CROP PROTECTION PROGRAMME

Epidemiology, vector studies and control of *Banana streak virus* in East African highland bananas

R7529 (ZA0365)

FINAL TECHNICAL REPORT

1st February 2000 – 31st March 2003

Natural Resources Institute – University of Greenwich
National Agricultural Research Organisation – Uganda
University of Reading – Reading, UK

Date FTR completed: 15 July 2003

This publication is an output from a research project funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID. *[R7529 Crop Protection Programme]*

Project details

Title of project:	Epidemiology, vector studies and control of banana streak virus in East Africa highland bananas.
R Number:	R7529
Project leader:	Lawrence Kenyon
Institution:	Natural Resources Institute
CPP Production System:	High Potential
CPP Purpose:	HP1. Yields improved and sustainability enhanced in high potential cropping systems by cost effective reduction in losses due to pests.
Commodity base:	Bananas and Plantains
Beneficiaries:	Banana growers and consumers in Uganda/East Africa.
Target Institutions:	NARO, Kawanda, Namulonge, INIBAP, BARNESA, IITA/ESARC, Makerere University
Geographic focus:	East Africa; Uganda (but findings are likely to be applicable more widely)

Project Partners	
Natural Resources Institute	Lawrence Kenyon, Timothy Chancellor, Richard Lamboll, (Nawshad Joomun MSc Student)
Uganda National Banana Research Programme	Wilberforce Tushemereirwe, Jerome Kubiriba, Charles Murekezi, Dezi Ngambeki
University of Reading	Tim Wheeler, Ermias Kebreab, Simon Gowen, Savitri Abeyasekera
International Institute of Tropical Agriculture	James Legg (and NRI), Jackie Hughes, Philip Ragama, Suluman Okech

Executive Summary

Before the start of this project, *Banana streak virus* (BSV) had been reported as being an increasingly important constraint to the cultivation of East African highland bananas in certain areas of Uganda. However, little was known about the epidemiology of the disease or its effect on the growth and productivity of banana plants.

Monitoring virus-indexed plants planted in small plots within areas of relatively high BSV incidence in Ntungamo and Rakai districts confirmed that there is natural, vectored, spread of BSV in the field in Uganda. The rate of spread appeared to be location and season-dependent, while the pattern of incidence in a larger planting of the local variety "Kisansa" in Rakai suggested that both longer distance primary spread and short-distance secondary spread (to near neighbours) occurred. Mealybugs were identified as the most likely vector of BSV and several different species were found colonising banana plants in Uganda; a key to the identification of the mealybugs found on banana plants in Africa was developed. Controlled transmission experiments have so far indicated that nymphs of a *Planococcus* species and *Dysmicoccus brevipes* cultured from banana on pumpkin fruits are capable of transmitting a strain of BSV (from Kawanda) from banana to banana. Associated work under project R7478 revealed that there are at least 12 different strains or species of BSV *Badnavirus* infecting banana in Uganda, and it is possible that these have different vector species specificities.

Data collected on the physiology and growth of bananas in trials at Kawanda and Mbarara indicates that even in the mother crop, infection with BSV can cause a significant reduction in photosynthetic ability and a consequent reduction in productivity (yield and quality). In these trials, providing good crop management generally resulted in a decrease in symptom severity in infected plants, and where symptoms were mild, good management gave a significant increase in yield. The indications are that this trend will be even more pronounced in subsequent crops (ratoons) from these trials. These findings were supported by observations from farmers' fields in Ntungamo where symptoms were generally more severe and bunch weights were generally smaller where the plants were less well cared for and under greater stress. Different farmer management practices also appeared to have an effect on mealybug abundance (average numbers of colonies per plant), but so far no association has been observed between mealybug prevalence and BSV incidence (proportion of plants infected).

Responses to a rapid rural appraisal questionnaire revealed that most banana growers in regions where BSV is present recognise and can accurately describe the symptoms, but tend to associate them with causes such as poor soils, drought and weevil infestation rather than with a viral cause. They thus tend to use inappropriate measures to try to control the symptoms. Most farmers obtained their agricultural information from local extension services, although neighbouring farmers, the radio and the church were also regular sources of information. Many farmers said that they would rogue plants with BSV symptoms, though this appears to be a practice of last resort.

The results from these studies indicate the potential benefit of simple cultural management practices in reducing the spread and mitigating the effects of BSV, and hence of improving banana productivity in parts of Uganda. The conclusions provide a foundation for developing strategies to combat the impact of BSV, but further work is required to determine which combinations of the cultural practices are most beneficial and under which circumstances.

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Associated Annexes

1. Epidemiology of *Banana streak virus* (BSV) in East African Highland Bananas (*Kubiriba Tushemereirwe and Kenyon*)
2. Identification of mealybugs on banana and plantain in Africa (*Watson and Kubiriba, 2003?*)
3. Impact of *Banana streak virus* on the growth and yield of banana var. Cavendish Williams (*Musa* AAA) and var. Mbwazirume (*Musa* AAA-EA) (*Wheeler, Murekezi, Gowen, Kebreab and Tushemereirwe.*)
4. Effect of farmers' cultural practices on *Banana streak virus* (BSV) expression in Ntungamo (*Murekezi and Kubiriba*)
5. Farmer knowledge and perceptions of *Banana streak virus* on East African highland bananas in villages with relatively high BSV incidence in south-western Uganda (*Ngambeki, Kubiriba, Murekezi, Ragama and Lamboll*)
6. Baseline survey report: *Banana streak virus* on East African Highland bananas in south and western Uganda (*Ngambeki, Kubiriba, Murekezi and Ragama*)
7. Detection of BSV in Ugandan bananas (*Kubiriba, Joomun and Kenyon*)

Background

Information should include a description of the importance of the researchable constraint(s) that the project sought to address and a summary of any significant research previously carried out. Also, some reference to how the demand for the project was identified.

At the start of this project, banana streak disease was becoming an important constraint to banana and plantain production in many banana growing regions of the world. Symptoms indicative of the disease had first been described from Côte d'Ivoire in 1979 (Lassoudière, 1979), though the causal agent, *Banana streak virus* (BSV; genus *Badnavirus*) was only identified in 1986 (Lockhart, 1986).

Banana streak has become an emotive issue in East Africa, and especially in Uganda where the disease had been called the 'AIDS of banana' in the national press. An epidemic was reported and studied in the Rakai District in the south of Uganda (Tushemereirwe *et al*., 1996). The causes of this sudden epidemic were unclear, but one suggestion was that it was associated with very poor plant husbandry as a result of high incidence of HIV/AIDS in the local human population, which had drastically reduced the labour force able to tend the banana plants.

Badnaviruses had been characterised as having non-enveloped bacilliform particles (120-150 x 30 nm) containing a circular double-stranded DNA genome (Lockhart, 1990) 7.4 - 7.8 kb in size. As well as BSV, definitive members of the group then included *Cacao swollen shoot virus* (CSSV), *Rice tungro bacilliform virus* (RTBV), *Commelina yellow mottle virus* (CoYMV), *Canna yellow mottle virus* (CaYMV), *Dioscorea bacilliform virus* (DBV), *Sugarcane bacilliform virus* (ScBV), *Piper yellow mottle virus* (PYMV), *Kalanchoë top-spotting virus* (KTSV) and *Schefflera ringspot virus* (SRV). It had been observed that many of the badnaviruses occurred in clonally propagated tropical crops and were probably most usually spread in nature by vegetative propagation of the host. However, transmission in a semi-persistent manner by mealybug (*Pseudococcidae*) vectors had also been demonstrated for several viruses in the group (Lockhart *et al*., 1997), with the exception of RTBV, which is transmitted by the leafhopper *Nephotettix virescens* and some other *Neohotettix* species (Hibino, 1983). A partial purification of BSV had been mechanically (sap) transmitted to sugarcane where it induced no symptoms (Lockhart & Autrey, 1988). BSV had also been transmitted under controlled conditions using the citrus mealybug *(Planococcus citri*) and the sugarcane mealybug *(Saccharicoccus sacchari*) in USA (Lockhart & Olszewski, 1993), and the Philippines (Magnaye & Herradura, *pers. com*.), and in Taiwan by *Pseudococcus comstocki* (Su, 1998). However, vector transmission had not been substantiated with mealybugs in Africa or in the field.

In their attempt to develop sensitive PCR-based diagnostic tools for BSV (as part of a Gatsby Charitable Foundation project) for use in screening IITA's Black Sigatoka-resistant hybrids, Thottappilly *et al* (1998) and Harper *et al* (1999a), had found that West African BSV-like DNA sequences were detectable in a high proportion of the breeding lines and progeny (LaFleur *et al,* 1996, Harper *et al,* 1999b). This severely restricted IITA's dissemination of improved germplasm. Subsequently it was shown that BSV-like sequences are integrated into the genomes of most, if not all, *Musa* genotypes (Ndowora *et al,* 1999). While integrated, these sequences cause no symptoms in the host, but it was suspected that some of the integrated forms could be activated (probably by some form of stress) to give rise to episomal BSV that then induced the characteristic disease symptoms. Symptom expression of BSV in field plantings was also thought to be influenced by climate (temperature, rainfall), plant nutrient status and crop management. For example, water stress and cool temperatures were suspected to be the cause of localised outbreaks of BSV. However, there was a lack of

information on precisely how symptom expression was related to poor conditions for crop growth, and whether symptoms could be countered using appropriate crop management. The mechanism of the activation of integrated forms was the subject of lab-based research funded by the Gatsby Charitable Foundation at the John Innes Centre (JIC, Norwich).

The rationale behind this project was that, if the interaction between BSV spread and symptom expression and crop-growing conditions could be better understood, then low-cost/sustainable technologies that would moderate symptom expression or reduce the rate of spread, and hence reduce the losses caused, might be identified. While a lot of resources were being spent on detailed molecular studies (at JIC and at the University of Minnesota), there was a dearth of information on the epidemiology of the disease, its effect on yield under different management regimes and its interaction with stresses and other pests and diseases.

Project Purpose

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

High Potential 1.9: The role of insect vectors in the epidemiology and ecology of banana streak virus disease determined and integrated disease management strategies developed and promoted.

High Potential 1.11: Virus diseases of banana, cassava, yam, sweet potato, Solanum potato and food legumes characterised and improved methods for diagnosis and management of virus diseases developed and promoted.

The purpose of this project was to gain a better understanding of the epidemiology and ecology of *Banana streak virus* disease, and its importance and effect on banana production in Uganda. The role of insect vectors in the spread of the virus in the field was explored, and putative vector species were identified. By focusing on the Ugandan banana benchmark sites, some of the factors influencing BSV symptom expression were determined and yield loss under different conditions was quantified. Using this information, cropping practices that could limit the spread of BSV and reduce the effect of the virus on productivity have been identified.

Research Activities

This section should include detailed descriptions of all the research activities (research studies, surveys etc.) conducted to achieve the outputs of the project. Information on any facilities, expertise and special resources used to implement the project should also be included. Indicate any modification to the proposed research activities, and whether planned inputs were achieved.

Activity 1: BSV epidemiology and vectors

A clear understanding of whether BSV spreads naturally under field conditions in Uganda and, if it does, an indication of which vector species is/are the most effective at transmitting the disease.

Activity 1.1 Determine if there is natural (vectored) spread of BSV, and if so, determine the rate and pattern of spread.

Activity 1.2 Assess the rate of spread of BSV in farmers' fields in different locations into small blocks of clean plants.

