efficacy and persistence of *B. bassiana* applied under three plant spacings was established at Kawanda. In 2002, a preliminary survey on farmers that would be selected to participate in the on-farm testing of *B. bassiana* was done at Masaka-Kisekka banana benchmark site. On-farm trials were initiated in mid 2003 and a preliminary survey of farmers perceptions done in early 2004 indicate that most farmers are happy with the technology and they would be willing to pay for this control method if it is available at a cost far cheaper than the current chemical control cost on the market. To disseminate the outputs of this project, two refereed articles were produced on work in this project and related to studies in the other DFID-CPP funded projects.

More than 6 papers were presented at national and international workshops conferences. Five progress reports were prepared and are included as an appendix to this report.

## **2. Background**

The banana weevil (*Cosmopolites sordidus* Germar) is a monospecific pest and infests bananas wherever they are grown. It originates in S E Asia and has been distributed on vegetatively produced planting material. It has for long been considered a major constraint for banana production in Uganda. It was first reported in Uganda in 1918 (Harris, W.V. *The East African Agricultural Journal*, 1947) and was probably already well established at that time. In the past, several control options were considered including the introduction of predators and the use of insecticides. The predators failed to establish and where insecticides were used, resistance soon developed. Since 1991, the Uganda National Banana Research Programme (UNBRP) in collaboration with its partners, the International Institute of Tropical Agriculture (IITA), CABI Biosciences, and The University of Reading have implemented an integrated pest management (IPM) approach to manage this pest which farmers have reported to be one of the major constraints to productivity. This project has put an emphasis on the use of the entomopathogen *Beauveria bassiana* as a major component of the likely IPM strategy.

Development of microbial control of the banana weevil has gone through a series of stages involving, isolation and characterisation, screening and pathogenicity testing, and evaluation of mass-production and delivery systems. Various strains of *B. bassiana* have been isolated from soil and insects hosts in Uganda, which can cause 50%-100% mortality in 2 weeks. Some of the *B. bassiana* isolates have shown good growth and spore production on locally available substrates such as cracked maize and maize bran. Field evaluation of possible delivery systems of *B. bassiana* showed that application of the entomopathogenic fungi with planting material, pseudostem traps or soil around the banana plants can be used to infect banana weevil in the field and also reduce the damage caused to the plant. These studies have demonstrated that good potential exists for the use of *B. bassiana* and would fit well with the broad IPM context being developed for the banana weevil. It was necessary to further investigate the applicability of this entomopathogenic fungus to the banana farming system in more ecological and complex farming conditions.

## **3. Project Purpose**

In this project further research was undertaken to integrate *B. bassiana* with other banana weevil IPM options, such as use of semiochemical-based traps and also developing economically viable delivery systems that will overcome the problems associated with fungal efficacy, persistence and transmission.

## **4. Research Activities:**

To achieve the above project outputs, a series of stakeholders meetings were conducted to plan for the activities. In Uganda two meetings were held, one in May 2001 between Drs Gold, Godonou and Nankinga. This meeting was to discuss work progress and activities planned for the project (Report filed in Appendix). The second meeting was in July 2001 and was held between Drs Gold, Moore, Gowen and Nankinga to discuss progress and design for on-station trials. A third meeting was held in Kenya in November 2001. At that meeting progress and delivery system of *B. bassiana* against banana weevil was discussed (report filed). The output of these meetings was to enable the project collaborators interact and jointly plan and review the project activities. Since 2002, various meetings and seminars have been conducted between the NBRP leader Dr Tushemereirwe, and Drs Gowen, Moore, Gold, Nankinga, Rutherford together with other National Banana programme research scientists, to review and plan research activities on *Beauveria bassiana* for banana weevil control and other joint banana IPM research activities at the benchmark sites.

Most of the project progress reported here has received contributions from and witnessed by a team of researchers from collaborating institutions Drs Simon Gowen and Savitri Abeyasekera (University of Reading), Dr. Mike Rutherford (CABI Bioscience), Mr Richard Lamboll (NRI) and Mr Philip Ragama (IITA) all gave their views on scientific, social economic and statistical matters on the activities. Additionally researchers from other countries working on the banana weevil; Dr

Altus Viljoen and Mr Edzard Grimbeek from the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria, South Africa joined the visiting team and were able to contribute. The project activities are reported according to the six outputs of the project as follows:

#### **4.1. Output 1: A technique of producing consistent good quality inoculum of *B. bassiana* and appropriate formulation developed and optimum conditions for deployment described**

For successful use of entomopathogenic fungi for insect control, it is important to select a strain that displays rapid growth, abundant sporulation and high virulence to the target pest and can be economically mass-produced. At Kawanda we adopted a two-phase system utilizing sucrose and yeast-based liquid media and various solid substrates for the production of *B. bassiana*. Previously we used cracked maize as a solid substrate for production of the fungus. Whereas the maize-based formulations produced satisfactory kill rates of banana weevils, the formulation cost of application was high and it was proposed to explore more substrates that could producing high fungal yields but lower costs. We therefore further screened other locally available substrates. It has been found that maize is still yielding better than all the substrates screened. Maize bran and 'machecha' (millet waste from local breweries) can provide adequate yields (e.g.  $2 \times 10^7$ -  $10^8$  conidia  $g^{-1}$  substrate), while cheaper materials such as bagasse (residues from extracted sugar cane) and cotton seed waste (following oil extraction) gave unsatisfactorily low yields. Though 'machecha' can produce satisfactory yields it not always available. A technical difficulty experienced with maize bran is caking caused when the bran is not thoroughly mixed and is too wet, this results in lower spore yields due to incomplete ramification of the mycelia of the fungus. Based on these preliminary studies a more detailed study was conducted as an Msc study component for Magara Everest of which summary is given below:

##### **4.1.2. Determination of conidial yields from selected *B. bassiana* substrates, and infectivity of different formulations against the banana weevil (part of MSc for Magara Everest).**

Eight substrates were evaluated for conidial yield: Cracked maize, maize bran, bagasse, "machicha"\*, cotton husks, maize bran + bagasse, maize bran + cotton husks and bagasse + spent. The substrates were cultured following the modified diphasic method, which entails preparation of sucrose yeast, fungal inoculation and incubation of the substrates. Both cracked maize and maize bran produced significantly higher conidia than the other substrates. Cracked maize yielded the highest amount of conidia per unit gram of substrate ( $3.2 \times 10^9$  conidia per gram), while cotton husks had the lowest ( $2.6 \times 10^8$  conidia per gram). However, the conidial yields from cracked maize and maize bran were not significantly different ( $P > 0.05$ ). It was generally observed that *Beauveria* on cracked maize sporulated more profusely than on other substrates.

Eleven *B. bassiana* formulations were evaluated under laboratory conditions; maize bran alone, maize bran + loam soil, maize bran + clay soil, "Machicha" ("bussa") alone, "machicha" + loam soil, "machicha" + clay soil, Cracked maize alone, cracked maize + loam soil, cracked maze + clay soil, Loam soil alone, clay soil alone, and control (nothing added). Significant differences ( $p \leq 0.05$ ) in weevil mortality were observed between the different formulations. Cracked maize-based *B. bassiana* formulations caused the highest weevil mortality (90%), while maize bran + loam soil, caused the lowest. Weevil mortality progressively increased with number of days with the lowest registered at 5 days, and highest at 30 days. It was only the cracked maize formulation that caused 50 % mortality at 15 days. It was also observed that soil formulations of "machicha" and maize bran reduced its infectivity except the clay-based formulation of cracked maize.

#### **4.2. Output 2: Method of production, harvesting and packaging of *Beauveria bassiana* refined.**

This activity was implemented at Kawanda in Uganda and also at CABI Kenya.

In Uganda the production procedure described by Nankinga (1999) was modified as follows: The maize substrate material was reduced from 250-500g to 100g per sterilizable polyethylene bag to improve aeration and reduce caking during growth. Accordingly, the inoculation liquid of sucrose-yeast media was reduced from 75 to 20 ml for 100g of substrate. This also provides ample moisture for growth of the fungus on the substrate. Evaluation of maize bran, cracked maize, bagasse, and cotton waste showed that cracked maize gave the best growth characters, good sporulation and no caking. *B. bassiana* hardly grows on bagasse unless if it is incorporated with an extra carbohydrate such maize bran. A ratio of 90g bagasse: 10g of maize bran or lower levels (<10g of maize bran) incorporated with 90-100g of bagasse give some growth but with insufficient sporulation.

The harvesting of conidia from the substrate was improved by a mycoharvester purchased from CABI. It was found in the implementation of the project, that it was not appropriate to apply conidia powder in the banana field, as the solid substrate based formulation was more persistent in the soil. The harvested conidia were only used for simple laboratory assays. The maize-based formulation was packaged in sterile polythene bags in 100g and 200g packs and applied in on-station trials. Before use, these packs were stored at laboratory temperature, under refrigeration or by deep freezing.

A study on the shelf life of the maize-based formulation was done at Kawanda by determining the viability and infectivity of the formulation over time. For the first three months (90days), after growth and drying under laboratory conditions, *B. bassiana* caused over 85% weevil mortality in 30 days. Thereafter, the fungus efficacy declined and by 180 days storage at room temperature, the maize-based formulation caused only 20% weevil mortality. The freshly prepared cracked maize formulation was white, however, as storage time increased, the formulation lost its colour due attack by to mites. The major deduction from this study is that cracked maize grains are ideal for *B. bassiana* production and the dried formulation is effective against the weevil but could only store for 3-4 months. This storage period could be improved by further drying of the grains to reach a moisture level in the conidia of less than 5%.

In most studies in Uganda *Beauveria bassiana* has been applied as a dry formulation of solid carbohydrate substrate used in culturing or the dry carbohydrate substrate-based fungus mixed with sterilised dry soil or clay to form dust formulations.

No packaging and storage of conidia was done in Uganda due to lack of a freezer and unstable electricity power supply. It is proposed to investigate the alternative ways of producing, packaging and transporting the *B. bassiana* used for on-farm applications. To reduce the adverse effects of the sun heat and sunrays, the on-farm fungus has been transported in coolers from Kawanda. This may not be a feasible way especially if large on-farm trials requiring big amounts of inoculum are to be implemented. One of the proposals to overcome this bulky transportation of the inoculum is to have a decentralised production centre near the site of operation. As a follow-up study from this project, it will require more investigation in the quality standards of the fungus being produced. Funds are being sought to investigate the feasibility of implementing on-farm cottage production and storage.

At CABI Kenya, waste solid substrates from local brewer industries (busaa), sugar factory (bagasse), rice husks were evaluated against rice grains for their potential in *B. bassiana* aerial conidia production. The waste solid product of busaa was tested under different moisture content for aerial conidia production. It was found that moisture content of the substrate affected *B. bassiana* conidia production.

#### **4.2.1. Production of trial quantities of *B. bassiana* of defined quality standards, conducted under approved conditions of safety.**

##### **Introduction**

Rice is typically used as the substrate for mass production of *B. bassiana* but there was a need to identify low-cost, sustainable alternatives. The experiments described below focused on the screening of cheap and locally available solid substrates for their potential for the mass production of *Beauveria bassiana*. The experiments were carried out sequentially to refine the methods of production, harvesting and packaging *B. bassiana*.

##### **Materials and methods**

A two stage mass production technique was used. The liquid phase was to produce active growing hyphae and blastospore biomass for inoculation in to the (second) solid phase, which supports growth and sporulation of the fungus. The two stages were prepared and inoculated as follows:

##### **Liquid phase**

A simple sucrose/brewer's yeast broth was prepared by mixing 20g sucrose and 20g brewer's yeast in one litre of tap water. This was heated in a water bath until boiling point. The resulting broth was homogenized in a warring blender at medium speed for one minute. About 75 ml of the homogenized broth was poured into 250 ml Erlenmeyer flasks. The flasks were plugged with cotton wool bungs and covered with aluminium foil. The flasks were then autoclaved at 121°C (15 psi) for 20-40 minutes.