By the start of this project, it had been demonstrated under laboratory conditions that as well as being perpetuated through vegetative propagation of the host plants, BSV could also be transmitted between plants in the lab using mealybug nymphs as vectors (Lockhart & Olszewski, 1993). However, there was no evidence that vectored spread was regularly occurring in the field since the distribution of infected plants in a field could have arisen from the planting of infected suckers, or from the activation of BSV sequences integrated in the host plant genome. Thus, the first two activities for this output were aimed to determine if vectored spread of the virus could be detected in the field in Uganda. Both activities used the Cavendish dessert banana c.v. "Williams" which had already been shown to be very susceptible to BSV in the laboratory, but which was believed not to contain activatable integrated BSV sequences.

Because so little was known about the vectored spread of BSV in Uganda at the start of the project, the expectation that "the rate and pattern of spread" could be determined was over-ambitious given the financial and time limitations of the project. Thus, activity 1.1 was limited to two on-station plantings (one at Kawanda and another at Mbarara) to see if BSV would spread **out** from a central block of infected plants **into** the surrounding plants of BSV-indexed "Williams". Activity 1.2 consisted of planting a small block (4 x 4 plants) of virus-indexed "Williams" in each of four farms with relatively high incidence of BSV in Kyangara village in Ntungamo district and a similar set of four blocks in Rakai district, and monitoring for spread of BSV **into** each block from the surrounding plants (Annex 1 [Epidemiology Report]). Because mealybugs are the putative vectors of BSV in the field, their species and number on the plants in the trials were also monitored.

Activity 1.3 Identify the mealybug species present in banana plantings in Uganda.
Activity 1.4 Test if any of the mealybug species identified in 1.3 can transmit BSV under controlled conditions.

By the start of this project, other workers had shown that strains of BSV from other parts of the world could be transmitted between banana plants under laboratory conditions using several different mealybug species. The aim of activities 1.3 and 1.4 was to determine which, if any, of the mealybug species found colonising bananas in Uganda might be responsible for transmitting BSV in the field in Uganda.

Samples of mealybugs were collected from banana plants in farms at various locations in the main banana-growing regions of Uganda. Following clearing and mounting on microscope slides, the mealybugs were identified with taxonomic assistance from Dr. Gillian Watson (Natural History Museum, London). The information gained was combined with previously published records of mealybugs identified from *Musa* species, and a taxonomic key for identifying the more common mealybugs found on banana in Africa was developed (See Annex 2, Watson & Kubiriba 2003?).

Initial attempts to establish pure (single-species) cultures of mealybugs from banana on young banana plants at Kawanda and in controlled environment rooms in UK were not successful. Later, following advice from Dr. John Thomas (QDPI, Brisbane, Australia) colonies were established from individual adult females on pumpkin fruits at Kawanda. Two simple screen-cages were constructed using local materials at the IITA farm at Sendusu, near Namulonge. Transmission experiments where mealybug nymphs from these 'pumpkin' colonies were allowed an acquisition-feeding period on pot-grown banana plants showing pronounced BSV symptoms, and were then transferred for inoculation feeding on virus-indexed test plants, were undertaken in the screenhouses.

Activity 2: Stress and BSV activation and expression.

A better understanding of the role of different stresses on the activation of the virus and its symptom expression.

Activity 2.1 Investigate the influence of various stresses on BSV activation and symptom expression in East African highland bananas.

When this activity was originally proposed, it was thought likely that there were activatable forms of BSV DNA sequences integrated in the genomes of East Africa highland (EA-AAA) bananas. There was also circumstantial evidence that the tissue culture procedure, hot-water treatment for nematode/disease control and the use of nematicides in banana plantations might be sufficient stresses to promote the activation of integrated forms of BSV. The plan was thus to plant replicated trials in association with Dr. P. Speijer (IITA/Sendusu) to test the effect of these stresses.

Activities 3 & 4: Management effects on BSV, physiology and growth.

Empirical data on the effect of climate on the disease, whether good management can alleviate the effects of the disease and restore yield to near normal and, based on these findings, formulation of a strategy for controlling, or at least managing, BSV in Uganda. The effects of BSV on the growth and yield of bananas quantified.

Activity 3.1 Trials at contrasting sites to assess the effect of minimal and good [optimal] crop management practices on BSV incidence and severity, and on crop productivity.

Activity 3.2 Experiments to elucidate the interactions between BSV [climate] and management on crop productivity using modern physiological techniques to quantify resource use by the crops.

Part of the justification for this project was that there was circumstantial evidence that BSV-infected plants growing under poor or stressed conditions showed more severe disease symptoms than diseased plants growing under better conditions. The suggestion was that poor conditions might increase the rate of spread of the disease and reduce the yield obtained from diseased plants by a greater proportion than they do for non BSV-infected plants. Given that there is high prevalence of BSV infection in some areas and that farmers are generally reluctant to remove (rogue) infected plants, the question was then "is it likely to be economical viable (sustainable) not to rogue infected plants but to improve cultural management to mitigate the effect of the virus?" The aims of the activities for Outputs 3 and 4 were primarily to measure how much BSV affected yield under good and minimal management and to explore how infection with BSV changes the physiology of the plant to cause that reduction in productivity. BSV can display a wide range of different symptoms in the different plant parts, at different stages in the plant growth and in different *Musa* genotypes (Daniells *et al* 1999). Since "symptom duration x symptom severity" is likely to be related to extent of yield reduction, these trials were also used to try to develop an easily usable and reliable symptom severity index (SSI) that might be used to predict likely yield reduction under different conditions in farmers' fields.

Although the initial intention was to conduct these trials at three sites with contrasting climates, the cost and logistics meant that the planned trial at the higher altitude site at Mbale on the lower slopes of Mt Elgon (Eastern Uganda) had to be abandoned and the trials were only planted at Kawanda and Mbarara stock farm (thus excluding the climate effect). Also, the original plan was to compare the effect of management on plants of

c.v. “Mbwazirume” that had tested negative for BSV by ELISA (Mbz-) with plants of the same variety that had tested positive by ELISA (Mbz+). However, once the trials had been established it became apparent that the ELISA testing had not been reliable (See additional activity below, and Annex 7 [Diagnostics Report]) since similar numbers of plants from both treatments showed symptoms of being infected with BSV.

4.1 On station field trial to determine the effects of disease incidence and severity on the growth, development and yield of local landraces and improved banana varieties.

Observations from advanced comparative agronomic trials of local landraces with the new *Musa* hybrids emanating from the breeding programmes in East and West Africa and Central America indicated that some of these lines were more susceptible to infection by BSV and developed more severe symptoms than others. This activity was to be a more systematic study of these apparent differences to see if disease phenotype could be associated with particular parental genotype or morphological/ agronomic characteristics of the banana varieties. It was to be led by Dr Vuylsteke, but following Dr Vuylsteke’s tragic death in January 2000, it was not taken forward as originally envisaged. However, discussion between project partners resulted in activities 3.2 and 4.2 being expanded in order to incorporate some of the objectives of this trial.

4.2 Use a benchmark site to obtain more information on the effect of cultural practices on BSV disease progression and yield.

This activity builds on work already established (by NARO and IITA) at the Ntungamo benchmark site to look at the effect of management practices on banana weevil incidence and damage severity (e.g. Ssali *et al*., 2003). The aim was to assess if any association between particular farmer crop management practices and incidence or severity of BSV infection could be demonstrated. Sixty farms in Ntungamo were assessed for BSV incidence and symptom severity; and each was classified as having a low, medium or high level of cultural management (using a scoring system adapted from the weevil studies). Thirty farms (10 from each management level) were then selected, and 40 plants in each farm marked. The variety name of each plant was recorded. These plants were assessed at regular intervals for BSV symptom severity and presence of mealybug colonies, and any changes in seven features of the management in each farm were also recorded. This activity involved some participation by the farmers, who kept records of bunch weights. (See Annex 4 [Ntungamo report]).

Activities 5 & 6: Benchmark sites and Socio-economic aspects.

Benchmark sites maintained and managed, and producing valid/reliable results from on-farm trials.
Base-line data on the socio-economic aspects and consequences of BSV and of the project.

Activity 5.1 Co-ordination of banana benchmark site activities.
Activity 6.0 Socio-economic and biometric study/survey to assess farmers' perceptions of BSV and strategies that might be used to control it.

Drs S Okech and D Ngambeki (both part-funded from this project) were responsible for co-ordination of activities at Mbarara/ Bushenyi/ Ntungamo and Luwero/ Masaka

benchmark sites, respectively. As well as benchmark site activities, Dr Okech was also involved with activities 1.1, 1.2, 3.1 and 6. Dr Ngambeki led activity 6.

A survey questionnaire to determine farmers' perceptions and understanding of BSV and the measures they might use to control it was developed and tested at 30 farms in Rakai district. Based on the findings at Rakai, the questionnaire was modified and used on 30 farms in Ntungamo and 30 in Masaka. Focus group discussions on the same theme were also held in Ntungamo and Masaka. (See Annex 4 [Survey Report] and Annex 5 [Focus Group Discussion Report]). At the same time as the sociological survey was being carried out, the crop management practices, BSV incidence and severity and mealybug abundance were also recorded for each farm surveyed.

Activity 7: Strengthening capacity.

Strengthening of the Ugandan national programme's capacity for plant virus epidemiology/vector research and disease management.

Research into combating pest and disease constraints to banana production is a major part of the work carried out by the Uganda National Banana Research Programme of NARO. Because it had only started emerging as an important constraint to banana production relatively recently, and because of its apparent complexity in terms of strain variability and vector specificity, studies on BSV in the field had been relatively limited. Part of the aim of this project was to build up the capacity within the UNBRP to carry out high quality research to understand the ecology of the virus and its vector and to develop sustainable control practices against it.

Additional activity: BSV diagnosis and variability.

Prior to development of the proposal for this project, antisera had been developed at IITA (Nigeria) that appeared to work reliably in detecting BSV in the Nigerian *Musa* breeding lines and hybrids (Dahal *et al*., 1998). Since it was anticipated that these would also work in Uganda, IITA were contracted to provide sufficient antiserum and reagents for use in this project (R7529). However, since it was known that in other areas BSV (and other badnaviruses) are very variable serologically (Lockhart and Olszewski, 1993), a sister project (R7478) was established with the John Innes Centre (JIC) to assess the molecular variability of the virus in Uganda. If needed, JIC would develop a diagnostic method for use in the epidemiology or transmission studies in Uganda.

It soon became apparent that the IITA antisera were giving inconsistent and unreliable results when used for virus-indexing Ugandan banana plants. Later, the JIC project showed that there are at least 12 different *Badnavirus* species associated with banana streak in Uganda and it is this high level of variability that led to the unreliability of the IITA (and other sources of antiserum) for detecting Ugandan BSV strains. Project R7478 then developed some protocols based on the Polymerase Chain Reaction (PCR) for more reliably detecting *Badnavirus* species/strains (Hull and Harper, 2001). However, these protocols required further development and adaptation to be useful in the Ugandan situation (See Annex 7 [Diagnostics report]).

Outputs

The research results and products achieved by the project. Were all the anticipated outputs achieved and if not what were the reasons? Research results should be presented as tables, graphs or sketches rather than lengthy writing, and provided in as quantitative a form as far as is possible.

Output 1: BSV epidemiology and vectors

A clear understanding of whether BSV spreads naturally under field conditions in Uganda and, if it does, an indication of which vector species is/are the most effective at transmitting the disease.

As yet, no movement of BSV has been observed in the on-station BSV-spread trials at Kawanda and Mbarara (See Annex 1 [Epidemiology report]). However, in Ntungamo, 11 of the 16 plants in one of the small blocks of virus-indexed c.v. "Williams" was infected with BSV within about 6 months of planting, while in the same period in the other three small blocks 1, 2 and 2 plants are now showing symptoms of infection. In Rakai, just one plant in one of the four blocks is now infected. These results indicate that **there is vectored spread of BSV at some locations in Uganda**. The observation that there has been a gradual increase in the number of BSV-infected plants in the block of 23 x 23 c.v. "Kisansa" planted in Rakai in 1998 further supports this. The first infection in this block was not observed until three years after planting and the incidence is now still only about 7%; while in Kyangera village, Ntungamo, infection was seen within six months. This indicates that the rate of spread is location-specific and probably depends on several factors. These factors could include: how much disease is already present at the location, the climatic conditions present and cultivation practices employed, the varieties of banana grown, the numbers and species of mealybug present and what strains/species of BSV are present.