Pure conidia of *B. bassiana* cultured on agar slant were suspended in sterile distilled water (SDW) containing 0.05% Tween 80. The spore concentration in the suspension was adjusted to 10<sup>7</sup> spores per ml. One ml of the spore suspension was used to inoculate each flask (75 ml of the sterilized and cooled liquid medium). The inoculated flasks were incubated on a rotary shaker at approximately 150 rpm for 3 days at room temperature (25-30°C)

##### **Solid phase**

The substrates were distributed in autoclavable plastic bags. Appropriate volume of tap water was added according to different treatments and bags massaged to mix the substrate and water. The bags were autoclaved at 121°C, 15 psi for 40 minutes then allowed to cool to room temperature before they were inoculated with 15 ml of the liquid culture and mixed properly to evenly distribute the inoculum over the substrate. Inoculated bags were folded loosely and then placed in plastic bowls of 14 cm diameter and 15 cm deep with three small holes (3.5 cm diameter) around the circumference, for aeration of the substrate as the fungus grows. The bowls were covered with lids and the three holes plugged with non-absorbent cotton wool bungs. The containers were kept closed for 7 days, in a room in which temperature fluctuated between 24 to 28°C. After 7 days incubation, some sporulation was noted over the surface of the substrate the containers were opened to allow drying of the sporulated fungus and the substrate. The substrates were dried for 21 - 42 days at room temperature (24 – 28°C) before aerial conidia extraction was done.

##### **Extraction**

In each container, 200 ml of SDW plus 0.05% Tween 80 was added to the substrate and the bowl gently shaken to dislodge all the-conidia from the substrate. The resulting suspension was passed

through a fine metal sieve with a mesh size of 106  $\mu\text{m}$ . and, thereafter, diluted for counting number per ml or g of substrate and viability test (percent germination) of conidia.

### Spore count

An improved Neubauer haemocytometer was used to determine the concentration of the spore suspension. A cover slip was firmly placed on the two sides of the counting chamber. The spore suspension was shaken for even distribution of spores. Using a sterile Pasteur pipette, a drop of the solution was picked and placed at the edge of the cover slip this was left for a few minutes until all the spores settled. All squares on the grid were counted in a weak suspension while five diagonal squares were counted in a very concentrated suspension.

The experimental design for all of the experiments was a randomized complete block design although the number of replicates varied in different experiments. In each experiment, data on the number of conidia per ml or g of solid substrate, together with the percentage viability were taken. Statistical analysis was carried out using an Analysis of Variance (ANOVA) or Generalised Linear Models (GLS) as appropriate, in GenStat Release 6.1 (GenStat, 2003)

### Experiment 1: Effect of using bagasse with different moisture contents as a substrate in the production of aerial conidia of *B. bassiana*.

“Bagasse”, a relatively cheap, waste product from sugar production was evaluated as a potential low cost substrate for the mass production of *B. bassiana*. The treatments tested were, bagasse + 0% moisture, bagasse + 25% moisture, bagasse + 50% moisture and rice. The treatments were replicated 4 times in a completely randomized design. After inoculation, they were incubated for 7 days to allow sporulation under room temperature (24 – 28°C). The bags containing the substrates were subsequently opened and allowed to dry for 21 days at room temperature after which the conidia were extracted and counted.

Significant differences ( $p = 0.026$ ) were observed in the number of conidia per unit volume of substrate, with bagasse + 0% moisture producing significantly fewer conidia. Interestingly, bagasse +25% moisture and bagasse + 50% moisture both resulted in a greater production of conidia than the control substrate (rice), but these increases were not statistically significant (Table 1).

**Table 1: Effect of bagasse with different moisture contents on the production of *B. bassiana* conidia.**

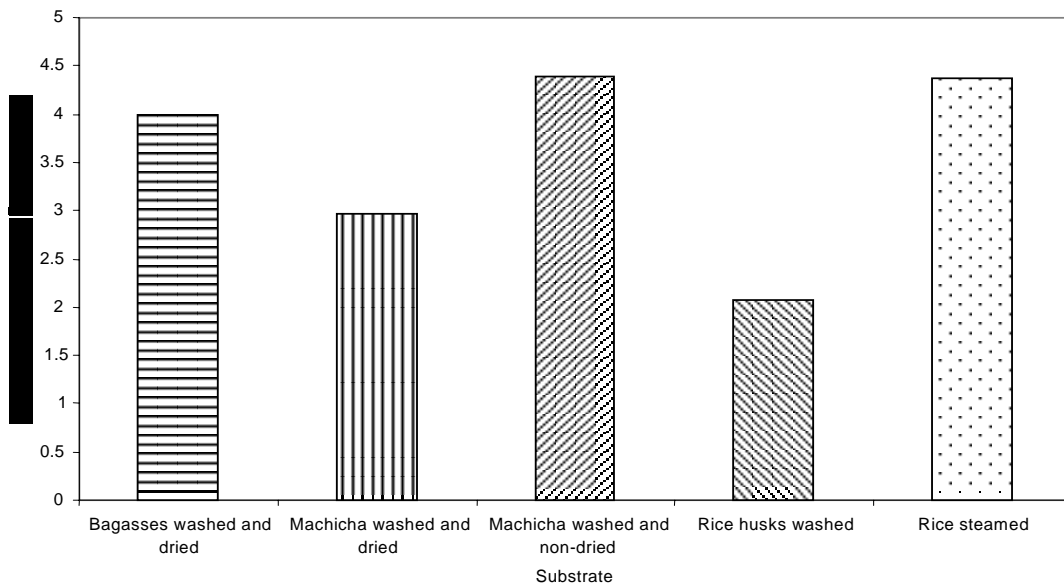
Treatment	Number of conidia x $10^8$ ml <sup>-1</sup> of bagasse
Rice	1.76
Bagasse + 0% moisture	0.99
Bagasse + 25% moisture	2.30
Bagasse + 50% moisture	1.92
Grand mean	1.74
P-value (Overall)	$p = 0.026$ (sed = 0.347)

In contrast, the viability of conidia produced on bagasse + 50% moisture was consistently lower than for the other substrates (Fig.2), although this effect was not statistically significant ( $p = 0.084$ ).

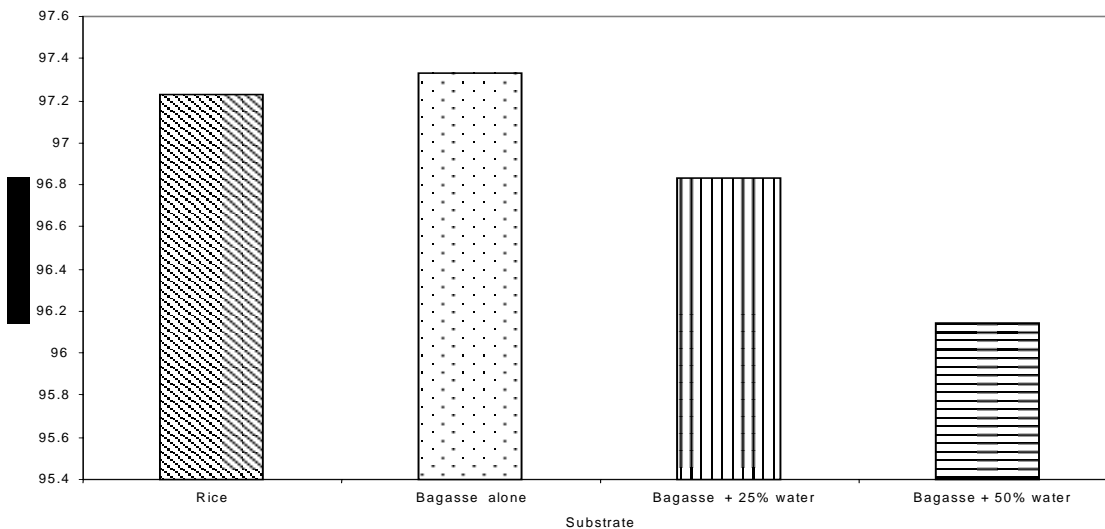
**Experiment 2: A comparison of bagasse, machicha and rice as substrate for the production of conidia of *B. bassiana*.**

Five treatments i.e. bagasse washed and dried, 'machicha' (a by-product from the local beer brewery), washed and dried, 'machicha' washed and non-dried, rice husks washed and rice were evaluated for production of *B. bassiana* aerial conidia. The treatments were replicated 4 times. Data were taken 21 days after drying.

There was no significant difference ( $p = 0.185$ ) in the number of conidia produced on the different substrates. Mean production of conidia among the substrates is  $4 \times 10^8 \pm 1.04 \times 10^8$  per gram of substrate (Fig 1).



**Figure 1. Effect of bagasse, machicha and rice on the production of *B. bassiana* conidia**



**Figure 2. Effect of bagasse with different moisture contents on the viability of *B. bassiana* conidia**

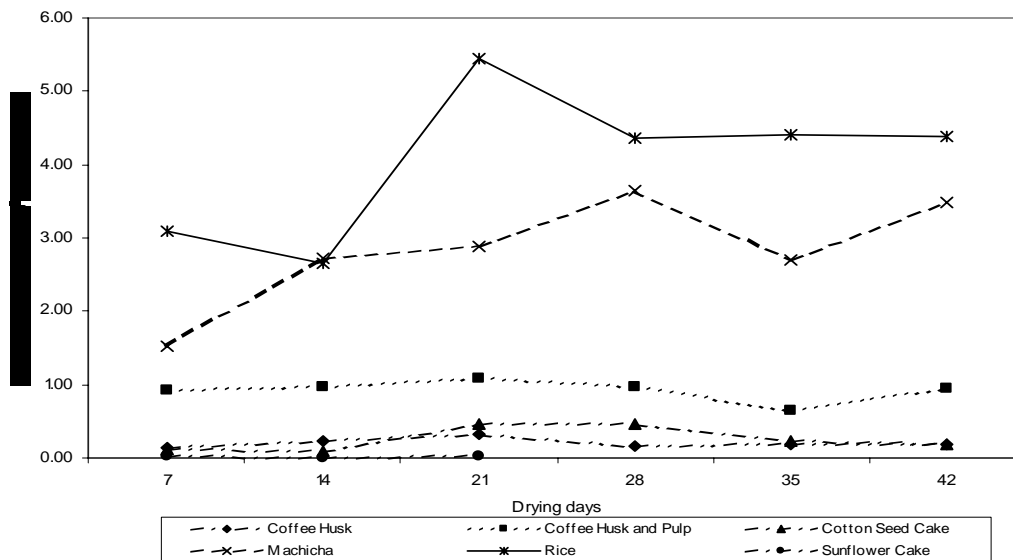


Similarly, there was no significant difference ( $p = 0.659$ ) in the viability of the conidia produced on bagasse, machicha or rice. The mean viability was 99% ( $\pm 0.55\%$ ) germination of the conidia.

**Experiment 3: A comparison of machicha, coffee husks, coffee husks plus pulp, cotton seed cake, sunflower cake and rice as substrates for the production of *B. bassiana* conidia.**

Six substrates were evaluated in this experiment and these included: ‘machicha’, a mixture of coffee husk and pulp, pure coffee husk, cotton seed cake, sunflower cake and rice. The treatments were replicated 4 times and data taken weekly for six weeks during the drying period.

All of the substrates sustained the production of *B. bassiana* except the sunflower cake. Peak production of *B. bassiana* conidia occurred 21 to 28 days after drying (Fig 3). Analysis of the data at day 21 revealed a significant difference ( $p < 0.001$ ) in the production of conidia on the different substrates although rice consistently resulted in the highest production (Table 2).