The four mealybug genera most often collected from banana plants in Uganda were a *Dysmicoccus* sp. (identified as a putative vector of BSV by Kubiriba *et al* 2001a), a *Planococcus* sp. (*Pl. citri* was identified as a vector of BSV in the laboratory by Lockhart and Olszewski (1993)) a *Paracoccus* sp. and a *Pseudococcus* sp. (*Ps. comstocki* was identified as a vector of BSV in the lab in Taiwan by Su (1998)). In all, twenty mealybug species were included in the identification key as having been collected from *Musa* plants in Uganda, or from there being reference to their collection from *Musa* plants elsewhere in Africa. A manuscript on this has been prepared and submitted to the Journal *African Entomology* (See Annex 2 [Watson and Kubiriba 2003?]).

A culture of each of *Dysmicoccus brevipes,* a *Planococcus sp.* and a *Pseudococcus sp.,* originally collected from banana plants, were established on pumpkin fruits at Kawanda Research Institute towards the end of this project. Second instar nymphs from these colonies were used in preliminary transmission experiments to test if they could transmit BSV from infected c.v. "Mbwazirume" plants to healthy c.v. "Williams" test plants. So far, transmission has only been observed in one experiment using nymphs of the *Planococcus* sp. and in one experiment using *Dysmicoccus brevipes* nymphs (See Annex 1 [Epidemiology Report]). These experiments should be repeated with these two mealybug species and the *Pseudococcus* sp. to obtain more conclusive information. During the course of this project, the sister project R7478 (Hull and Harper 2001) showed that there are at least 12 different quasi-species of *Badnavirus* infecting banana in Uganda (Harper *et al*., 2002), and it may be that these have different vector species specificities. It would be interesting to test the ability of each of the mealybug cultures to transmit each of these *Badnavirus* species.

Output 2: Stress and BSV activation and expression.

A better understanding of the role of different stresses on the activation of the virus and its symptom expression.

The trials to investigate the role of stress in virus activation and symptom expression were not set up owing to the tragic death of Dr Speijer in January 2000. However, more recent results from the sister project (R7478, John Innes Centre) indicated that though there are *Badnavirus*-like sequences integrated into the *Musa acuminata* genome (EA Highland bananas are triploid *M. acuminata* [AAA]), the sequences cannot be activated (excised and recombined) to give rise to infective episomal forms of the virus. Thus, the research question had been answered and this activity was not taken forward. (NB. This finding makes us more confident that the infections observed in the block of c.v. "Kisansa" in Rakai (Activity 1.2 above) are due to spread of the virus by a vector).

Outputs 3 & 4: Management Effects on BSV, Physiology and Growth.

Empirical data on the effect of (climate on) the disease, whether good management can alleviate the effects of the disease and restore yield to near normal and, based on these findings, formulation of a strategy for controlling, or at least managing, BSV in Uganda. The effects of BSV on the growth and yield of bananas quantified.

In the on-station trials at Kawanda and Mbarara to explore the effect of crop management on BSV and banana productivity (See Annex 3 [Physiology Report]), BSV symptoms in the local East African Highland c.v. "Mbwazirume" growing under optimal management (with mulch and fertiliser) were less frequent and less severe compared to those found in plants grown under a minimal management regime (infrequent weeding and pruning). BSV disease development (measured as area under the disease symptom progression curve) for infected plants was reduced by between 25 – 58% under optimal management in the mother crop and first ratoon crop. Optimal management increased bunch weights by 62% and 51% in the mother plant crop at Kawanda and Mbarara, respectively. In the first ratoon crop, at Kawanda, bunch weights were increased by 106%. BSV reduced bunch weights by 1.2 % under optimal management and by 13.5% under minimal management in the mother plant crop at Mbarara. At Kawanda, BSV reduced bunch weights by 16.6% under optimal management compared with 22.8% under minimal management in the mother plant crop. In the first ratoon crop, BSV reduced bunch weights under optimal management by almost the same magnitude as in the mother crop. Under minimal management, however, bunch weights were reduced by 29%, a further reduction compared to the mother plant crop. This indicated that the effects of BSV on crop productivity and the difference between optimal and minimal management could increase with successive crop cycles.

The effects of BSV on important growth and development processes were also assessed (See Annex 3 [Physiology Report]). Results showed that BSV reduced the rate of leaf photosynthesis, biomass accumulation and growth, and increased the duration of development stages of East African Highland bananas. Therefore, BSV reduced the productivity of banana grown under Ugandan conditions. The negative impact of BSV on bunch yield reported here is greater than previously reported for well-fertilised and irrigated conditions in Australia (Daniells *et al*. 2001), and tended to be greater under minimal compared with optimal management.

The effect of farmers' cultural practices on BSV symptom expression, mealybug abundance and BSV effects on growth and bunch weight was also explored in farmers' banana fields at Ntungamo, southwestern Uganda (See Annex 4 [Ntungamo Report]). Farmers commonly used one or a combination of seven different cultural practices in their management of their banana crops. These practices were 1) provision of soil and

water conservation structures (water bunds), 2) application of an organic mulch, regular desuckering, regular detrashing, manure application, regular weeding and intercropping or monocropping.

The practices of detrashing and desuckering that improve illumination and aeration in the plantations reduced mealybug abundance (Table 1), possibly because dark and warm, humid conditions are favourable for the reproduction and colonisation of mealybugs. However, banana fields with intercrops supported more mealybug colonies than those with banana alone, probably because the intercrops acted as alternative hosts for the mealybugs. Practices targeting improving soil and water conservation and soil nutrition such as application of mulch or manure, or provision of water bunds, appeared not to affect mealybug abundance. Most of the management practices appeared not to influence the proportion of plants infested with mealybugs; but the slow generation time and slow movement of mealybugs may mean that there is a lag period and it is the management practices undertaken in previous seasons that have the more major effect on current mealybug numbers and distribution.

Table 1. Effect of different crop management practices on mealybugs and BSV in farmers fields in Ntungamo

Cultural management Practice	Mealybug abundance	Mealybug incidence	BSV Severity	BSV incidence
Water conservation structures (provision of)	[a]		**0**[b]	
Mulch (application of)			[c]	**0**
Desuckering (regular)			**0**	
Detrashing (regular)			**0**	**0**
Manure (application of)				
Weeding (regular)	**0**		**0**	**0**
No intercropping	**0**	**0**	**0**	

[a] **=** had the effect of reducing that feature, [b] 0 = has no effect on that feature, [c] = had the effect of increasing that feature (the greater the number of arrows in either direction the greater the extent of the effect observed)

Farms in which manure was applied appeared to have increased incidence of BSV-infected plants. However, this probably arises because farmers' tend to apply manure to BSV-diseased plants, perceiving the symptoms to be caused by nutrient deficiency. BSV incidence was less in farms where several cultural practices were used; though, because incidence was measured as the proportion of plants with visible symptoms, this effect may not have been on the rate of spread of the disease, but rather through an effect on symptom expression.

The cultural practices that reduced crop stress tended to have a positive effect on growth and bunch weights in the farmers' fields in Ntungamo (Table 2). These practices included frequent weeding, provision of soil and water conservation structures, improving plant nutrition through application of mulch, and appropriate desuckering and detrashing. Plants with symptoms of BSV infection tended to be shorter and have smaller girths than plants without symptoms, irrespective of which cultural management practice was being assessed for. The only yield response for which there was a significant practices x BSV interaction was application of manure, and this probably came about again because of the farmers' tendency to target manure application to plants with BSV symptoms as mentioned above. Thus, banana growth

and bunch weights were improved and the effects of BSV reduced in relatively intensively managed farms. However, it was apparent that farmers' mulching practice did not reduce BSV disease severity or its effects bunch weight. This is contrary to observations made of on-station experiments at Kawanda and Mbarara (See Annex 3 [Physiology Report]) where application of mulch was a treatment. This disparity is probably because farmers tend to apply less mulch than recommended. Thus, it may be that other practices on the study farms in Ntungamo were not carried out sufficiently frequently or well for their full potential effect on BSV or productivity to be seen.

Table 2. Effect of cultural management practices and BSV on growth and yield of bananas in farmers' fields in Ntungamo

Cultural management Practice	Growth parameter	BSV effect	Practice effect	Interaction (practice x BSV)
Water conservation structures (provision of)	**Height**	[a]	[b]	**0**[c]
	Girth			**0**
	Bunch wt.			**0**
Mulch (application of)	**Height**[a]			**+**[d]
	Girth			**+**
	Bunch wt.	**0**		**0**
Desuckering (regular)	**Height**[a]			**++**
	Girth			**++**
	Bunch wt.		**0**	**0**
Detrashing (regular)	**Height**[a]		**0**	**0**
	Girth			**0**
	Bunch wt.	**0**		**0**
Manure (application of)	**Height**[a]		**0**	**+**
	Girth	**0**	**0**	**+**
	Bunch wt.		**0**	**++**
Weeding (regular)	**Height**[a]			**+**
	Girth			**0**
	Bunch wt.			**0**
No intercropping	**Height**[a]		**0**	**0**
	Girth		**0**	**0**
	Bunch wt.	**0**	**0**	**0**

[a] **=** had the effect of reducing that feature, [b] 0 = has no effect on that feature, [c] = had the effect of increasing that feature (the greater the number of arrows in either direction the greater the extent of the effect observed), [d] + = significant interaction

Nonetheless, the results from these studies indicate the potential benefit of altered cultural practices in mitigating the effects of BSV and improving banana productivity in parts of Uganda. The conclusions provide a foundation for developing strategies to combat the impact of BSV; but further work is required to determine which

combinations of the cultural practices are most beneficial and under which circumstances. For example, at which level of BSV severity do crop management practices cease to be beneficial; and under what circumstances would roguing of infected plants be the most beneficial and cost-effective option for control?

Output 5 & 6: Benchmark sites and Socio-economic aspects

Benchmark sites maintained and managed, and producing valid/reliable results from on-farm trials.
Base-line data on the socio-economic aspects and consequences of BSV and of the project.

In the socio-economic survey in Rakai, Masaka and Ntungamo (See Annex 5 & 6), weevils were ranked by the farmers questioned as the most important constraint to banana production. This was followed by "*Yellowing of leaves/black spots on fruit/ heart of plant rotting*" which was interpreted by the scientists on the survey team as being the manifestation of disease caused by BSV. The group discussions and questionnaire responses showed that many farmers are aware of the most characteristic symptoms associated with BSV infection and associate them with reduced yields. However, most farmers do not know the cause and often attribute the symptoms to weevil damage, drought or decline in soil fertility. Knowledge of BSV was greatest in areas of highest disease incidence. The extension services were ranked as the most usual source of crop protection and banana cultivation information. However, there were significant differences between districts in ranking of other information sources; for example, fellow farmers and family ranked as unimportant in Rakai but relatively important in Ntungamo and Masaka. This may be a result of the impact of the HIV/AIDS epidemic that has removed much of the working-aged adult population from Rakai. Radio ranked highly across all districts. In both the questionnaire survey and the focus group discussions, the farmers often cited roguing as a useful means for managing the disease signalled by BSV symptoms, although it was unclear how many were using this in practice.

The farms included in the survey were chosen because they had plants showing symptoms of BSV infection present either in the farm or very close to the farm. Generally, the incidence of BSV was lower in the farms classed as having high levels of management. Although the incidence of plants with observable mealybugs and the average number of mealybugs observed on each plant were different for each farm, there was no obvious relationship between these numbers and farm management level. Similarly there was no obvious correlation between BSV incidence or severity and mealybug numbers or incidence.

Output 7: Strengthening Capacity

Strengthening of the Ugandan national programme's capacity for plant virus epidemiology/vector research and disease management.

Two Uganda banana research programme staff members, Jerome Kubiriba and Charles Murekezi, undertook the bulk of the epidemiology/vector studies and plant physiology/crop management components of this project respectively, with guidance and training from NRI, the University of Reading and IITA staff. They were registered for split-PhD programmes at University of Greenwich and University of Reading respectively. They in turn trained banana programme technicians in a range of procedures including mealybug handling and identification, BSV symptom recognition,

and use of photosynthesis measurement equipment. Moses Ekwaro, a NARO plant pathology technician, received some training in molecular plant virus diagnostics when the immunocapture- polymerase chain reaction test for BSV (developed by the John Innes Centre project [R7478]) was adapted and transferred to Uganda.

Mr Nawshad Joomun, a University of Greenwich MSc (Natural Resources) student from Mauritius, conducted his research project on further developing and adapting the diagnostic tools for BSV in association with this project (Joomun, 2002).

Additional Output: BSV diagnosis and variability

Initial attempts to transfer the diagnostic procedures developed by project R7478 to Uganda were not successful, probably because the PCR primers specified were not sufficiently universal and did not detect many of the strains of BSV *Badnavirus* present in Uganda (See annex 7 [Diagnostics report]). Later a different pair of PCR primers were developed (Yang *et al*. 2003) which were shown to be less *Badnavirus* strain/species-specific and thus capable of detecting a much wider range of the BSV strains in Uganda. However, these had the problem that they would detect both episomal (true) virus sequences, and the *Badnavirus* sequences integrated into the banana plant genome and so would show a positive response when used with DNA from any banana plant whether infected or not.

To develop a PCR-based test that would only detect true episomal virus, an immunocapture step was re-introduced to the procedure. However, as had been shown previously, the *Badnavirus* strains in Uganda are serologically very heterogeneous (Harper *et al.,* 2002) and so IC-PCR using just one antiserum to capture the virus particles was very strain specific. To try to make the IC-PCR less strain specific, different combinations of antiserum were tested. By the end of this phase of the project, the most reliable diagnostic for *Badnavirus* in Banana was to use a mixture of three antisera (Agdia-BSV, Agdia ScBV and PMx R2-2C) to capture any virus particles from banana sap extracts, and then to amplify a portion of the DNA from these particles using PCR with the universal primers (Badna FP and Badna RP). This system appears to work well for many Ugandan BSV strains, though it should be tested on a wider range of strains/species from Uganda and elsewhere. This procedure and the equipment and reagents to perform it were transferred to Uganda, initially to Namulonge and Sendusu-IITA farm, and then to the biotechnology laboratory at Kawanda. Attempts are now under way to see if the procedure can be further extended by addition of restriction fragment polymorphism profiling of the PCR amplicons in order to differentiate between different strains of BSV and mixtures of strains.

Contribution of Outputs to developmental impact

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

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

HP1.9: The role of insect vectors in the epidemiology and ecology of banana streak virus disease determined and integrated disease management strategies developed and promoted.
HP1.11: Virus diseases of banana, cassava, yam, sweet potato, Solanum potato and food legumes characterised and improved methods for diagnosis and management of virus diseases developed and promoted.

East African Highland bananas are a major food crop and source of income for over 70% of farmers in Uganda (IITA, 1995), and they are estimated to provide 30% of the calories for the entire population. Probably more than 90% of production, covering 30% of utilized agricultural land, is by peasant farming communities for food security and income generation. In recent years, the productivity of banana plantations, particularly in central, eastern and parts of western Uganda has been in rapid decline. The causes of this reduction in yield are believed to be declining soil fertility, less intensive management through reduced availability of labour, and a complex of pests and diseases. Results and observation from this project and sister project R7478 indicate that increasing incidence of *Banana streak virus* (BSV) throughout the banana-growing regions of Uganda, and specifically in certain districts such as Rakai and Ntungamo, may constitute one of the constraints leading to the decline in productivity. Through the joint effort of these two projects, there is a better understanding of how genetically and serologically variable the strains or species of *Badnavirus* infecting banana in Uganda are, more reliable diagnostic tools have been developed, and the capacity and technology to perform these procedures has been transferred to NARO staff in Uganda.

This project has confirmed that natural, vectored, spread is a component of the epidemiology of BSV in Uganda, and that several mealybug species may be implicated as vectors. A key to the identification of the mealybugs found on banana plants in Africa has been developed, and this will be a useful tool for technicians and researchers studying the epidemiology of the virus in other banana growing areas of Africa. Data collected on the effect of BSV infection on the physiology and growth of bananas indicates that even in the mother crop there can be a significant reduction in photosynthetic ability and a consequent reduction in productivity, and this effect is likely to increase in subsequent ratoon crops. Thus it is important to detect the disease early in its onset and to implement measures to eliminate it or reduce its effect on productivity as soon as possible.

The results also confirm that improved crop management can reduce both BSV disease severity and some of the negative effects of BSV on growth and yield of plants with symptoms. This conclusion provides a foundation for developing strategies to combat the impact of BSV. However, future work needs to address several issues:-

- The effect of BSV on growth and productivity in further ratoon crops in relation to crop management and nutrition practices needs to be defined. It is suspected that both symptoms and their impact on bunch yield will increase in further crop cycles.

- Now that the effects of BSV alone on banana productivity are becoming clearer, the impact of BSV in the presence of other pests and diseases found in Uganda should be studied.
- Strategies for farmers to mitigate the effects of BSV through the better management of their plantations need to be explored through farmer participation.

With this knowledge it will be possible to better formulate integrated disease management practices for controlling the spread and/or impact of the disease. Participatory rural appraisal should be used to assess the feasibility and sustainability of implementing these management practices. Pathways for disseminating and promoting to banana growers and other concerned agencies those practices identified as being most appropriate and beneficial should also be explored.

A proposal for a follow-on project, joint with the Integrated Banana Crop Management project, is being developed for submission to CPP. It will include aspects of the research and dissemination activities outlined above.

Project publications/outputs.

CHANCELLOR, T.C.B. (2000): Managing Banana Streak Virus. *CABI-Biocontrol News and Information 21(3) September 2000* (http://pest.cabweb.org/Journals/BNI/Bni21-3/IPM.htm)

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KUBIRIBA, J. TUSHEMEREIRWE. W. and KENYON, L. (2003): Epidemiology of *Banana streak virus* (BSV) in East African Highland Bananas (Annex 1 of this report)

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MUREKEZI, C. and KUBIRIBA, J. (2003): Effect of farmers' cultural practices on banana streak virus (BSV) expression in Ntungamo (Annex 4 of this report)

NGAMBEKI, D., KUBIRIBA, J., MUREKEZI, C., and RAGAMA, P., (2002): Baseline survey report: Banana streak virus on East African Highland bananas in south and western Uganda (Annex 6 of this report)

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WATSON, G. and KUBIRIBA, J. (Submitted): Identification of Mealybugs on Bananas and Plantain in Africa. *African Entomology*. (Annex 2 of this report)

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Biometricians Signature

The projects named biometrician must sign off the Final Technical Report before it is submitted to CPP. This can either be done by the projects named biometrician signing in the space provided below, or by a letter or email from the named biometrician accompanying the Final Technical Report submitted to CPP. (Please note that NR International reserves the right to retain the final quarter's payment pending NR International's receipt and approval of the Final Technical Report, duly signed by the project's biometrician)

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:

Signature:
Name (typed):
Position:
Date:

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Associated Annexes

1. Epidemiology of *Banana streak virus* (BSV) in East African Highland Bananas (*Kubiriba, Tushemereirwe and Kenyon*)

2. Identification of mealybugs on banana and plantain in Africa (*Watson and Kubiriba, 2003?*)

3. Impact of *Banana streak virus* on the growth and yield of banana var. Cavendish Williams (*Musa* AAA) and var. Mbwazirume (*Musa* AAA-EA) (*Wheeler, Murekezi, Gowen, Kebreab and Tushemereirwe.*)

4. Effect of farmers' cultural practices on banana streak virus (BSV) expression in Ntungamo (*Murekezi and Kubiriba*)

5. Farmer knowledge and perceptions of Banana streak virus on East African highland bananas in villages with relatively high BSV incidence in south-western Uganda (*Ngambeki, Kubiriba, Murekezi, Ragama and Lamboll*)

6. Baseline survey report: Banana streak virus on East African Highland bananas in south and western Uganda (*Ngambeki, Kubiriba, Murekezi and Ragama*)

7. Detection of BSV in Ugandan bananas (*Kubiriba, Joomun and Kenyon*)

NATIONAL AGRICULTURAL RESEARCH ORGANISATION (NARO)

REPORT

EPIDEMIOLOGY OF BANANA STREAK VIRUS (BSV) IN EAST AFRICAN HIGHLAND BANANAS

June 2003

CONTENTS

SUMMARY

The work presented in this report constitutes part of a larger study on the ecology and epidemiology of *Banana streak virus* (BSV) and its effect on growth and productivity of East African Highland bananas (AAA-EA) in Uganda.

Cultures of three different mealybug species (*Dysmicoccus brevipes*, a *Planococcus* sp. and a *Pseudococcus* sp.) collected from banana were established on pumpkin fruits from single females. Preliminary results from transmission experiments indicate that nymphs of both the *D. brevipes* and the *Planococcus* sp. are capable of transmitting a Kawanda strain of BSV from banana to banana.

BSV was not observed to spread from a central block of infected plants to the surrounding virus-indexed cv "Williams" plants in two on-station field trials at Mbarara and Kawanda. However, anincrease in mealybug numbers and mealybug movement from the infected sources plants was observed. Water pan traps were more reliable than sticky traps for monitoring mealybug nymph numbers in established banana plantations. Pseudostem counts were the most appropriate method of estimating adult mealybug population size.

BSV was observed to spread to infect plants in small (4x4) plots of virus-indexed cv "Williams" planted within farms with high incidence of BSV within 6 and 17 months after planting in Ntungamo and Rakai respectively. This indicates that there is natural vectored spread of BSV in Uganda. In Rakai, in a larger plot (23x23) of the local variety "Kisansa" (AAA-EA) established in September 1997, the first BSV infections were observed 38 months after planting, and to date the disease has built up to an incidence of about 7%. The pattern of infection within this plot suggests that both primary spread into the plot and secondary spread between near neighbours within the plot is occurring.

BSV spread seems to be slow in some areas, roguing may be effective in reducing the spread of the disease in farms with low incidence in some areas provided that infected plants can be identified at an early stage of infection.

BACKGROUND

Banana streak virus (BSV; genus *Badnavirus)* was first reported in Uganda in 1990 (Dabek and Waller, 1990). A severe outbreak of the disease was reported from the Rakai District in the early 1990's and later other areas of Uganda (Tushemereirwe, *et al.*, 1996). BSV isolates are both genetically and serologically very diverse (Lockhart & Olszewski, 1993; Ndowora & Lockhart, 1997).

BSV was one of the first plant viruses where it was found that parts of the virus DNA genome could integrate into the host chromosomes, and under certain circumstances these integrated sequences can be excised and recombine to form active virus (Lockhart *et al.*, 1998). However, it is believed that it is only the episomal (non-integrated) forms of BSV that are infectious and can be transmitted by the activity of a vector. Several mealybug species have been identified colonising bananas (Matile-Ferrero & Williams, 1995), and three species of mealybug, *Planococcus citri* (Risso), *Dysmicoccus brevipes* (Cockerell) and *Saccharicoccus sacchari* (Cockerell), have been reported to be capable of transmitting BSV from plant to plant under screenhouse conditions (Lockhart and Olszewski, 1993; Kubiriba *et al.*, 2001b). However, the preferred hosts for these mealybugs are citrus, pineapples and sugarcane respectively. There is a need for more detailed transmission studies to be conducted with mealybugs found on bananas in order to clearly establish which species are BSV vectors in Uganda.