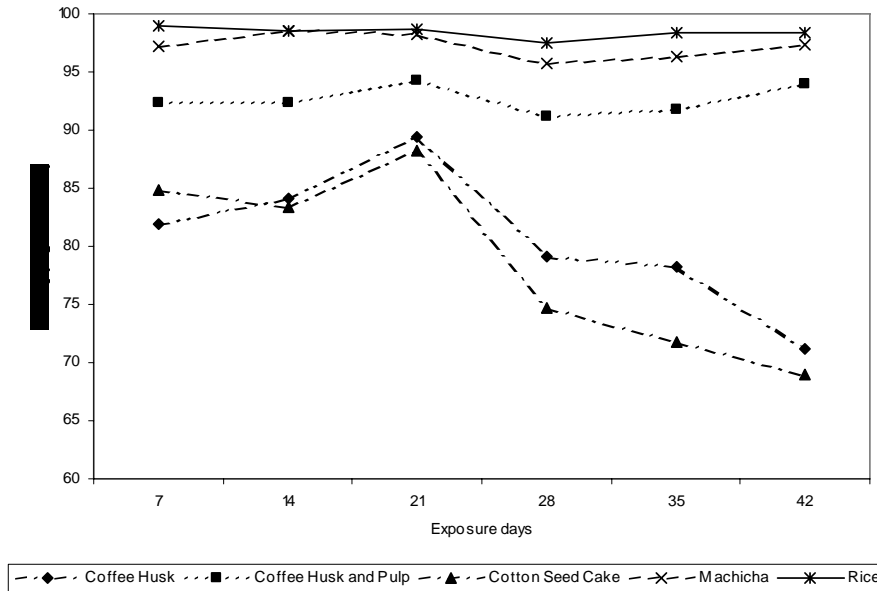


**Figure 3. Effect of rice, coffee husk, coffee husk plus pulp, cotton seed cake, machicha and sunflower cake on the production of *B. bassiana* conidia over time**

**Table 2. Effect of rice, coffee husk, coffee husk plus pulp, cotton seed cake, machicha and sunflower cake as substrates for the production of *B. bassiana* conidia**

Substrate	Number of conidia g <sup>-1</sup> of substrate
Rice	$5.45 \times 10^8$
Coffee husks	$3.28 \times 10^7$
Coffee husk and pulp	$1.09 \times 10^8$
Cotton seed cake	$4.56 \times 10^7$
Machicha	$2.89 \times 10^8$
Sunflower cake	$1.68 \times 10^6$
Grand mean	$2.00 \times 10^8$
P-value (Overall)	$p < 0.001$ ( $sed = 1.332 \times 10^7$ )

The viability of conidia produced on the substrates tested was assessed and found to be significantly different ( $p < 0.001$ ), with the viability of conidia on Rice and Machicha being significantly higher than on coffee husk or cotton seed cake (Fig 4).



**Figure 4. Effect of rice, coffee husk, coffee husk plus pulp, cotton seed cake and machicha on the viability of *B. bassiana* conidia.**

**Experiment 4: A comparison of machicha, coffee husks, coffee husks plus pulp, cotton seed cake and rice as substrates for the production of *B. bassiana* conidia.**

This experiment was similar to Experiment 3 but excluded sunflower cake as a substrate. So, the five treatments tested were; ‘machicha’, coffee husk and broken coffee pulp, washed coffee husk and broken coffee pulp, cotton seed cake and rice. The treatments were replicated 3 times and data taken for at weekly intervals for a period of six weeks.

There was a significant difference ( $p < 0.001$ ) in the number of conidia produced on the different substrates (Fig. 5), with rice consistently producing the highest number of conidia. At the time of peak conidial production i.e. 21 days after drying, mean conidial production on rice was  $9.1 \times 10^8 (\pm 0.539 \times 10^8)$ . In contrast to Experiment three, conidial production on Machicha was not significantly higher than on coffee husks and pulp, coffee husks, cotton seed cake.

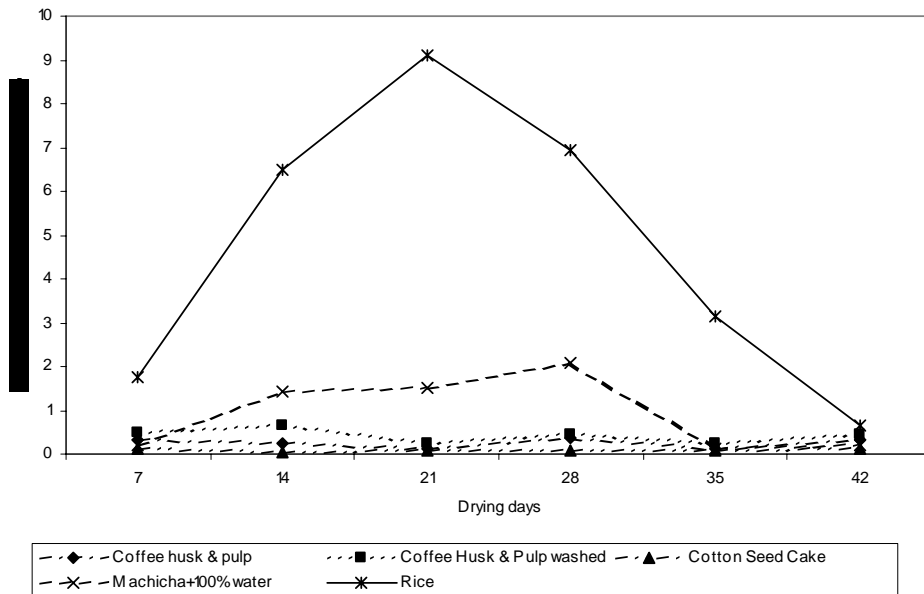


Figure 5. Effect of rice, coffee husk, coffee husk plus pulp washed, cotton seed cake and machicha as substrates on the production of *B. bassiana* conidia

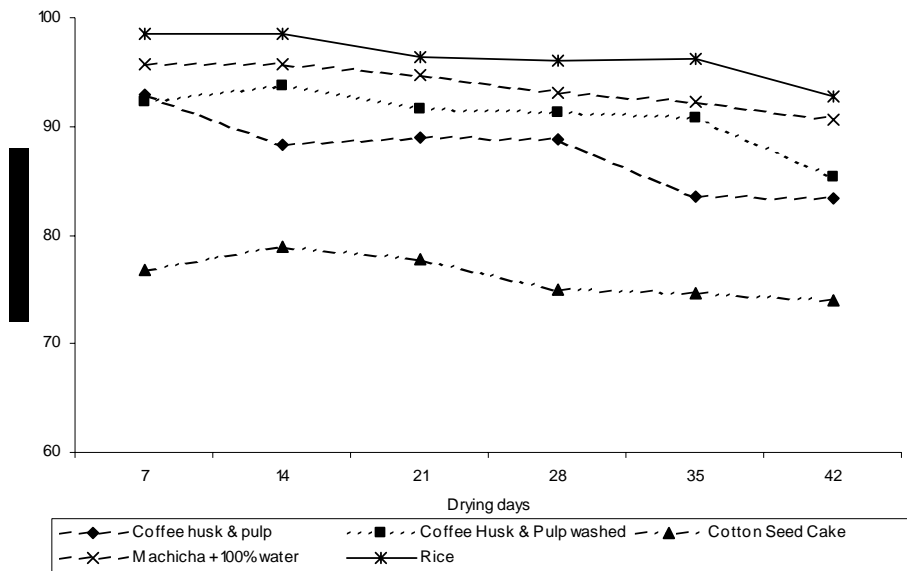


Figure 6. Effect of rice, coffee husk, coffee husk plus pulp washed, cotton seed cake and machicha as substrates on the viability of *B. bassiana* conidia

There was a significant difference ( $p < 0.001$ ) in the viability of the conidia produced on the different substrates (Table 3).

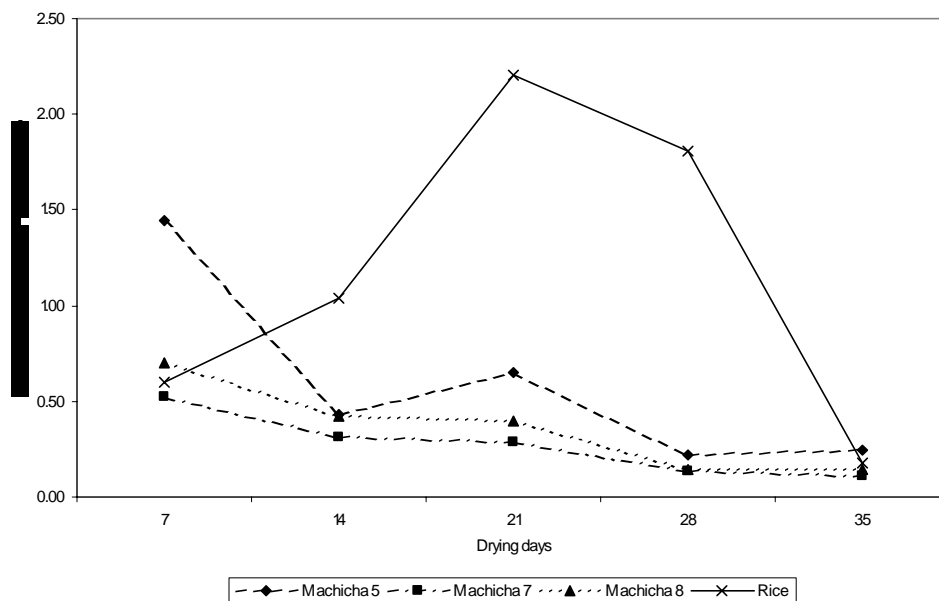
**Table 3. Effect of rice, coffee husk, coffee husk plus pulp washed, cotton seed cake and machicha on the viability of *B. bassiana* conidia.**

Substrate	Viability (% germination)
Rice	96.4
Coffee husks and pulp	89.0
Coffee husk and pulp washed	91.6
Cotton seed cake	77.8
Machicha + 100% water	94.8
Grand mean	89.9
P-value (Overall)	p < 0.001 (sed = 0.990)

**Experiment 5: Effect of different types of machicha on the production of *B. bassiana* conidia.**

This experiment was set to test the potential of different types of machicha based on colour and method of preparation (ratio of different ingredients). The treatments included machicha 5, machicha 6, machicha 8 and rice, which were replicated 4 times and incubated for 7 days. The substrates were then dried for 35 days during which data was taken at weekly intervals.

There was a significant difference in the number of conidia produced on the different substrates ( $P < 0.01$ ) with rice resulting in the highest production of conidia. There was no difference in the number of conidia produced on the different types of machicha (Fig. 7).



**Figure 7. Effect of rice and three different types of machicha on the production of *B. bassiana* conidia**

The effect of substrate on the viability of the conidia was also statistically significant ( $p = 0.003$ ) although the differences between the substrates were extremely small (Table 4).

**Table 4. Effect of rice and three lots of machicha on the viability of *B. bassiana* conidia**

<b>Substrate</b>	<b>Viability (% germination)</b>
<i>Rice</i>	97.8
<i>Machicha 5</i>	96.6
<i>Machicha 6</i>	95.2
<i>Machicha 8</i>	96.8
Grand mean	96.6
P-value (Overall)	p = 0.003 (sed = 0.538)

**Experiment 6: A comparison of rice, machicha and maize as substrates for the production of *B. bassiana*.**

Machicha, broken maize and rice were tested for their potential to produce high quality *B. bassiana*. The treatments were replicated five times, which were then incubated for 7 days and dried for 35 days with weekly data collection. There was no significant difference ( $p = 0.069$ ) in the production of conidia among the substrates (Table 5).

**Table 5: Effect of rice, machicha and maize on the production of *B. bassiana* conidia**

<b>Substrate</b>	<b>Number of conidia x 10<sup>8</sup>/ g of substrate</b>
<i>Rice</i>	1.50
<i>Machicha</i>	4.19
<i>Maize</i>	3.16
Grand mean	2.95
P-value (Overall)	p = 0.069 (sed = 0.986)

There was no significant effect of the substrates on the viability of conidia (Table 6).

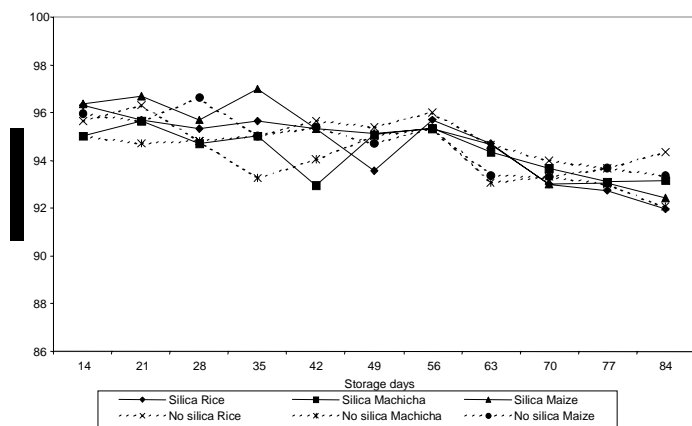
**Table 6. Effect of rice, machicha and maize on the production of *B. bassiana* conidia**

Substrate	Viability (% germination)
Rice	93.8
Machicha	93.8
Maize	94.8
Grand mean	94.2
P-value (Overall)	p = 0.560 (sed = 1.032)

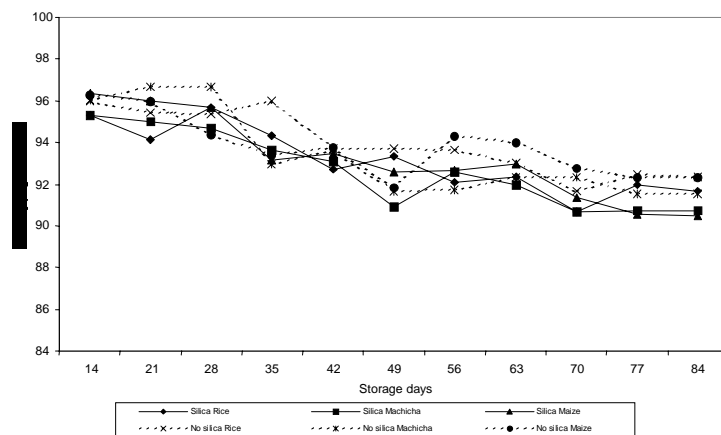
**Experiment 7: Assessment of the longevity of *B. bassiana* conidia produced on different substrates (machicha, broken maize and rice) at different temperatures.**

Survival rates of conidia produced on “machicha”, rice and broken maize were assessed in the following temperature levels -5°C (freeze), 4°C (fridge), 15°C (incubator) and 25°C (incubator). A pre-test of moisture content and viability was done before exposing the samples to the different temperature regimes. Each treatment was split into two sets, one set was placed with non-indicating silica gel and another set without silica gel in each temperature regime. The viability of samples from each treatment was monitored fortnightly.

The pre-test results showed that the moisture content and viability of the samples from the three substrates averaged 7% and >90% respectively. There was no significant decline in the viability of the conidia stored in -5°C and 4°C 70 days after storage (Fig 8 a and b). However, there was a significant reduction in the viability of the conidia stored in 25°C and 15°C 70 days after storage (Fig. 8 c and d). The inclusion of silica did not affect the viability of conidia.



**Figure 8 a. Viability of conidia stored in -5°C**



**Figure 8 b Viability of conidia stored in -4°C**

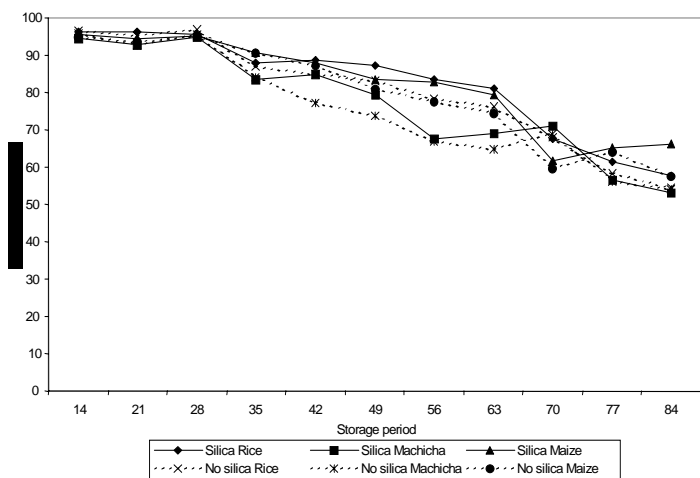


Figure 8 c. Viability of conidia stored in 15°C

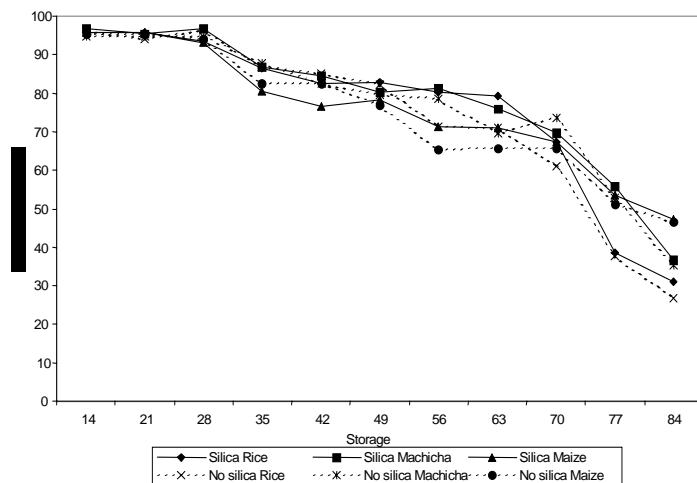


Figure 8 b Viability of conidia stored in 25°C

**Experiment 8: Evaluation of the effect of different sizes of pumice in supporting production of *B bassiana* conidia.**

Pumice, a volcanic rock from Kenyan Rift Valley was tested for its potential to support the growth of *B bassiana*. Different sizes of the rock particles with the following diameters 3.0 cm (large), 2.3 cm (medium), 1.0 cm (small) and 0.6 cm (fine) were evaluated against rice. 200g of each treatment was weighed and soaked in 100ml of tap water and replicated 4 times. The treatments were incubated for 7 days and dried 35 days. Data was taken at weekly interval throughout the drying period, starting from the time of opening (0 drying).

There was a significant difference ( $p < 0.001$ ) in the production of conidia (Table 7), with rice resulting in a significantly higher number on conidia than the pumices. Differences between the different sizes of pumice were not statistically significant.

Table 7. Effect of rice and different pumice sizes as substrates for the production of *B. bassiana* conidia

Substrate	Number of conidia x 10 <sup>8</sup> / g of substrate
Rice	6.043
Fine pumice	0.043
Small pumice	0.041
Medium pumice	0.068
Large pumice	0.081
Grand mean	1.255
P-value (Overall)	$p < 0.001$ (sed = 0.3103)

There was also a significant difference ( $p < 0.001$ ) in the viability of conidia produced on the different substrates although again, the differences were small (Table 8).

**Table 8. The effect of rice and different sizes of pumice on the viability of *B. bassiana* conidia**

<b>Substrate</b>	<b>Viability (% germination)</b>
<i>Rice</i>	96.8
<i>Fine pumice</i>	88.8
<i>Small pumice</i>	88.3
<i>Medium pumice</i>	94.7
<i>Large pumice</i>	94.8
Grand mean	92.7
P-value (Overall)	p < 0.001 (sed = 1.210)

These therefore show that pumice cannot support the conidial production, but can be used as a carrier of *B. bassiana*.

### **Conclusion**

There is no real advantage of using the alternative substrates. Although they are more cost effective, however the drop in the production and loss in viability of conidia does not warrant the promotion of their use in the mass production of *B. bassiana* conidia.

### **4.3. Output 3: *B. bassiana* delivery systems evaluated under farmers' conditions**

*Beauveria bassiana* will be used in banana fields under various environmental conditions and banana management practices, such as application of organic manure, various types of mulch and different plant densities. The practices in turn can influence the soil pH, temperature, moisture, soil fauna and flora, which directly or indirectly influence the efficacy and persistence of the applied entomopathogenic fungus. To address this output, studies were designed to evaluate the performance of *B. bassiana* applied in soil incorporated with commonly used organic amendments in banana fields and in a banana field with varying plant densities and mulching practices.

This output had on-station and on-farm studies. On-station studies were accomplished as previously planned but it was not possible to get the on-farm studies initiated in time due to failure of raising sufficient quantities of *Beauveria* inoculum for large scale field application. During March-July 2002, we reported limited laboratory space as a major constraint to this mass production. We expected to have overcome this problem by expanding the insect pathology laboratory at Kawanda during the August-December 2002, but it was not possible due to delayed acquisition of funding to get this expansion work initiated.



## On-station pot experiments

### 4.3.1. Evaluation of efficacy and persistence of cracked maize-based *B. bassiana* formulation under different soil amendments (Part of MSc thesis for Magara Everest).

Pot experiments were conducted to evaluate the efficacy and persistence of *B. bassiana* in soil amended with different levels of coffee husks, decomposed cow dung manure, and artificial fertilizers. *B. bassiana* was applied as a maize-based formulation (found most effective formulation under laboratory tests) to soil amended with different levels of coffee husks, decomposed cow dung and artificial fertilizers and planted with suckers of local cooking banana 'Mpologoma'. The treatments involved applying *B. bassiana* to 10 treatments; loam soil not mixed with any organic amended or soil mixed with coffee husks, cow dung and inorganic fertilizers in the ratios of 1:1, 1:2, 1:3; and where the coffee husks, cow dung were used in pure stand without soil. The coffee husks were purchased from commercial coffee processors, moistened and incorporated in the soil as per the ratios mentioned above. Completely decomposed cow dung was obtained from local farmers' kraal at Kawanda. The artificial fertilizer used was NPK (Nitrogen, Phosphorous, and Potassium), applied at a rate of 69g of NPK (28g of Urea, 31g of KNO<sub>3</sub>, 3g of MgSO<sub>4</sub>) per plant per year. Soil characteristics (Nitrogen, Phosphorous, and Potassium), soil macrobiota, organic matter and pH, temperature and moisture content were determined at the beginning of the experiment. Soil pH, temperature and moisture content were monitored every 10 days to check on change in these characteristics that are likely to influence the performance of the fungus.

The pots were planted with pared corm suckers of a local cooking banana cultivar "Mpologoma" and 100g of maize-based *B. bassiana* formulation applied in the pots 44 days after planting and when all the plants had established. To test the infectivity and persistence of the fungus in the soil, 10 adult banana weevils were released into each treatment immediately after *B. bassiana* application. To check on the persistence of the fungus, weevils were subsequently released into the pots at 4-week intervals for a period of 16 weeks. Pots were monitored for dead *B. bassiana* infected weevils. Samples were also picked from the pots and tested for infectivity in the laboratory by introducing live weevils and monitoring mortality and *Beauveria* infection.

The highest mortality (30-70%) was obtained for weevils released in pots immediately after *B. bassiana* application and steadily decreased thereafter with lowest mortality rates (5-40%) for weevils released 90 days after applying *B. bassiana*. *Beauveria bassiana* applied to soils without amendments caused significantly higher mortality than other treatments for all release periods. Some of the dead *B. bassiana* infected weevils were discovered from the banana sheaths. For the samples picked from pots and tested in the laboratory, highest banana weevil mortality (75-100%) was obtained for samples taken after 30 days and declined thereafter. Samples collected 120 days after treatment, caused the lowest mortality of 10-20%. *B. bassiana* collected from un-amended soils consistently caused higher weevil mortality in the laboratory although treatments were not significantly ( $P>0.05$ ) different for the 30 and 60 sample periods.

There was more weevil damage on the lower than than upper sections of the corm. Also, the plants planted in soil with amendments exhibited more damage than those planted in un amended soils. Plants with coffee husk based amendments showed the highest weevil damage. Soils with coffee husks or cow dung manure exhibited significantly higher levels of mineral content, organic matter and macrofauna. The macro fauna recorded from the samples were essentially of the following orders; Isoptera (ants), Nematoda and Annelida (worms), Mallophaga (centipedes and millipedes), Archinida (spiders) and Collembolans (spring tails; parainsects) and Gastropoda (snails and slugs). *Aspergillus flavus* and *Penicillium crysogenum* were the dominant fungal colonisers, with the latter being more prevalent. These two fungi were observed in all treatments from 28 days after treatment. However, the rate of substrate colonisation and abundance of the colonisers was observed to be more in amended than un-amended soils. Temperature did vary between 20-34°C throughout the study period. This study demonstrated that organic soil

amendments have a degradative effect on the maize based formulation of *B. bassiana*. The high moisture levels and microbial attack on the maize-based formulation associated with organically amended soils might have led to reduction in efficacy and persistence of *B. bassiana*.

#### **4.3.2 Effect of banana spacing on efficacy and persistence of *B. bassiana* for control of the banana weevil**

Manipulation of plant spacing can minimize both the impact of solar radiation reaching the soil surface, and enhance optimal soil moisture and temperature required in propagation of *B. bassiana* in soil and may increase the persistence of *B. bassiana* in the banana field. Also, repeated applications of *B. bassiana* can help replace the degraded *B. bassiana* within the soil ecosystem, and hence ensure prevalence of microbial inoculum within the soil ecosystem. An on-station trial was designed to test whether different plant densities could influence the soil environment (sunlight penetration, moisture and temperature, micro-organism activity), and growth characteristics of the plant, which would in turn directly or indirectly influence the performance of *B. bassiana* in the field. An additional objective of this study was to generate baseline information on how the different plant densities would affect the banana agronomic performance.