The development and implementation of successful disease management strategies necessitate a more thorough understanding of the factors that contribute to the development of plant disease epidemics (Campbell and Madden, 1990). Hughes (1998) reported that there was no conclusive evidence of BSV spread in the field. Kubiriba *et al.*, (2001a) reported that BSV–infected plants are clustered at disease foci in banana fields in Uganda and that incidence was lower away from these foci. Little information is available about the rate of disease spread within or between banana plantations and the role of putative mealybug vectors remains unclear. Consequently, it has not been possible to develop strategies to manage BSV effectively.

This study aimed at providing a better understanding of the natural spread of BSV under field conditions in Uganda, including the vector species that are effective in transmitting the disease.

RESEARCH ACTIVITIES

Mealybugs and Screenhouse Transmission

Rationale

A number of mealybug species have been identified as colonising banana plants in the field (Dahal, *et al*., 1998) but only a few have been associated with transmission of BSV, and then only in the laboratory/screenhouse. Those mealybug species used for transmission experiments in the laboratory/screenhouse were obtained from hosts other than bananas. *Cocoa swollen shoot virus* (CSSV), another *Badnavirus* closely related to BSV, is reported to be transmitted by at least nine different mealybug species, though some species appear to be more efficient vectors than others (Posnette, 1950). It is possible that several different mealybug species transmit BSV and that some have not been identified under screenhouse conditions. The study was therefore undertaken to verify the role of different mealybug species present in banana plantations in Uganda as vectors of BSV.

Materials and Methods

Identification key for mealybugs

The morphological features of all the mealybug species that have been collected and identified from bananas and plantain in Africa were collated from published papers (e.g. Cox, 1989; Ezzat and McConnell, 1956; Williams, 1958 & Williams and Granara de Willink, 1992) (Table 1). Mealybugs were also collected and preserved in 70% alcohol from banana plants at four farms in each of Rakai, Masaka, Ibanda and Ntungamo districts. They were then cleaned and cleared in preparation for microscopic examination and identification by the method described by Watson and Chandler (2000). Based on both the published morphological features and those observed in the laboratory, a dichotomous key for all the mealybug species encountered on *Musa* spp in Africa was constructed (Watson & Kubiriba, 2003 submitted). The key was designed to act as aid to the identification of mealybug samples collected from banana fields.

Table 1. Distribution of mealybug species recorded on *Musa* in Africa

Mealybug species	Distribution	Author
Cataenococcus ensete	Ethiopia	Williams & Matile-Ferrero, 2000
**Dysmicoccus brevipes* (Cockerell)	Eritrea, Ghana, Nigeria, Sierra Leone, Uganda	Williams & Watson, 1988; Williams & Granara de Willink, 1992
*Dysmicoccus grassii (*Leornadi*)*	Tropicopolitan- in most parts of Africa	Williams & Granara de Willink, 1992
Ferrisia virgata (Cockerell)	*Ghana*	Williams & Matile-Ferrero, 2000; Williams & Watson, 1988.
Geococcus coffeae (Green)	Ghana, Nigeria, Uganda, Kenya , Zanzibar	Williams, 1958; Williams & Watson, 1988; Williams & Granara de Willink, 1992
Maconellicoccus hirstus (Green)	Benin, Burkina Faso, Cameroun, Central Africa Republic, Chad, Congo Republic, Gabon, Cote d'Ivoire, Kenya, Liberia, Niger, Nigeria, Senegal, Somalia, Sudan, Tanzania, Democratic Republic of Congo, Zambia	Williams, 1996
Nipaecoccus nipae (Maskell)	South Africa, Tanzania, Zanzibar, Zimbabwe	Ben-Dov, 1994; Williams & Granara de Willink, 1992
Paracoccus burnerae (Brain)	Kenya, Angola, Zimbabwe, Democratic Republic of Congo	Ben-Dov, 1994; De Lotto, 1967
Paraputo anomalus (Newstead)	Uganda, Ghana, Kenya, Tanzania	Williams, 1958
Phenacoccus parvus (Morrison)	*Gabon, Senegal, Congo Republic*	Williams & Granara de Willink, 1992
Planococcus njalensis (Laing)	*Benin, Cameroun, Cote d'Ivoire, Liberia, Nigeria, Ethiopia, Guinea, Principe, Sao Tome, Togo, DRC*	Ezzat & McConnell, 1956
**Planococcus citri* (Risso)	*Angola, Cote d'Ivoire, Liberia, Nigeria, Ethiopia, Ghana, Kenya, Malawi, Swaziland, Tanzania, Guinea, Principe, Sao Tome, Togo, DRC, Zambia, Zimbabwe*	Cox, 1989; Watson *et al.*, 1995
Planococcus ficus (Signoret)	*Angola, Egypt, Libya, South Africa, Sudan*	Cox, 1989;
Planococcus musae (Matile-Ferrero& Williams)	Nigeria	Matile-Ferrero & Williams, 1996
**Pseudococcus comstocki* (Kuwana)	Ghana, Kenya,	Williams & Granara de Willink, 1992
Pseudococcus cryptus Hempel	*Tanzania, Zanzibar*	Williams & Granara de Willink, 1992
Pseudococcus longispinus (Targioni Tozzetti)	*Ghana, Kenya, Malawi, Nigeria, Soa Tome, South Africa, Tanzania, Togo, DRC, Zimbabwe*	Williams & Granara de Willink, 1992; Williams & Watson, 1988
Rastrococcus iceryoides (Green)	*Kenya, Tanzania, Zanzibar*	Williams, 1989
Rastrococcus invadens (Williams)	*Ghana, Togo, Benin, Nigeria, Sierra Leone, Congo Republic*	Williams, 1989
**#Saccharicoccus sacchari* (Cockerell)	*Ghana, Angola, Namibia, South Africa, Zimbabwe, Malawi, Tanzania, Uganda, Kenya, Somalia, Ethiopia, Sudan, Egypt*	Williams & Granara de Willink, 1992

*These are the mealybug species that have been reported to transmit BSV under screenhouse conditions

Has not been identified on bananas but also reported to have transmitted BSV under screenhouse conditions

Raising mealybug cultures

Live mealybugs were collected from farmers' fields in ventilated plastic tubs (lids tightly screwed on) on sections of fresh banana pseudostems. Individual female mealybugs were placed on a pumpkin fruit (Plate 1) and placed in a rearing cage in a dark, shady area of the laboratory at Kawanda at room temperature (about 27^0C). The mealybug cultures were then identified in the laboratory as described above.

Plate 1. A culture of mealybugs growing on a pumpkin fruit

Preparation of source plants and test plants for transmission

One sucker from a plant of cv. "Mbwazirume" showing clear BSV symptoms was collected from a BSV-management trial at Kawanda. This was multiplied by the "split-corm" method and the resulting plantlets grown on in buckets of soil in the screenhouse and used as virus-source plants in the transmission experiments. Occasionally, when they got too big, these source plants were cut back and transferred to fresh soil to allow new, tender leaves, suitable for mealybug feeding, to emerge. Farmyard-manure was also occasionally applied to the buckets to maintain the vigour of the plants.

Plantlets of virus-indexed cv. "Williams" (imported from South Africa) were micro-propagated by tissue culture, and then hardened in the weaning sheds at Kawanda before transplanting in buckets for use as test plants. They were used for transmission when about 20 cm tall with 4 open young tender leaves. The cv. "Williams" (AAA) was used as test plants because the BSV DNA sequences integrated in the genome appear stable and not activatable to episomal forms that cause disease. Any BSV symptoms showing on this variety would therefore be caused by infection due to transmission by a mealybug vector rather than from within the plant.

Transmission of BSV by mealybugs in the screen-house

Transmission experiments were carried out in the purpose-built screen-houses at the IITA Namulonge-Sendusu farm. For each experiment, more than 200 second instar mealybug nymphs from one of the pumpkin cultures were allowed to feed on young leaves of the cv. "Mbwazirume" virus source plants (contained within clip-cages) for four days. The nymphs were then transferred to 10 cv. "Williams" test plants; 20 nymphs (in a clip-cage) per plant. As the control, the procedure was replicated with another 200+ nymphs and 10 test plants, but here the nymphs were allowed to feed for four days on non-infected leaves of cv. "Williams" prior to transfer to the test plants. Both sets of test plants were sprayed with Chloropyrifos 48% E.C 24 hr after introduction of the nymphs for inoculation feeding. To avoid bias during assessment, each test and control plant was given a concealed label (a marked piece of polythene buried in the soil of the pot) and the plants were placed in a randomised pattern in the screenhouse. All plants were scored for appearance and severity of BSV symptoms at regular intervals for up to 4 months after inoculation feeding.

Results and Discussion

Identification of mealybugs on bananas in Uganda

Four mealybug genera, *Dysmicoccus* sp., *Planococcus* sp., *Paracoccus* sp., and *Pseudococcus* sp. were identified from the samples collected in Uganda in banana plantations. Although *Planococcus* sp. had not been reported on bananas in Uganda before, *Planococcus citri* has previously been reported to transmit BSV under screenhouse conditions in USA (Lockhart and Olszewski, 1993; Su, 1998). *Paracoccus* sp., *Pseudococcus* sp. and *Dysmicoccus* sp. had previously been identified on bananas in Uganda, and *Dysmicoccus brevipes* was associated with BSV transmission under screenhouse conditions (Kubiriba *et al*., 2001b.). *D. brevipes* and *Pseudococcus comstocki* have been reported to transmit BSV in the laboratory in Taiwan (Su, 1999).

Raising cultures of mealybugs

After failure to get mealybug cultures to establish on small banana plants in the screenhouse, a culture each of the three different mealybug species, *Dysmicoccus brevipes*, *Planococcus* sp. and *Pseudococcus* sp., was established on pumpkin fruits in screen cages in the laboratory at Kawanda.

Screenhouse transmission tests

These experiments are still on-going. So far, one out of ten plants inoculated using *Planococcus* sp. nymphs has developed symptoms of BSV, and one out of 10 plants inoculated using *Dysmicoccus brevipes* nymphs has developed symptoms. The former first developed symptoms 12 weeks after inoculation and later 8 weeks after inoculation.

Natural field spread of BSV

Rationale

BSV is a *Badnavirus* (or group of badnaviruses) and most badnaviruses are transmitted by mealybugs. Transmission of BSV by mealybugs has only been reported under laboratory and screenhouse conditions (Jones and Lockhart, 1993; Su, 1998; Kubiriba *et al.*, 2001b). Trials at IITA-Nigeria with foci of BSV–infected plants planted in the field surrounded by non-BSV-infected plants did not show any evidence of radial spread of the virus (Hughes, 1998). However, in farmers' fields in Uganda, BSV–infected bananas were observed to occur in clusters and incidence of infected plants reduced with distance from these infection foci (Kubiriba *et al.*, 2001a). This suggests the likely involvement of a slow moving vector in BSV spread; though it does not provide definitive proof for field spread. The main objective of this component of the work was to study the spread dynamics of BSV in relation to mealybug movement under field conditions.

Materials and method

Development of trapping procedures for monitoring mealybug populations

Two types of traps were tested for monitoring mealybug nymph numbers; water pan traps and sticky traps. Fourteen of each type of trap were placed in random locations within a long-established block of banana at Rakai and the mealybug numbers caught in each counted every three days for a period of about a month.