#### **Experimental design**

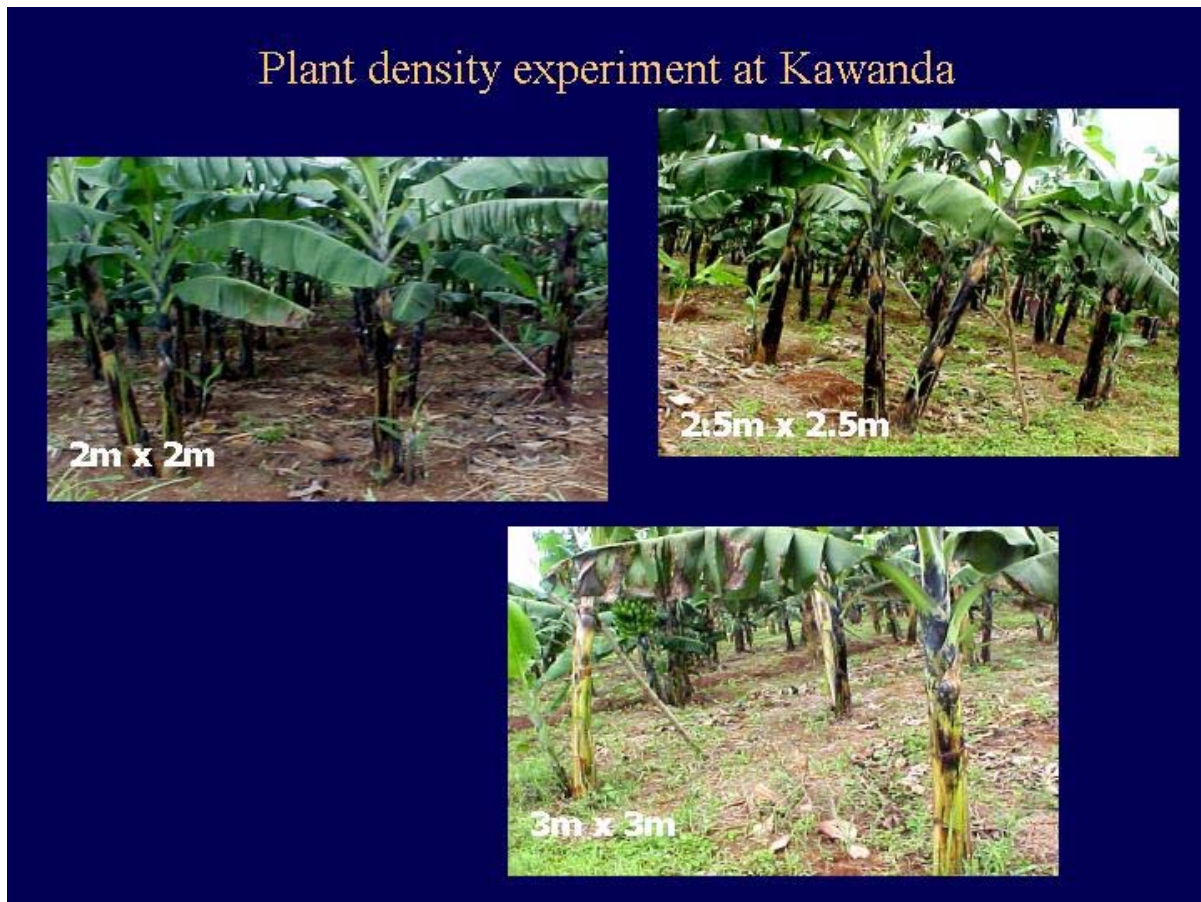
The study was undertaken at Kawanda Agricultural Research Institute. Banana corm suckers of a local cooking banana cultivar 'Mpologoma' were established under three plant spacings: 2 m x 2 m, 2.5 m x 2.5 m, and 3 m x 3 m, corresponding to 126 plants/plot (equivalent to 2500 plants/hectare), 90 plant/plot (equivalent to 1600 plants/hectare, and 60 plants/per plot (equivalent to 1110 plants/hectare) respectively.

To enhance growth and vigour of the plants, artificial fertilizers were applied four times; (June and October, 2002) and (April and October, 2003). Fertilizers were applied per mat at the recommended rates; 69g of NPK, 28g of urea, 31g of KNO<sub>3</sub>, and 3g of Mg SO<sub>4</sub>. The plantation was mulched mainly with dry banana leaves cut from the plants. Established banana fields normally exhibit varying plant densities depending on age of the plantation, cultivars used and agronomic practices employed on the farm.

These spacings were chosen to simulate possible plant densities under farmers' conditions. Two application rates of *B. bassiana* (100g and 200g per mat) were imposed under the different plant spacing applied at 2-monthly and 3-monthly intervals. Application of 100 grams of maize-based *B. bassiana* formulation correspond to approximately  $3.25 \times 10^{13}$  conidia/hectare,  $2.08 \times 10^{13}$  conidia/hectare and  $1.44 \times 10^{13}$  conidia/hectare respectively. Application of 200 grams of maize-based *B. bassiana* formulation correspond to approximately  $6.5 \times 10^{13}$  conidia/hectare,  $4.16 \times 10^{13}$  conidia/hectare and  $2.88 \times 10^{13}$  conidia/hectare respectively in the 2m x 2m spacing, 2.5m x 2.5m spacing and 3m x 3m spacing respectively. *B. bassiana* was produced by the Insect Pathology Laboratory at Kawanda Agricultural Research Institute. The fungus was produced on cracked maize as the substrate following the modified diphasic method as described by Nankinga (1999).

The experiment design was a randomised complete block design (CRBD), in a split plot arrangement, with three replicates. Each replication had as main units, the three spacings, the two *B. bassiana* applications rates, and a control without *B. bassiana* application. Split units arose because the *B. bassiana* plots were further vertically split into two equal parts to accommodate the two *B. bassiana* application regimes. Due to limited space, it was not possible to produce enough *B. bassiana* for application in all the experimental plots at the same time. *B. bassiana* application was therefore staggered (at approximately 2-3 weeks intervals) among the three replicates. The first applications in replicates 1, 2 and 3 were done between late October and November 2002. Since then, six and five *B. bassiana* applications have been administered at 2-month and 3-month

intervals respectively. In each experimental plot, 60, 36 and 24 middle banana mats were selected as experimental plants from the spacing of 2 m x 2 m, 2.5 m x 2.5 m, and 3 m x 3 m respectively. Two lines of bananas were left at the top, bottom, right and left edges of the plot as guard rows. The experimental plants from each spacing were split into two, on which plants i.e. 30 for 2m x 2m, 18 for 2.5 m x 2.5m, and 12 for 3 m x 3m, received *B. bassiana* at 2 and 3 months respectively.



To investigate the influence of plant spacing on agronomic and yield parameters, data were collected on: plant girth, plant height, number of suckers per mat, number of leaves per plant (mother and daughter), flowering and harvesting dates, bunch weight, and number of clusters per bunch. Plant girth in cm was taken at 1m from the ground using a tape measure; plant height in cm was defined as the measurement from the ground level to the cigar leaf, and this was taken using a marked wooden stick; flowering dates defined as the number of days from planting to shooting. Days to harvesting were recorded as days from planting to physiological maturity and filling of the banana fingers. Bunch weights were measured using a spring balance. Data for the first cycle has been completed; currently data for the second, third harvests per banana stool is being collected. The efficacy and persistence of *B. bassiana* was assessed by monitoring corm damage of harvested banana stumps (up to 2 weeks after harvest) and weevil populations per plot estimated at monthly intervals. Weevil populations were monitored using pseudostem traps made from the harvested stumps in the field. One pseudostem trap per mat was placed at the base of each experimental mat and banana weevil counts done after 5 days. Corm damage was assessed on both the inner and outer cylinders of the upper corm section cut at the core (soil level) of the harvested banana corm.

To check changes in some of the physical and chemical features that could be influenced by plant spacing and management practices, data was collected on soil temperature, moisture and micro nutrients. Soil temperature was monitored weekly by placing clinical thermometers into the soil from

23 months after planting. Five readings were taken at a depth of 2 cm in the ground next to the base of five mature experimental banana mats in each experimental plot. The final figure recorded per experimental plot is the average of the five readings. For soil moisture, five soil samples were randomly collected per plot, bulked, and 20 g sub-samples were dried to constant weight in an oven at 27°C. Soil moisture and temperature measurements commenced in September 2003 (23 months after planting), and thereafter on a monthly basis. Soil samples collected from 0-20 cm and 20-40 cm were separately analysed for pH, organic matter (%), nitrogen (%), phosphorus (ppm), potassium (mg/100g), sodium (mg/100g), calcium (mg/100g), sand (mg/100g), clay (%), and silt (%). Soil analysis was done twice, June 2002 and August 2003. Similarly, nutrient uptake in the plants at the different spacing was done by analyzing the nutrient content of the banana leaves for percentage nitrogen, phosphorus, and potassium in June 2002 and August 2003.

**Weevil abundance under the different spacing and doses:** Table 9 shows preliminary weevil counts from the experimental field at Kawanda. Generally, bananas established at spacing of 3m x 3m and receiving *B. bassiana* at 3 months intervals registered the lowest weevil counts of 0.63 weevils/mat, while banana at 3m x 3m with no *B. bassiana* application registered the highest weevil counts of 1.36 weevils/mat. Generally banana plots that received 200g of *B. bassiana* registered lower weevil numbers (0.63-0.74 weevils/mat) as compared to banana plots with no *B. bassiana* application (1.06-1.36 weevils/mat). Weevil counts here were from natural infestations, which normally take long to build up. From these observations, it is advisable to continue monitoring these weevil populations for another 12 months so as to derive more conclusive data from the trial.

**Corm weevil damage under the different plant spacing and doses:** Generally, highest corm damage (1.98%) was registered in banana plots established at spacing of 2m x 2m and lowest in bananas established at spacing of 3m x 3m (1.03%). Banana weevil upper cross section corm damage was generally lower in banana plots receiving *B. bassiana* every two months as compared to those plots receiving *B. bassiana* every three months. This analysis is based on the upper section weevil damage of the first plant crop harvest and the second banana plant harvest. It was also not possible to assess the lower cross section damage of the harvested plants because normally the first and the second plants corms are deep in the ground and cannot be easily uprooted to check the lower sections, as this would weaken the whole banana mat, leading to toppling. For more conclusive analysis, more data on the third crop plant and at both upper and lower cross sections of the plants should be collected in the next 8-12 months of running the trial.

**Table 9. Banana weevils per mat as influenced by spacing, *B. bassiana* application doses and frequencies<sup>1</sup>**

Spacing (m)	Treatment	2-month interval	3-month interval	Mean
2 x 2 (n=2700) Time (T): P= 0.16 Treatment (trt): P= <0.001	Control	1.28	1.12	<b>1.20</b>
	<i>B. bassiana</i> (100g)	0.76	0.73	<b>0.70</b>
	<i>B. bassiana</i> (200g)	0.72	0.71	<b>0.72</b>
2.5 x 2.5 (n=1620) Time (T): P= 0.20 Treatment (trt): P= 0.0009	Control	1.16	0.85	<b>1.00</b>
	<i>B. bassiana</i> (100g)	0.91	0.91	<b>0.91</b>
	<i>B. bassiana</i> (200g)	0.63	0.74	<b>0.71</b>
3 x 3 (n=1050) Time (T): P= 0.193 Treatment (trt): P= <0.001	Control	1.36	1.06	<b>1.21</b>
	<i>B. bassiana</i> (100g)	0.83	0.92	<b>0.88</b>
	<i>B. bassiana</i> (200g)	0.73	0.63	<b>0.68</b>

<sup>1</sup> Pooled data for five sampling occasions.

**Banana agronomic performance under the different spacing and doses:** Table 10 shows a summary of the agronomic performance of the experimental field. Bananas established at a spacing of 2m x 2m had lower bunch weight (12.8kg) and number of clusters (6.6/bunch), compared to bananas established at 3m x 3m which had a higher bunch weight (14.8kg) and number of clusters (6.9/bunch). However, days to flowering, stem girth, height and number of leaves under the different plant spacing showed no significant variation. In the plant crop, the plants took between 13.9-14.1 months to flower in the three spacings (Table 10). They had 8.2-8.8 leaves at flowering in the first crop and had 6.4-6.7 leaves at flowering in the second crop. The pseudostem girth at 1 m above the ground ranged between 46.4-47.5 cm in the first crop and was between 53.2-54 cm in the second crop. The plant height at flowering was between 230-233 cm while it increased to between 256-260 in the second crop. The number of banana clusters per inflorescence ranged between 6.6-7 in plants. These preliminary observations have not showed remarkable variation in the growth parameters apart from the yield per unit area. The yields demonstrate that lower spacing of 2m x 2m, or 2.5m x 2.5m may give a higher yield per unit area than the currently recommended (conventional) spacing of 3m x 3m (Table 11). Though the banana bunches at closer spacing looked small, a farmer who wish to have continuous supply of food/many banana bunches in his/her garden might opt for these closer spacings. More data will be collected for the third and fourth crop to monitor a more conclusive trend in the agronomic performance of this banana plantation and also evaluate whether these spacing can have effects on the lifetime of the banana plots under the different plots.

**Foliar analysis, soil nutrients, temperature and moisture levels, sun light penetration under the three banana plant spacing:** Preliminary observations have not shown significant variation in the nutrient uptake, soil nutrients, temperature and moisture levels under the three plant spacings. Tables 12, 13, 14 and 15 show summaries of the status of nutrients in the leaves, soil and moisture

and temperature levels and sun light penetration on in the field. Nitrogen, phosphorus and potassium varied between 3.01-3.59%, 0.17-0.26%, 2.36-2.70% in the 2m x 2m, 2.25m x 2.5m and 3m x 3m spacing respectively in the first 20 months after establishing the trial. On the other hand, lowest nitrogen, phosphorus and potassium levels were during 2002 and 2003 respectively observed in bananas established at spacing of 2 x 2 (N=3.01%; P=0.19%; K=2.36%) and 2.5 x 2.5 m (N=3.19%; P=0.17%; K=2.64%). Soil analyses indicate no appreciable variation in soil chemical characteristics among the three plant spacings. Soil temperature between the different banana spacings were not significantly different, and varied between 21.0 to 24.4°C. Soil moisture content although, not varying considerably between the different banana spacing, was lowest in banana established at 3 x 3 m (18.5%), and highest (19.4%) in bananas established at either 2 x 2 m or 2.5 x 2.5 m. Although the leaf area index was lower in the 3m x 3m spacing, there was no noticeable variation in the penetrating light to soil and that might explain why the temperature and moisture levels in the different spacing were not different (Table 15). More data on these parameters will be collected in the third and fourth cycles for more conclusive analysis.

**Table 10. Influence of plant spacing on the agronomic and yield components of banana**

F								
First crop								
Spacing (m)	MTF	No. of leaves	Girth (cm)	Height (cm)	No. of clusters	Bunch weight (kg)	Range of Bunch weight (kg)	Range of No. Clusters
2 x 2	13.9(739)	8.2 (932)	46.4 (932)	233.4 (933)	6.6 (894)	12.3 (750)	2-28	2-10
2.5 x 2.5	14.0(563)	8.5 (693)	46.8 (694)	230.2 (694)	6.7 (648)	13.8 (565)	2-36	2-9
3 x 3	14.1(406)	8.8 (491)	47.5 (491)	229.5 (491)	6.9 (474)	14.9 (408)	2-30	4-10
<b>Mean</b>	<b>14.0</b>	<b>8.5</b>	<b>46.8</b>	<b>231.5</b>	<b>6.7</b>	<b>13.4</b>		
LSD	1.49	0.12	0.48	2.13	0.10	0.51		
Second crop (ratoon)								
	MTF	No. of leaves	Girth (cm)	Height (cm)	No. of clusters	Bunch weight (kg)	Range of Bunch weight (kg)	Range of No. Clusters
2 x 2	23.5	6.4 (619)	54.0 (619)	264.9 (619)	6.8 (278)	13.3 (212)	2-24	3-12
2.5 x 2.5	22.1	6.5 (525)	53.7 (526)	260.1 (526)	6.7 (348)	13.5 (266)	0.3-27	3-9
3 x 3	21.3	6.7 (385)	53.2 (387)	256.9 (387)	7.0 (275)	14.7 (222)	4-29	4-10
<b>Mean</b>	<b>22.5</b>	<b>6.5</b>	<b>53.8</b>	<b>261.6</b>	<b>6.8</b>	<b>13.6</b>		
LSD	1.19	0.10	1.06	2.76	0.09	0.43		

Data in parentheses indicates number of observations; MTF months from flowering to harvest.

**Table 11. Actual and adjusted banana yields as influenced by plant spacing**

First plant	Spacing (m)	Actual yield per plot (kg)	Expected yield (kg/ha)
First plant	2 x 2	9225	30750
	2.5 x 2.5	7797	22080
	3 x 3	6079	16553
Second plant	2 x 2	2819	33250
	2.5 x 2.5	3591	21600
	3 x 3	3263	16331
		Total yield (kg) after two years	
	2 x 2		64000
	2.5 x 2.5		43680
	3 x 3		32884

**Table 12: Leaf analysis under three plant populations**

Spacing (m)	2002 <sup>1</sup>			2003 <sup>2</sup>		
	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
2 x 2	3.01	0.19	2.36	3.14	0.25	2.70
2.5 x 2.5	3.19	0.17	2.64	3.05	0.24	2.58
3 x 3	3.59	0.19	2.70	3.14	0.26	2.61

<sup>1</sup> Assessment done nine months after planting; <sup>2</sup> assessment done twenty two months after planting.

**Table 13: Soil characteristics under three plant populations**

Soil characteristic	Spacing (2002) <sup>1</sup>			Spacing (2003) <sup>2</sup>		
	2 x 2	2.5 x 2.5	3 x 3	2 x 2	2.5 x 2.5	3 x 3
PH	4.6	4.6	4.6	4.8	4.8	4.9
Organic matter (%)	3.6	3.6	3.3	3.5	3.5	3.6
Nitrogen (%)	0.1	0.1	0.1	0.1	0.2	0.2
Clay (%)	35.5	33.8	36.9	37.9	35.8	36.2
Silt (%)	12.1	11.5	11.3	8.7	9.1	8.9
Phosphorus (ppm)	10.5	9.4	6.7	16.0	12.6	17.2
Potassium (mg/100g)	19.8	20.6	18.1	12.7	13.0	14.8
Sodium (mg/100g)	0.4	0.4	0.5	0.8	0.8	0.7
Sand (mg/100g)	52.4	54.8	51.9	53.5	55.9	54.9

<sup>1</sup> Assessment done eight months after planting; <sup>2</sup> assessment done nineteen months after planting



**Table 14. Summaries of soil moisture and temperature as influenced by banana plant spacing during 2003-January 2004.**

Spacing (m)	Temperature					
	September	October	November	December	January 2004	Mean
2 x 2	21.0	22.0	23.7	22.0	23.1	22.3
2.5 x 2.5	21.2	22.1	24.4	22.5	24.0	22.8
3 x 3	21.3	22.1	24.4	22.6	23.2	22.7
Spacing (m)	Moisture					
	September	October	November	December	January 2004	Mean
2 x 2	18.8	21.6	17.7	13.8	6.5	15.6
2.5 x 2.5	19.9	20.6	17.8	19.3	6.6	16.8
3 x 3	18.6	21.1	15.8	15.2	7.3	15.6

**Table 15. Transmitted solar radiation to the soil surface and leaf area index as influenced by plant spacing.**

Spacing	Transmitted light	Leaf area index
2 x 2	0.58	1.26
2.5 x 2.5	0.56	1.22
3 x 3	0.56	0.87
LSD	0.17	0.66
P value	0.520	0.87

#### **4.3.3. On-farm evaluation of the performance of *Beauveria bassiana***

Preliminary on-farm studies investigating *Beauveria bassiana* persistence and infectivity under farmers' management conditions are being conducted at Kisekka benchmark sites in Masaka District. The trial was established in July 2003, and so far implemented on 10 farmers' fields, and more trials will be established. Maize formulated *B. bassiana* at a rate of 200g per mat was applied in two different ways: 1) applied around the banana mats without mulching and 2) applied 30-45 cm away from banana mats and then mulched.

When applying mulch, some farmers place it away from the banana mats while others just put blanket mulch in the field covering up to the banana stools. This trial was intended to investigate how *B. bassiana* would perform under these farmers' practices. In each farmer's field 20 mats were used for each treatment and control. Before the initial treatment, a baseline on weevil damage for each farm was done on 7 to 10 recently harvested plants. Weevil damage assessments were done on the upper cross sections, on both the inner and the outer parts of the corm, and an overall corm damage established. After *B. bassiana* application, sword suckers (less than 3 months), on each of the 20 mats per treatment were tagged, for monitoring bunch weight and corm damage at harvest. Additionally, soil samples were also taken from each farm at two depths (0-10 cm and 10-20 cm).

Data is being collected on chemical (organic matter, pH, nitrogen, phosphorus, potassium, sodium and calcium) and physical properties (soil textural classes) varied between the farmers' fields. Records are also being taken on cultivars grown and agronomic management practices. *Beauveria* applications are being done at 3-month intervals, and this will be done for a period of 3 years.

Because this is a new technology being introduced to the farmers, it was necessary to continuously assess the farmers' perceptions on the technology and seek ways of improving the approaches used in its evaluation and dissemination. A preliminary survey was conducted in January 2004. A structured questionnaire was developed and used to gather information from the ten farmers who are currently participating in the trial. Additionally, for each farmer, the neighbouring farmer was interviewed to establish whether or not they are aware of research activities within their area. Key findings were that: 1) most farmers have seen the weevils killed by *B. bassiana* under field conditions and appreciated the effect of the technology in killing and/or reducing weevil populations, 2) if commercialized, the farmers would be willing to pay between 0.25 to 1.25 US\$ per kg of the formulation of *B. bassiana*, and 3) they recognized the slow action of the fungus as compared to the commonly used insecticides, and requested that the technology be refined to be more effective. During the survey the scientist sensitised the farmers on the mode of action of *B. bassiana* and participating farmers are now more aware of the fungus, how it kills weevils and how it should be applied having been involved in the establishment of the trials.

#### **4.4. Output 4: A protocol for integrating different banana weevil control options with *B. bassiana* developed and evaluated.**

Although candidate strains of *B. bassiana* have been identified for use against the banana weevil, an economic and effective strategy to maximise their field effects is still a major challenge in the use of this technology. Its therefore necessary to integrate one or more weevil control measures with *B. bassiana*; one such strategy is the use of pheromones. With this package, it is hypothesized that pheromones attract and enhance aggregation of the weevils at *Beauveria* delivery sites, where they get infected and killed.

Indeed, the use of pheromone lures in the dissemination of *B. bassiana* has been reported for other insects. In this output, studies were designed to explore the possibility of using semiochemical-based trapping (kairomones and pheromones) as a delivery system of *B. bassiana* in the management of the banana weevil in the banana farming system.

##### **4.4.1. Integration of *B. bassiana* with pheromones**

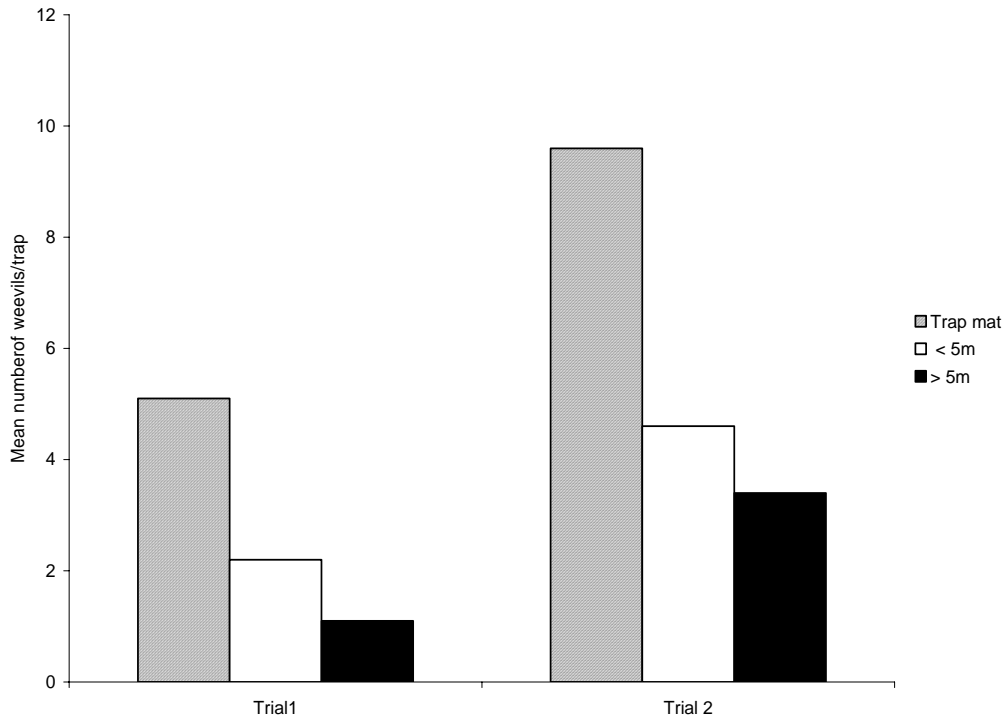
##### **Determination of potential for the pheromone-baited traps to aggregate *C. sordidus* around the trap mat (part of PhD thesis for William Tizaara)**

The experiment was conducted at Sendusu and Kawanda Agricultural Research Institute (KARI). The experiment at Sendusu was conducted in an 8-year old banana plantation of cultivar Atwalira (*Musa* spp, AAA-EA). Plots measured 25 x 25m, and were separated by 10 m alleys. Each plot was kept weed free, but not mulched, and had an average of 40 plants. Prior to treatment application, trapping was done to establish the natural weevil infestations; an average of 2.1 weevils were captured per trap. All the captured weevils were thereafter released on the mat of capture. On the other hand, the trial at Kawanda was established in a 3-year old plantation of cultivar Atwalira.