On station BSV spread trials

The trials were set up in October 2001 at Kawanda Agricultural Research Institute and Mbarara Stock farm. A square block of 20 x 20 banana plants (making a total of 400 plants) was planted at each site. In the middle of the block, 16 infected Mbwazirume were planted surrounded by virus indexed cv. "Williams" (Figure 1). The variety "Williams" (AAA) is reported to have stable integrated BSV sequences that are probably not readily activatable to episomal (infectious) forms (Geering et al., 2001) so any infection must either have arisen by transmission through the planting material or by the activity of a vector. When infected with BSV it shows clear symptoms. The cv. Mbwazirume is one of the numerous East African highland bananas (AAA-EAs), which is popular with the farmers. It is also susceptible to BSV showing clear symptoms. An unidentified mixture of species of mealybugs were collected from infected plantations in Rakai and introduced to the virus source plants in the

centre of each block 3 months after planting. Disease assessment of all the 384 'virus tested' plants was based only on observation of symptoms since the ELISA using the antiserum from IITA had been shown not to give reliable results and a more reliable diagnostic method had not yet been developed. Adult mealybug colonies were counted on the pseudostems (above ground plant parts up to about 1.8m) of each of the 400 plants at both sites. Mealybug colonies below the ground and above 1.8m of plant height were not estimated. Both disease and mealybug assessment was done monthly from the time of first assessment 3 months after planting. Assumed mean distance moved by the mealybugs within the spread trials starting from the middle point of the trials was estimated by use of the SADIE PC programme (Perry *et al.*, 1996).

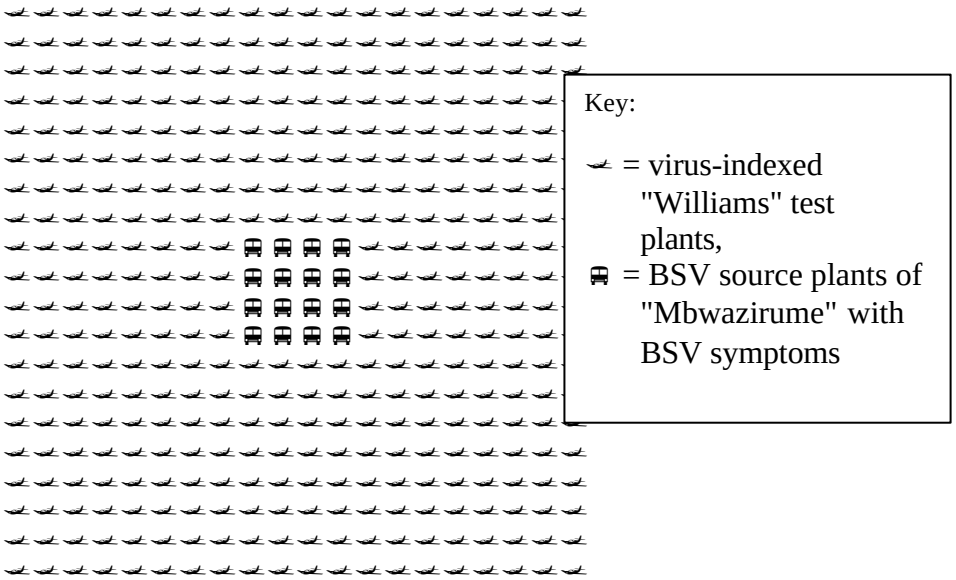


Figure 1. Diagrammatic representation of the layout of the BSV spread trials at Kawanda and Mbarara

Spread trials on farmers' fields

Three different sets of trials were set up on farmers' fields in Ntungamo and Rakai (both sites with high BSV incidence and assumed well established mealybug populations. The hypothesis was that the chances of transmission would be higher in farmers' fields than under controlled experimental conditions on station.

The first trial, which was similar to the on-station trials, was set up in Rakai using the AAA-EA cv "Kisansa" planted in a 23 plants by 23 plants block in September 1998. The planting material was obtained from a farm where no symptoms of BSV had been observed (the site had been under observation since 1995). The assumed sources of infection were the fields surrounding the trial, which had incidences up to 90%. Incidence in the surrounding fields was estimated by taking a zig-zag walk through the fields and assessing every fifth plant for BSV symptoms.

In May 2001, small plots (4 x 4 plants) of cv. Williams were also planted in the middle of eight farms where BSV had been observed to be at relatively high incidence (>80%); 4 in Rakai and 4 in Ntungamo. The hypothesis was that BSV would be spread from the surrounding infected bananas to the newly planted cv. Williams by a vector; possibly nymphs of the mealybugs already established in each farm. Positions where the test plants did not survive at first planting were replanted with virus indexed plants from the same source. BSV symptom severity, incidence and mealybug abundance were all recorded.

In order to try to better characterise the pattern and rate of spread of BSV in Ntungamo, another spread trial (12 x 12 plants) was set up in April 2002 at the Kyangara farm where the small plot described above had the greatest incidence of BSV. The planting material used was of the local AAA-EA cv. Kisansa and was obtained from a farm where no BSV symptoms had been observed since 1995 (the same source as for the larger block in Rakai (described above). Although some Ugandan BSV isolates in Hull and Harpers (2001) "Super group I" are closely related to strains that are activatable from integrated sequences, there is no evidence that activatable BSV sequences are present in these AAA-EAs (Harper and Hull, unpublished). This is in accord with the finding that the activatable integrants appear to be limited to the B-genome (Geering *et al*., 2001).

Results and Discussion

Development of trapping procedures for monitoring mealybug nymphs

Data obtained from water pan traps and sticky sheet traps deployed in the established banana plantations in Rakai revealed that the water traps are the better method for monitoring the numbers and movement of the motile mealybug instars (Figure 2). Water traps were subsequently used in trapping mealybug nymphs in the spread trials.

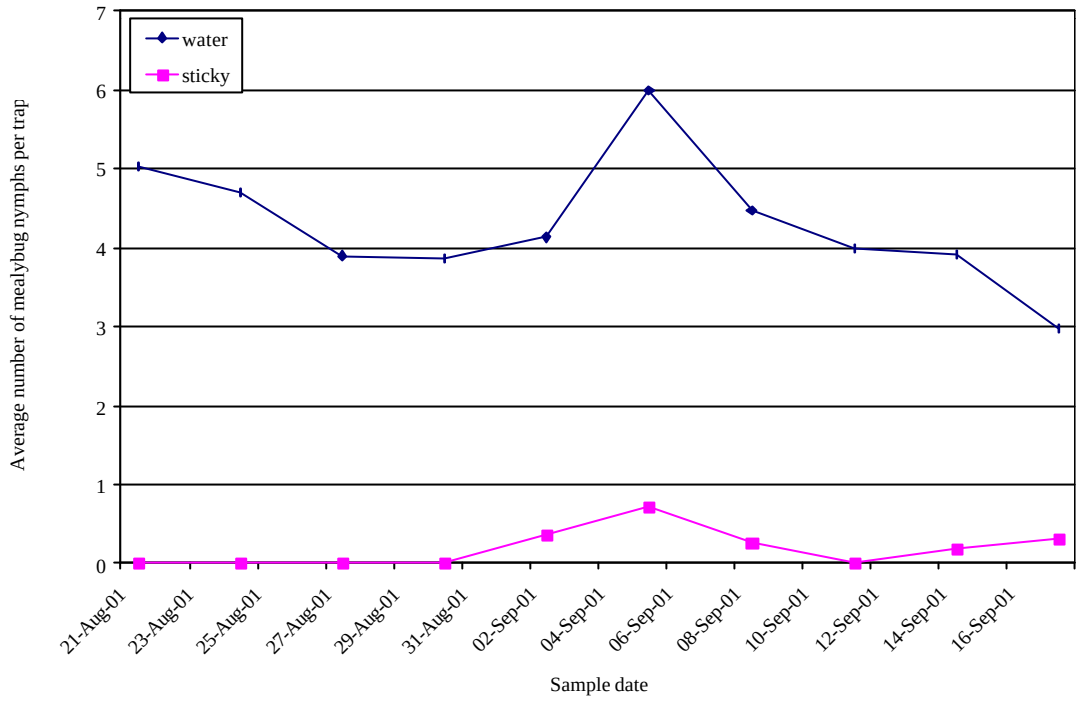


Figure 2. Comparison of mealybug nymph catches in water pan- and sticky-traps in an established banana plantation in Rakai

On-station spread trials

No test plants in either of the on-station-spread trials at Kawanda or Mbarara has developed symptoms of BSV infection in the 25 months since planting. This is possibly because the virus-source plants planted at the centre of each trial were poor to establish and remained stunted – one of the symptoms of the BSV infection they contained. Apart from these source plants, there were no other sources of infection near the trials. Other factors (environmental, virus strain and mealybug species) could influence acquisition of the virus by the vectors (assumed to be mealybugs) from infection sources, subsequent inoculation to the recipient plants and expression of the symptoms after inoculation under field conditions. A small number of mealybug nymphs were caught in the water traps on both sites, but these only appeared nearly 2 years after planting the trials. Adult mealybugs have been observed on the test plants (cv. Williams) that initially did not have mealybugs on them. Since mealybugs were only introduced to the virus source plants at the centre of each trial, this suggests movement of mealybugs from the source plants to the test plants, but does not exclude the possibility that some of these mealybugs may have been carried into the trial from further

afield.. The mealybug counts data from the two sites are being analysed using the **S**patial **A**nalysis by **D**istance **I**ndic**E**s (SADIE; IACR Rothamsted) programme. During the first 6-7 months after introduction to the BSV source plants, the mealybugs multiplied and moved away from the middle of the trial (point assumed by SADIE programme) a distance of approximately one plant per month along the row (= total average distance over 6-7months = 21m in Kawanda and 23 m in Mbarara) (Figure 3 and Figure 4).