The experimental plots consisted of 36 mats. Plots were kept weed free, mulched with elephant grass (*Pennisetum purpureum*), and separated with 5m grass alleys. Similarly, natural weevil infestations were recorded and released as described earlier; trap captures averaged six weevils per plot. Plots were stratified on the basis of numbers of weevils captured, and treatments were assigned to plots with similar numbers of weevils. A total of 12 pheromone-baited pitfall traps were placed in each of the fields for 30 days. After the pheromone-trapping period, three pseudostem traps were then placed at the base of each mat, trapped weevils counted and recorded after three days. Distance moved from the pheromone lure, i.e. 0 m (trap mat), < 5 m and > 5 m were recorded. Data was square root transformed and analyzed using ANOVA of SAS (SAS® Institute Inc., 1999), and means were separated using the Student-Newman-Keuls test (SNK).

**Results:** Twice as many *C. sordidus* were captured in pseudostem traps at the base of the trap mat (pheromone-baited mat) than at mats < 5 m from the trap mat, and four times as many at mats > 5 m away (Fig. 9).

**Figure 9. Weevil numbers recovered at different distances from positions where pheromone lures had been placed at two field sites**



Both experiments at Sendusu and KARI showed similar trends of the number of weevils captured in pseudostem traps at the different distances from the pheromone-baited trap. The results have demonstrated the potential of pheromone lures to aggregate the banana weevil.

**Field transmission of *Beauveria bassiana* from infected to non-infected adults of the banana weevil (part of PhD thesis for William Tizaara)**

The experiment was conducted at Sendusu in a banana plantation established with cultivar Nabusa (*Musa* AAA-EA group). The experimental plots were kept weed free, but unmulched, consisted of 42 mats at a spacing of 3m x 3m. Weevils were collected from Masaka, marked according to sex, and grouped into two categories; infected and non-infected. For the infected weevil, this was achieved by placing them in 3cm diameter Petri dishes containing 2g of maize formulated *B. bassiana* (approx.  $3 \times 10^9$  conidia/g) for 6 hours. Thereafter, the infected weevils were transferred to Petri dishes (9cm diameter) lined with moist tissue paper for 3 days before releasing them in the field. The infection process was done in the Insect Pathology Laboratory at Kawanda.

Prior to weevil releases in the field, pseudostem trapping was done to determine the resident pathogen prevalence. Two pseudostem pieces were placed per mat and checked after three days. The captured weevils (at least 3 per mat) were placed in Petri dishes and taken to the laboratory for incubation and observed for any signs of mycosis at 3 day intervals for 21 days. Incubation was carried out in Petri dishes lined with moist tissue paper. In the field, sixteen uninfected weevils (8 males: 8 males) were released per mat. After one week, ten infected weevils (5 males: 5 females) were released on the same mats; weevil releases were done at dawn. Thereafter, pseudostem

trapping was conducted after 7, 14, 21, 35 and 42 days of releasing infected weevils. Two pseudostem pieces were placed per mat and inspected after 3 days. Weevils captured were placed in vials according to mat of capture, observed to verify whether released infected or uninfected, and taken to the laboratory for incubation. The distances moved by the recaptured marked weevils (released infected and uninfected) were computed. At the time of checking pseudostem traps, searches for dead weevils were conducted. Searching was done at four locations: (i) trash, (ii) residues (iii) soil surface, and (iv) plant base in leaf sheath. Weevils recaptured were recorded and those which died without mycosis or were live were placed in Petri dishes and incubated in the laboratory. Infected weevils (with mycosis) from each plot were recorded at 3 day intervals for 15 days.

**Results:** Within field transmission of *B. bassiana* from infected to uninfected weevils was low, and ranged between 13.7 to 7.1 percent depending on the recapture method (Table 16). The dead weevils were found at various sites (Table 17). These included: plant base leaf sheath (49.6), soil surface (23.5%), residues (13.4%), trash (12.6%), and on pseudostem traps (0.9%). The study also established that the *Beauveria bassiana* infected weevils moved to various lengths with most of them confined to the mats where they were infected (Table 18).

**Table 16: Percentage mortality of recaptured weevils released infected and non-infected with *B. bassiana*.**

Weevil status	Weevil recaptured method					
	Searching			Pseudostem traps		
	Initial <sup>1</sup>	Dead	% Mortality	Initial	Dead	% Mortality
Released infected	85	85	100.0	9	6	66.7
Released non-infected	51	7	13.7	154	11	7.1
Unmarked	65	2	3.1	256	3	1.8

<sup>1</sup>Weevils that were released; Data based three sampling occasions.

**Table 17: Field location of *Beauveria bassiana* infected weevils**

Location	Number of recaptured weevils			% weevils with mycosis
	Males	Females	Total	
Plant base- leaf sheath	35	24	59	49.6
Soil by mat	16	12	28	23.5
Residues	6	10	16	13.4
Trash	7	8	15	12.6
Pseudostem trap	1	0	1	0.9

The numbers of weevil indicated in the table are of five sampling/searching occasions.

**Table 18: Relative movement of *B. bassiana* infected and non-infected weevils recaptured by pseudostem trapping and searching in the banana field at Sendusu.**

Distance ranges (m) moved	Percentage number of weevils per distance range			
	Infected		Non-infected	
	No. Recaptured	% Weevils	No. Recaptured	% Weevils
0	53	49.1	61	25.0
0.1-3	21	19.4	37	15.2
3.1-6	17	15.7	55	22.5
6.1-9	7	6.5	49	20.1
9.1-12	4	3.7	22	9.0
>12	6	5.6	20	8.2

The mean distances (m) moved per week by *B. bassiana* infected ( $1.17 \pm 0.07$ ) and non-infected ( $0.96 \pm 0.15$ ) weevils were not significantly different (ANOVA,  $P < 0.05$ )

#### 4.4.2. Integration of *B. bassiana* with kairomones

##### **Banana weevil attractivity to traps made from various banana tissues (Part of M.Sc. thesis for Venasio Tumuhaise)**

This experiment sought to examine the attractivity of the banana weevil to different pounded banana tissues; the best material could thereafter be used in combination with *B. bassiana*. Fresh pounded tissues of a weevil susceptible banana cultivar “Atwalira” (*Musa* AAA-EA) and the resistant banana cultivar “Kayinja” (*Musa* ABB) were placed at 0, 5, 10 and 15 cm below the soil surface in 6-litre buckets. The experiment was a two factorial with eight treatments. The buckets were grouped into four subsets of three buckets per treatment. Ten adult field-collected banana weevils were released per bucket in the subsets at 0, 1, 2 and 3 weeks. After weevil placement, weevil movement to the pounded tissues placement sites were recorded. There was a progressive decline in the percentage of weevils attracted to processed banana tissues, from 95.4 % to 1.7% as the depth at which the tissues were placed increased from 0 cm to 15 cm respectively. Correlation analysis revealed a strong negative relationship ( $r = - 0.86$ ;  $P < 0.0001$ ) between the percentage of banana weevils recovered from pounded banana tissues and the depth at which the pounded tissues were placed. Pounded banana tissues remained attractive to banana weevils up to three weeks, with one-week-old tissues attracting the highest percentage of the released weevils (i.e. 59%), though not significantly ( $P > 0.05$ ) different from the fresh (49%) and two-week-old tissues (45%). However, a significant ( $P < 0.05$ ) difference was observed between the percentage of weevils attracted to the one-week and three-week old banana tissues (i.e. 35%). Correlation analysis revealed a weak negative relationship ( $r = - 0.14$ ;  $P > 0.05$ ) between the percentage of weevils in pounded tissues and age of the tissues. However, weevil attraction to banana tissues grouped by cultivars showed no significant difference between the two cultivars tested i.e. Kayinja (46%) and Atwalira (48%).

Follow-up field experiments were conducted to further examine the attractivity of the processed banana tissues to the banana weevil. The first experiment was conducted in May 2003 in which the processed banana tissues were buried at 5 cm under the soil while the second trial was conducted in December 2003 with the pounded tissues placed at the soil surface. Test materials included: fresh pounded corm, chopped corm, pounded pseudostem and chopped pseudostem from the susceptible cultivar Mpologoma (AAA-EA) and the resistant cultivar Kayinja (ABB). Thus, a total of eight processed banana tissues were evaluated. Results indicated that banana tissues placed at the soil surface attracted more weevils (2.0 – 2.7 weevils/trap) than the buried tissues (0.08 – 0.54 weevils/trap). Moreover, banana tissue traps at the soil surface were more attractive than conventional split pseudostem traps as compared to the buried tissues that were less attractive than split pseudostem traps. However, no significant ( $P > 0.05$ ) differences were observed in weevil catches among the various processed tissues of the two cultivars.

**Integrated management of the banana weevil using *Beauveria bassiana* delivered with semiochemical traps (Part of MSc thesis for Venasio Tumuhaise)**

This experiment sought to examine the potential of semiochemicals (pheromones and kairomones) in the delivery of *B. bassiana* to the banana weevil. A laboratory study was conducted in an olfactometer in a darkroom at Sendusu to determine the quantity of *B. bassiana* conidia picked by a weevil from a semiochemical trap treated with the fungus and also determine the ability of infected weevils to transmit the fungal conidia to their uninfected counterparts. Three combinations were evaluated: (i) pheromone + *Beauveria* vs *Beauveria* alone, (ii) pounded banana pseudostem + *Beauveria* vs *Beauveria* alone (iii) split pseudostem + *Beauveria* vs *Beauveria* alone. Results indicated that the pheromone attracted the highest number of weevils (3.5), followed by the split pseudostem (2.8) and then the freshly pounded pseudostem (1.5). Weevils spent more time on the olfactometer side with pheromones (24 min.), followed by the pounded pseudostem (19 min.) and least time under the split pseudostem (16 min.). However, no significant differences ( $P > 0.05$ ) were observed in the amount of *B. bassiana* spores picked by the weevils. Nevertheless, weevils attracted to the pseudostem apparently exhibited the highest number of spores per weevil ( $2.8 \times 10^7$  conidia/weevil). This experiment has demonstrated the potential of both pheromone and kairomone based traps in the delivery of *B. bassiana* to the banana weevils.



A field trail was conducted to evaluate the ability of *B. bassiana* treated kairomone-based traps to attract banana weevils from various distances, and to assess weevil infection from the traps. The following treatment traps were evaluated: (i) covered pounded corm (covered with split pseudostem), (ii) exposed pounded corm, (iii) split pseudostem, and (iv) control (empty pitfall trap). Ten banana weevils (5 males: 5 females) were released on mats at 0, 3, 6, and 9 m from the trap mat. Weevils

collected from the traps were placed in Petri dishes lined with tissue paper and monitored for signs of mycosis under laboratory conditions. Generally, percentage weevil recapture was very low, ranging between 3 – 10% for the various treatment traps. Nevertheless, pounded corm tissues covered with split pseudostem attracted the highest numbers of weevils (i.e. 10%), while the control had the lowest recapture rate of 0.5%. For all the traps, there was higher recapture rate from the trap mat (i.e. 0 m) as compared to other release positions. The pounded corm tissues covered with split pseudostem had the highest overall percentage recapture value, and it recaptured weevils from all distances. Exposed corm tissues never attracted weevils beyond 3 m from the trap. A total of 227 weevils (including the recaptured and the natural population) were collected from all the traps over a one-month trapping period, and 127 (i.e. 56%) of these weevils got infected with *B. bassiana* under moist chambers in the laboratory. This study has further demonstrated that *B. bassiana* applied around kairomone traps can successfully infect and kill weevils that are aggregated by the traps.