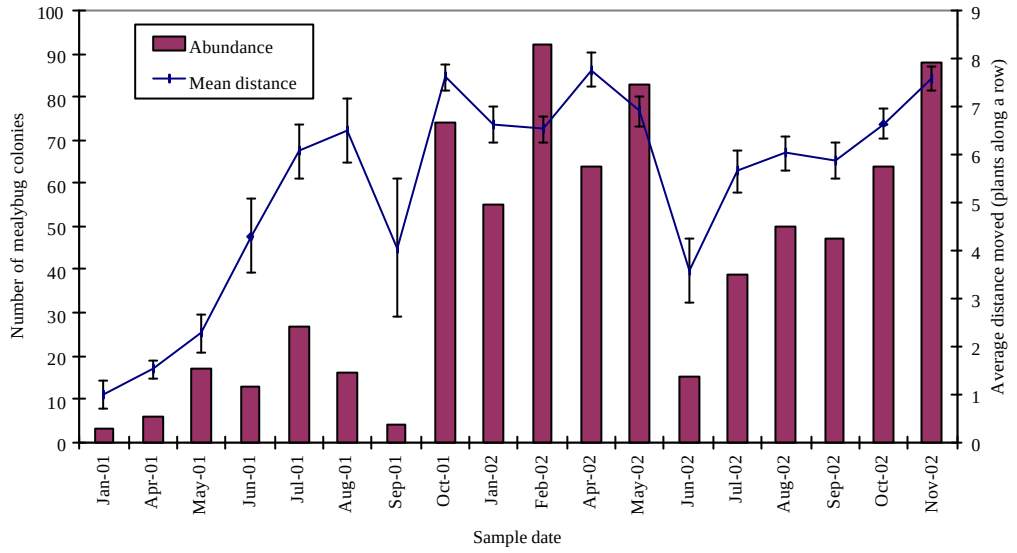


Figure 3. Mealybug colony numbers and movement in the Mbarara trial

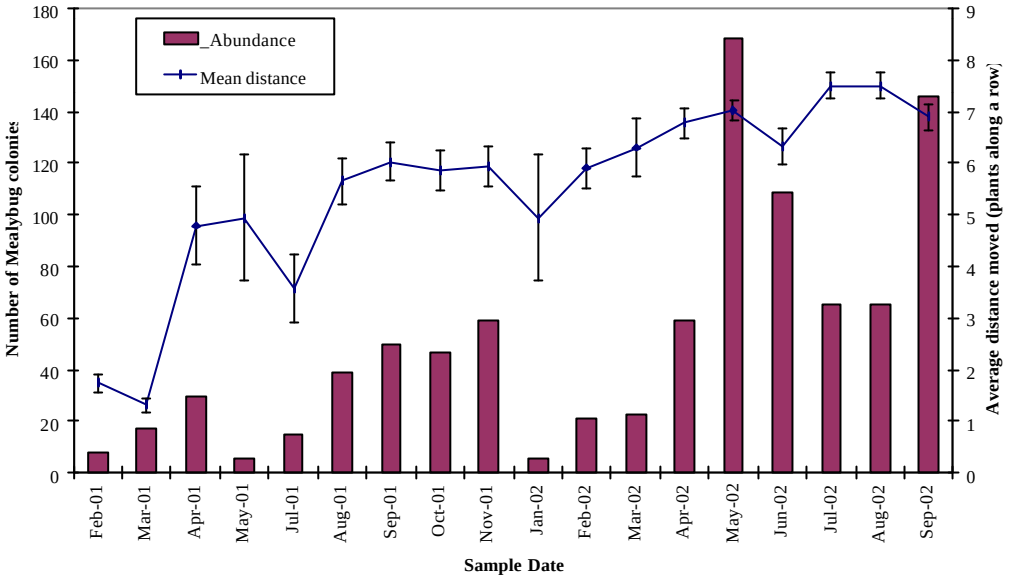


Figure 4. Mealybug colony numbers and movement in the Kawanda trial

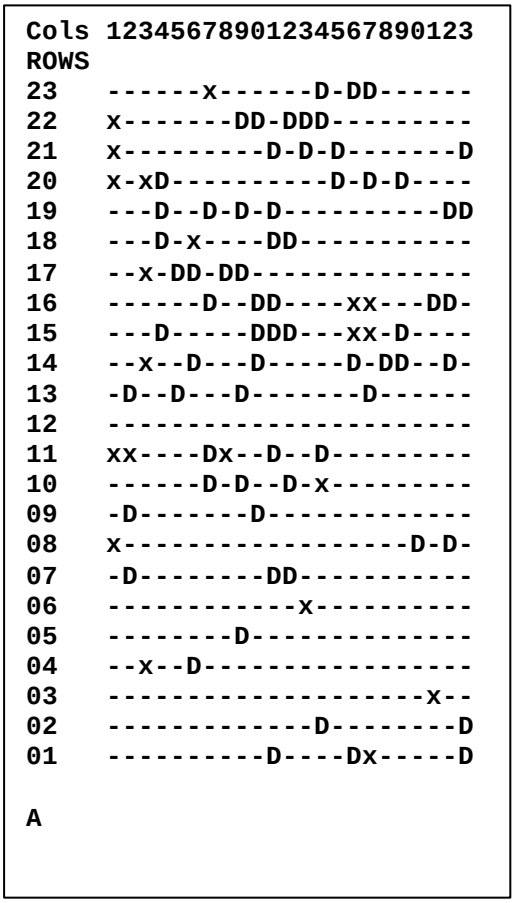
On farm spread trials

Some of the cv. Kisansa plants in the spread trial (23 x 23 plants) in Rakai started showing BSV symptoms about 3 years after planting (Figure 5A). The disease incidence had increased to about 7% by October 2002. Spatial analysis of the distribution of infected plants in the plot using the programme 2D-Class (Nelson *et al*., 1992), suggests that BSV spread to from plant to plant from without and within the trial; i.e. both longer distance primary spread and shorter distance secondary spread was occurring (Figure 5B). Other types of analysis, including ST-Class (Nelson 1995), 2d-CORR (Ferrandino, 1998) and semi-variogram (Gottwald *et al*., 1996) will be tested with this data set to see if any of these describe the types of spread more informatively.

Some of the cv. "Williams" plants in the small spread trials in Ntungamo showed BSV symptoms as soon as six months after planting, and there are now 11 infected plants out of the 16 planted in the plot with most spread. This plot is in the farm where the BSV has the most severe symptoms and is present at the greatest incidence, and where the leaves of the surrounding plants overhang the test plants (Table 2). In many cases, the plant bordering the plots were the first one to get infected (Table 2). This suggests spread of BSV to the c.v. Williams plants from the severely infected surroundings. Mealybug adults were also observed on the test plants in the small spread in Ntungamo and Rakai but data is still too

scanty to enable analysis and therefore relating mealybug movement to BSV spread. In Rakai, however, only 1 plant has been observed to develop symptoms of infection so far, 17 months after planting.

Since the incidence of BSV appears equally high in both Rakai and Ntungamo, the explanation for this apparent difference in rate of spread in the two areas could be differences in environmental conditions, differences in virus strains and/or differences in mealybug species presence. To investigate this further, a new trial has been planted at Kawanda. This is designed to compare the performance and BSV symptom expression of BSV-infected banana material obtained from Rakai, Ntungamo and Kawanda in the same environment (location). If the plants from the different locations continue to exhibit different disease phenotypes in this trial, it will indicate that there are probably virus strain/species differences between the locations (This may then be investigated further using molecular biology techniques). If the virus species/strain composition appears to be similar at the different locations, then more detailed investigation of the mealybug species composition and subtle differences in the environmental conditions at the different locations may be warranted.



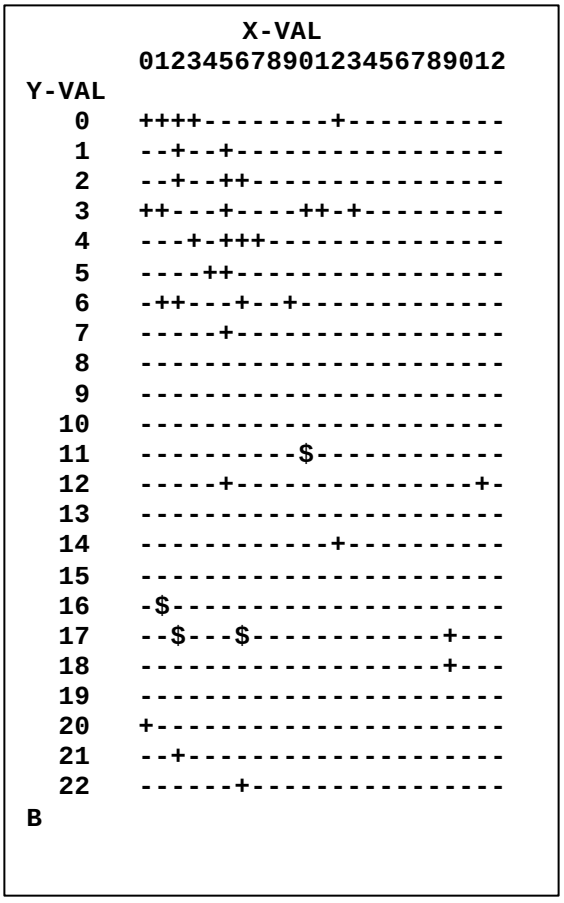


Figure 5. A) Diagrammatic plan of the 23x23 plot of banana (cv. Kisansa) in Rakai showing location, based on symptom assessment, of diseased (D), not diseased (-) and missing (x) plants, 5 years after planting (1st infection was seen 4 years after planting) B) Distance-class matrix output from "2DCLASS" analysis of situation represented in (A). Most [*X,Y*] distance-classes with standardised count frequencies (SCF) significantly greater than expected (*P* £ 0.05) through random chance (+) are located in the top-left of the matrix indicating that many of the infections probably occurred through the short distance spread of the virus within the plot ($= SCF less than expected, - = SCF as expected)

Table 2. Observations on the small plots of cv. Williams planted on farmers' fields

Site	Farmer	[2]Initial BSV incidence (%) of surrounding farm	Initial average no. of mealybug colonies per infested plant in surrounding farm	Time (months after planting) taken for the first symptoms to show	[1]Max. no. of mealybug colonies per infested plant in the plot	*[1]Number of test plants (Williams) infected	General description	Location of infected plants in each 4x4 plot
Ntungamo	Rwamafa	100	10	6	5	*11	Close spacing, closed canopy , leaves hovering over the test plants	X X X X X X X X X X X
Ntungamo	Katureebe	95	12	7	4	*3	Spacing not so close, canopy not so closed	X X X
Ntungamo	Tumusiime	92	5	8	3	*3	Wide spacing and open canopy	X X X
Ntungamo	Kyebitaama	100	2	7	8	*1	Spacing not so close, canopy not so closed	X
Rakai	Sekyondwa	100	9	17	6	*1	Spacing not so close, canopy not so closed	X
Rakai	Salongo	82	3	-	2	0	Spacing not so close, canopy not so closed	
Rakai	Kawalabu	100	6	-	7	0	Spacing not so close, canopy open	
Rakai	Muyonga	71	5	-	1	0	Close spacing, closed canopy, leaves not hovering over test plants	

- *Number of infected plants of the 16 plants in the small spread trials planted in each of the 4 farms in the 2 sites and their location is illustrated in the right most column.
- [1]Maximum number of mealybug colonies was recorded instead of means because of mobile nature of mealybugs. [2] Percentage BSV incidence was calculated using n/N* 100, where n= number of infected plants and N = 30 total number sampled randomly in an acre of banana field.

GENERAL DISCUSSION AND CONCLUSIONS

The distribution/pattern of infected plants in some banana plantations in Uganda suggests that some relatively slow moving vector is responsible for spreading BSV (Kubiriba *et al*., 2001a). This was supported by the findings from the small spread trials at Rakai and Ntungamo where BSV was observed to spread to the young, virus-indexed plants that had been planted within the established plantations where there was high incidence of BSV. One or more mealybug species is/are likely to be the vector of this group of badnaviruses in the field since transmission previously has been demonstrated in the lab using at least three different mealybug species, and in this study has so far been demonstrated with nymphs of *Dysmicoccus brevipes* and a *Planococcus* sp. Several different mealybug species were found colonising banana plants in Uganda and trap counts and stem counts indicate that there can probably be both long distance movement of nymphs on air currents and short distance movement of older stages by crawling.

Evidence so far suggests that there is BSV spread by mealybugs in the field. However, time of first infection appears to be location specific. Reasons behind this specificity (variation in virus strain, mealybug species, and environmental factors) require further investigation. The indication is that roguing of infected material from fields with low BSV incidence is likely to reduce BSV incidence and spread. However, this is only likely to be effective where farmers are reliably able to identify BSV symptoms at very early stages of infection. Roguing in fields with high incidence would be prohibitively expensive in terms on labour (R7529 Final Technical Report Annex 5 &6; Baseline survey data) and would therefore not be readily acceptable to the farmers. As to at which level of incidence roguing becomes impractical to manage BSV has not been investigated, but should be studied to complete the story. The costs (monetary and non-monetary) involved in roguing are not known.

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IDENTIFICATION OF MEALYBUGS ON BANANA AND PLANTAIN IN AFRICA

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Banana and plantain are important sources of carbohydrate and small farmer income in parts of Africa, and banana streak virus (BSV) causes serious loss of yield in some areas. Mealybugs (Hemiptera: Pseudococcidae) have been implicated in the transmission of BSV in the region. The identity of the mealybug vector is not known, and the methods involved in mealybug identification are not widely available in Africa. This paper provides methods of collection and preservation, information on mealybug morphology, and an identification key to adult females of 20 species and 14 genera that may be found on Musaceae in Africa. Brief notes are given on the species, most of which are important pests on other crops.