Another field experiment was conducted to further evaluate banana weevil attractivity to kairomone traps made from banana tissues and treated with *B. bassiana*, and also to assess the dissemination of *B. bassiana* by the weevils infected from the traps to their uninfected counterparts. Pre-treatment pseudostem trapping (2 traps/mat) was conducted in all plots, targeting the selected trap mats and a sample of five mats around each of the selected mats.

Samples of the trapped weevils per plot were taken to the laboratory to monitor for weevil mortality and growth of *B. bassiana* due to natural infection from the field. Treatments were then laid in the field on the selected mats and included (i) disk-on-stump trap (with one disk) + *B. bassiana* (ii) disk-on-stump (with two disks) + *B. bassiana* (iii) pounded pseudostem tissues + *B. bassiana* (iv) split pseudostem trap + *B. bassiana* (v) *B. bassiana* alone, which constituted the control. The numbers of weevils collected per trap were recorded, and the weevils were taken to the laboratory to monitor for mortality and *B. bassiana* growth. In addition, 2 g samples of *B. bassiana* were collected from around each treatment trap and taken to the laboratory. Ten adult weevils were released into the Petri dishes containing *B. bassiana* and left in contact for 24 hours. The weevils were transferred to Petri dishes lined with moist tissue paper and monitored for mortality and mycosis. Post-treatment saturation trapping was conducted at 2-week intervals for 2 months on the trap mat and 4 mats randomly selected around the trap mat. A record was taken of the total number of weevils trapped per mat, and weevils collected from the trap mat and from the neighbour mats were taken to the laboratory to monitor for mycosis. Results indicated that disk on stump trap (with two disks) attracted the highest number of weevils (3 weevils/mat), while pounded pseudostem tissues captured the lowest (1 weevil/trap). Weevils captured at the various treatment trap mats exhibited higher rates of mycosis, ranging between 9% and 22% as compared to weevils trapped from mats at 3 m from the trap (range 6 – 13%).

## 5. Contribution of outputs to project goals

**5.1. Improving the production and formulation of *B. bassiana*:** The production and infectivity of *B. bassiana* was tried with various local substrates and cracked maize emerged out best in terms of easy of production, sporulation and conidia yield, handling and economic availability and shelf life. It was found that the substrate best formulation is more persistent in the soil than the conidia based formulation.

**5.2. Performance of *B. bassiana* under farmers' management conditions.** The study where *B. bassiana* was applied to soil amended with commonly used organic mulches and manures such as cow dung and coffee husks, demonstrated that organic soil amendments have a degredative effect on the maize based formulation of *B. bassiana*. The high moisture levels and microbial attack on the maize-based formulation associated with organically amended soils might have led to reduction in efficacy and persistence of *B. bassiana*. A remedy to this efficacy would a frequent application of the fungus at 3 monthly intervals and application of the fungus away from the amendments.



More data on weevil population, weevil damage and agronomic performance of the plants need to be collected to give conclusive analysis on the effect of the different plant densities on the performance of *B. bassiana*. In this regard, an extension in the data collection is required for the third and fourth cycles of the banana plant to full fill the project goals.

Preliminary yields of the experimental field on performance of *B. bassiana* under different plant spacing have demonstrate that lower spacing of 2m x 2m, or 2.5m x 2.5m may give a higher yield out per unit area other than the current recommended convention spacing in Uganda of 3m x 3m. Though the banana bunches at lower spacing looked small, a farmer who wish to have continuous supply of food/many banana bunches in his/her garden might opt for these lower spacing. More data will be collected for the third and fourth crop to monitor a more conclusive trend in the agronomic performance of this banana plantation and also evaluate whether these spacing can have effects on the lifetime of the banana plots under the different plots.

**5.3. Integrating *B. bassiana* with other banana weevil IPM options:** The studies have demonstrated that the pheromone and kairomone based traps facilitate the delivery of *B. bassiana* under field conditions and weevil get infected and killed from *B. bassiana* applied with pheromones traps. Follow up studies will have to be conducted to establish the economic feasibility of using these delivery systems.

**5.4. Disseminating the technology to the end users:** A quick survey on the perception of the new technology at on-farm trial in Masaka banana bench mark site has shown three key findings: 1) most farmers have seen the weevils killed of *B. bassiana* under field conditions and appreciated the effect of the technology in killing and/or reducing weevil population, 2) if commercialized, the farmers would be willing to pay between US\$ 0.25 to 1.25 per kg of the formulation of *B. bassiana*, and this price should be far less the current chemical prices for weevil control in Uganda and 3) they recognized the slow action of the fungus as compared to the commonly used insecticides, and requested that the technology be refined to be more effective. During the survey the scientist sensitised the farmers on the mode of action of *B. bassiana* and participating farmers are now more aware of the fungus and how it might be deployed having participated in the establishment and monitoring of the trials.

### **5.5. Follow up activities from this project**

Research gaps have been identified from the outgoing project and follow up activities have been proposed.

### **5.5. Publications**

#### **5.5a. Book Chapter**

GOLD, C. S., NANKINGA, C., NIERE, N. & GODONOU I. (2003) IPM of the banana weevil in Africa with Emphasis on Microbial Control pp243-257. In: Neuenschwander, P. Borgemeister, C. and Langewald, J. (Eds) *Biological Control in IPM Systems in Africa* CAB International, Wallingford.

#### **5.5b. Journals**

GOLD, C.S., PENA J.E. & KARAMURA. E.B. (2001) Biology and integrated pest management for the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera:Curculionidae). *Integrated Pest Management Reviews* 6:79-155.

TINZAARA, W., NANKINGA, C., KASAHUJA, I. and TUSHEMEREIRWE. W. (2002) Studies on the efficacy of some biorational insecticides against the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Uganda Journal of Agricultural Sciences* 7: 31-35



TINZAARA, W., NANKINGA, C., TUSHEMERERWE, W. and KASHAIJA, I. (2002). Comparative studies on chemical, hot and cold-water treatments of banana suckers to control the banana weevil, *Cosmopolites sordidus* and the effect of paring suckers on banana nematodes in Uganda. *Uganda Journal of Agricultural Sciences* 7: 43-47

### 5.5.c. Proceedings

NANKINGA, C., TUSHEMERERWE W., NAMAGANDA J., BAGAMBA F., KATUNGI E. and NGAMBEKI D. (2003) Experiences of on-farm participatory research for improved productivity in Uganda. Paper presented at 6<sup>th</sup> Biennial Conference of African Crop Science Society, the Hilton Hotel Nairobi, Kenya October 12<sup>th</sup>-17<sup>th</sup>, 2003. Abstracts of the conference proceedings Page 254. African Crop Science Society Proceedings. In Press

BAGAMBA, F., NANKINGA C., TUSHEMERERWE, W.K. and KALYEBARA R. (2003) The economics of herbicide application in banana production. The case of Kisekka subcounty, Uganda. Paper presented at 6<sup>th</sup> Biennial Conference of African Crop Science Society, the Hilton Hotel Nairobi, Kenya October 12<sup>th</sup>-17<sup>th</sup>, 2003. Abstracts of the conference proceedings Page 286. African Crop Science Society Proceedings. In Press

TUMUHAISE, V., NANKINGA, C., GOLD, C.S., KYAMANYWA, S., TUSHEMERERWE W. & RAGAMA P. (2003) Kairomone trapping for delivery of *Beauveria bassiana* to control the banana weevil, *Cosmopolites sordidus* (Germar). Paper presented at 6<sup>th</sup> Biennial Conference of African Crop Science Society, the Hilton Hotel Nairobi, Kenya October 12<sup>th</sup>-17<sup>th</sup>, 2003. African Crop Science Society Proceedings. In Press

MAGARA, E., NANKINGA, C., GOLD, C.S., KYAMANYWA, S., TUSHEMERERWE, W. & RAGAMA P. (2003) Influence of soil amendments in the delivery of *Beauveria bassiana* for control of the Banana weevil, *Cosmopolites sordidus* (Germar). Paper presented at 6<sup>th</sup> Biennial Conference of African Crop Science Society, the Hilton Hotel Nairobi, Kenya October 12<sup>th</sup>-17<sup>th</sup>, 2003. African Crop Science Society Proceedings. In Press

NANKINGA, C.M. GOLD, C.S. MOORE, D. GOWEN S.R.. TUSHEMERERWE, W. K. GODONOU, I. (2002) Prospects of using *Beauveria bassiana* in the IPM of the banana weevil, *Cosmopolites sordidus* (Germar). In: Tenywa *et al.* (eds) Proceedings of the Integrated Pest Management Workshop, 8-12 September 2002, pp 224-229.

NANKINGA, C. M., TUSHEMERERWE, W.K., GOLD, C.S., TINZAARA, W., BALAKYE, A. and KAGEZI, G. (2002) Experiences of participatory IPM research at three banana benchmark sites in Uganda In: Tenywa J.S. *et al.* (eds.) Proceedings of the Integrated Pest Management Workshop, 8-12 September 2002.

GOLD, C.S. NANKINGA, C.M. DOCHEZ, C. TUSHEMERERWE, W. K. KARAMURA, E.B. and KARAMURA, D.A. (2002) Pests threatening *Musa* biodiversity in the Great Lakes Region of Eastern Africa. Paper presented at the INSITU Workshop 22-26 July 2002, Hotel Equatoria, Kampala Uganda In: Tenywa J.S. *et al.* (eds.) Proceedings of the Integrated Pest Management Workshop, 8-12 September 2002.

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BAREKYE A., TUSHEMERIRWE, W. K NANKINGA, C.. and KASHAIJA I. N (2002) Preliminary observations on resistance/tolerance of introduced banana germplasm to nematodes, weevils and black sigatoka as an IPM component. In J.S Tenywa *et al.* (edit.) Proceedings of the Integrated Pest Management Workshop, 8-12 September 2002, pp 256-261

KAGEZI, G.H., TINZAARA , W., GOLD, C.S., NANKINGA, C.M., TUSHEMERIRWE, W. and RAGAMA, P.E. (2002) Field evaluation of pheromone-enhanced traps for management of the banana weevil, *Cosmopolites sordidus*, in Uganda. In Tenywa, J.S. *et al.* (eds.) Proceedings of the Integrated Pest Management Workshop, 8-12 September 2002, pp 94-120

TINZAARA, W., GOLD, C.S., DICKE, M., VAN HUIS, A and NANKINGA, C. (2002) Use of infochemicals for the management of the banana weevil *Cosmopolites sordidus* (germar) in Uganda. In Tenywa, J.S. *et al.* (eds.) Proceedings of the Integrated Pest Management Workshop, 8-12 September 2002, pp 244-255.

NANKINGA, C.M. GOLD, C.S. and TUSHEMERIRWE, W. (2002) Overview of *Beauveria bassiana* for microbial control of the banana weevil in Uganda. Proceedings of the International workshop on the banana weevil a programme of PROMUSA 28<sup>th</sup> February to 2<sup>nd</sup> March, Tenerife, Canary Island, Spain INFOMUSA 11:XI

C NANKINGA (2002) Integrating indigenous knowledge and improved natural resource management in banana participatory research. Proceedings of the International Follow-up Workshop Strategies of Ecofarming promotion in Africa 14-25 October 2002, Uganda

#### **5.5.d. National Papers and Reports**

NANKINGA C., C. GOLD, GODNOU I. MOORE D, GOWEN, S, and TUSHEMERIRWE, W. (2003) Prospects of using *B. bassiana* for biological control of the banana weevil in Uganda. Paper presented at the Review and Planning meeting for DFID-CPP Banana Cluster funded projects in Eastern Africa, 9-12 January 2003, University of Reading UK.

#### **5.5e. Project Progress Internal reports (attached as annexes CD)**

DFID Progress Report 1	26 Feb, 2002
DFID Progress Report 2	March-July 2002
DFID Progress Report 3	August- Dec. 2003
DFID Progress Report 4	January-July, 2003
DFID Progress Report 5	August-Dec, 2003.

#### **5.5f. Other Technology dissemination tools where this work is reported:**

- Highlights of Banana Research Activities in Uganda. Research Highlights Series No. 1 Revised September 2002
- Sensitisation workshop for farmers on the use of *Beauveria bassiana* for the control of the banana weevil in Masaka district, Uganda. March, 2003.
- Farmers open day on banana utilisation and pest management technologies in Luwero district. March 2003.

#### **9. Name of author of this report**

Dr Caroline Nankinga and Dr Simon Gowen

Date: 10 May 2004