Key words: Hemiptera, Pseudococcidae, identification, banana, plantain, virus transmission, banana streak virus.

INTRODUCTION

Banana and plantain (Musaceae: Musa spp.) are the world's fourth most important commodity and are grown in more than 120 countries (Harper and Hull, 1998), providing a significant source of carbohydrates for over 400 million people in tropical regions (Anon., 1992). In the humid forest and mid-altitude zones of sub-Saharan Africa, banana and plantain supply over 25% of the carbohydrate requirement for about 70 million people (Anon., 1992). These crops are a major source of income for small-scale farmers; any loss attributable to insect damage can seriously affect the local economy (Matile-Ferrero & Williams, 1996).

During the last 15 years, plantain and banana production has been intensified and new pests and diseases have arisen that cause significant yield losses (Wilson, 1988). Banana streak virus (BSV; Genus Badnavirus) is one of the most widely distributed viruses of Musa spp. (Lockhart, 1994; Su et al., 1997) and can cause considerable yield loss, especially when bananas are affected at the early stages of growth. Jones and Lockhart (1993) reported up to 90% loss of yield in "Poyo" plants with severe BSV symptoms. In severely infected areas in Uganda, plantations suffer almost 100% loss of saleable yield (Tushemereirwe et al., 1996).

All the forms of BSV can be transmitted to daughter plants during vegetative propagation from an infected host, but it is believed that only the episomal forms can be transmitted by a vector. Virus transmission by mealybugs has been little studied but two species, Planococcus citri (Risso) and Saccharicoccus sacchari (Cockerell), have been reported transmitting BSV between banana plants under screenhouse conditions (Lockhart and Olszewski, 1993). However, these mealybug species are not found commonly on banana or plantain in the field. More virus transmission studies of mealybugs on Musaceae in Africa are needed.

Little has been published on the identification of mealybugs in Africa in recent years; Millar (in press) provided a key to the mealybug genera of South Africa. Several mealybug species have been identified on Musa spp. in Africa (Matile-Ferrero & Williams, 1995), but the methods involved in their identification are not widely available in the region. It is important to be able to identify the mealybug vectors of BSV in order to facilitate their control. This paper provides methods of collection, preservation and an identification key to adult females of 20 species of mealybug in Africa that may be found on Musaceae. Many of the species discussed are important pests on other crops also; brief notes on the species are provided.

METHODS

Mealybug species on plantain mentioned in Matile-Ferrero & Williams (1995), and samples collected on Musaceae from Africa in The Natural History Museum, London, UK collection

were listed. In addition, samples from Musaceae in Africa (mainly Uganda) were collected, prepared and identified.

Collection of mealybugs

Mealybugs avoid sunlight and occur on leaf undersides or in crevices, axils, under bark or even on the roots (Watson and Chandler, 2000), so plants were examined very closely and in good light. On banana and plantain, mealybugs were found under the pseudo-stem leaf sheaths, on the roots and sometimes in banana weevil tunnels in the pseudo-stem. Ants often attended the colonies to collect their honeydew, and sooty mould often grew on honeydew deposits that had accumulated on surfaces below the colony. To ensure collection of adult females, specimens of various sizes were collected. Pieces of infested plant were collected into polythene bags because picking individual insects off the plant often damaged them. Samples were kept in a well ventilated, shady, cool place for transport back to the laboratory. In the laboratory, the appearance of the mealybugs in life was noted or recorded by macrophotography, using a dissection microscope and incident illumination. The appearance of the insects in life was lost once they were immersed in alcohol.

Preservation of mealybugs

Live mealybugs are soft and easily damaged with forceps, so small pieces of infested plant were placed in a tube of 80% ethanol to kill and preserve specimens of a variety of sizes. The collection data was written in pencil on paper that was then inserted into the tube with the specimens. Each tube of newly killed mealybugs was immediately stood in a bowl of recently boiled water for 15-20 minutes to speed fixation and denature enzymes that might otherwise turn the body contents black. Good fixation, and storage in ethanol for 1-3 weeks to partly dissolve the waxy covering and toughen the cuticle, made it easier to produce good slide preparations.

Selection of specimens for identification

Mealybug species are identified using minute details on the cuticle of the adult female (Figure 1), because male mealybugs (which are winged) have not been widely studied. The best specimens to prepare for identification are young adult females with small bodies, as they contain few eggs and require less manipulation to clean out the body contents. Each adult female is larviform but possesses large legs, a vulva and usually has multilocular disk pores present. Immature females have smaller legs and fewer antennal segments and pairs of cerarii than adults; they also lack a vulva or multilocular disk pores, so they cannot be identified with confidence using keys to the adult females.

Preparation of mealybugs for identification

Recipes of the reagents required for the preparation of slide-mounts of mealybugs are given in Table 1. The schedule used for preparation of microscope slide mounts of adult female mealybugs for identification is in Table 2. Specimens were processed in solid glass cavity blocks with lids and were never allowed to dry out, to avoid air becoming trapped in the body. Slide preparation was carried out using a dissection microscope with a glass stage and transmitted light from below; samples were heated in covered cavity blocks on a thermostatically controlled dri-block, or in test-tubes in a water bath.

RESULTS

Nineteen species of mealybug, belonging to 13 genera, have been collected from Musaceae (mainly banana and plantain) in Africa. Adult females of these species may be identified using the key below. Another genus and species, Planococcoides njalensis (Laing), is included in the key even though it has never been collected from Musaceae, because it is a highly polyphagous species and a known virus vector (on cacao).

Key to mealybug species on Musaceae in Africa (adult females)

1. *Anal lobes strongly projecting, heavily sclerotised, each tipped with a spine-like anal seta; circuli numbering 2 or 3; eyes absent; venter of head bearing 2 large, thick setae* *Geococcus coffeae* *(Green)*
-- *Anal lobes only moderately developed, no more than partially sclerotised, each tipped with a slender anal seta; a single circulus present; eyes present; extra-thick setae absent from venter of head 2*

2. Antennae each with 9 segments 3
-- Antennae each with 6-8 segments 6

3. Cerarian setae with truncate tips.....genus Rastrococcus 4
-- Cerarian setae with sharp tips.....5

4. Ventral multilocular disc pores present as far forward as thorax; dorsal setae beside anal ring much longer than on other segments; anterior ostioles present; circulus usually about 7x as wide as long..... Rastrococcus iceryoides (Green)
-- Ventral multilocular disc pores only present posterior to circulus; dorsal setae near anal ring approximately the same length as those on other abdominal segments; anterior ostioles absent; circulus usually about 5x as wide as long..... .....Rastrococcus invadens Williams

5. Oral rim tubular ducts present in several rows across dorsum of each segment; cerarii numbering 4-6 pairs, situated on posterior segments of abdomen; anal lobe bar present (not always well developed); quinquelocular pores absent .... Maconellicoccus hirsutus (Green)
-- Oral rim tubular ducts absent; cerarii numbering 18 pairs, distributed around entire margin; anal lobe bar absent; quinquelocular pores numerous on venter ....Phenacoccus parvus Morrison

6. Antennae each with 6 or 7 segments.....7
-- Antennae with 8 segments 9

7. Antennae each with 6 segments; legs short and very robust, with tibia + tarsus shorter than trochanter + femur; cerarii present on head; anus situated more than 1.5x its own length from apex of abdomen; anal lobe bar present ....Paraputo anomalus (Newstead)
-- Antennae each with 7 segments; legs quite slender, with tibia + tarsus longer than trochanter + femur; cerarii absent from head (although single enlarged setae may be present); anus apical or situated no more than 0.5x its length from apex of abdomen; anal lobe bar present 8

8. Cerarii numbering 4-7 pairs, situated on posterior abdominal segments; circulus small, situated within the boundaries of abdominal segment 3; dorsal setae conical; multilocular disc pores only present posterior to circulus; minute thick-rimmed pores absent from around base of hind coxa ..... Nipaecoccus nipae (Maskell)
-- A single pair of cerarii present, situated on anal lobes; circulus very large and dumbbell-shaped, situated at the boundary between abdominal segments 3 and 4; dorsal setae robust flagellate; multilocular disc pores present on all segments; minute thick-rimmed pores present on cuticle around base of hind coxaSaccharicoccus sacchari (Cockerell)

9. Large oral rim ducts present on dorsum, each duct with several setae located within the sclerotised rim .....Ferrisia virgata (Cockerell)
-- Oral rim ducts, if present, each without any setae located within the rim 10

10. Cerarii forming an almost continuous marginal band, due to the presence of numerous intermediate cerarii; dorsal setae each with a curved tip; anal lobe cerarii each containing 3 or more cerarian setae, and venter of anal lobe widely sclerotised Cataenococcus ensete Williams & Matile-Ferrero

-- Cerarii distinct, numbering 17 or 18 pairs; dorsal setae not strongly curved at tips; if anal lobe with more than 2 cerarian setae, then venter of lobe with an anal lobe bar ... 11

11. Cerarii numbering 18 pairs (with 3 pairs present anterior to the eyes); anal lobe bar present..... 12
-- Cerarii numbering 17 pairs (with only 2 pairs anterior to the eyes); anal lobe with ventral sclerotisation triangular or oval or bar-shaped..... 15

12. Abdominal cerarii each with 3 or more conical setae; legs short and very robust, with tibia + tarsus shorter than trochanter + femur; dorsal and ventral setae very long. Not recorded from Musaceae so far ..... Planococcoides njalensis (Laing)
-- Abdominal cerarii each with only 2 conical setae; legs moderately robust or slender, each with tibia + tarsus usually as long as or longer than trochanter + femur; dorsal setae short ..... genus Planococcus 13

13. Eye associated with disc pores; circulus small, 40-50 m wide; disc pores each almost as large as a multilocular disc pore... Planococcus musae Matile-Ferrero & Williams
-- Eye not associated with any disc pores; circulus more than 100 m wide; disc pores each same size as or slightly larger than a trilocular pore..... 14

14. Oral collar tubular ducts present on margins of head and thorax; small group of disc pores present on dorsal midline of anterior abdominal and thoracic segments; hind femur never with translucent pores ..... Planococcus citri (Risso)
-- Oral collar tubular ducts absent from margins of thorax, and scarce or absent from head; disc pores never forming groups on midline of abdominal or thoracic segments; hind femur sometimes with translucent pores present distallyPlanococcus ficus (Signoret)

15. Oral rim tubular ducts present on dorsum (check head)..... 16
-- Oral rim ducts absent from dorsum..... genus Dysmicoccus 19

16. Anal lobe bar present; cerarii each without any auxiliary (flagellate) setae; hind femur without any translucent pores ..... Paracoccus burnerae (Brain)
-- Ventral sclerotisation of anal lobe oval or triangular; cerarii each containing auxiliary (flagellate) setae; hind femur always with translucent pores present 17

17. Most cerarii each with 2 or 3 dorsal oral rim tubular ducts present nearby; penultimate and anal lobe cerarii large and sclerotised, other cerarii not sclerotised; multilocular disc pores present only immediately next to vulva Pseudococcus longispinus (Targioni Tozzetti)
-- Each cerarius with no more than 1 dorsal oral rim duct present nearby; if penultimate cerarius sclerotised, then so are all the other cerarii; multilocular disc pores present at least as far forward as circulus..... 18

18. All cerarii sclerotised to some degree; multilocular disc pores present only posterior to circulus; dorsal oral rim ducts numbering fewer than 7, never present on midline of posterior abdominal segments ..... Pseudococcus cryptus Hempel
-- Only anal lobe cerarii sclerotised; multilocular disc pores present on thorax as well as abdomen; dorsal oral rim ducts numbering more than 7, some present on midline of some posterior abdominal segments ..... Pseudococcus comstocki (Kuwana)

19. Eye associated with disc pores; dorsum of abdominal segment 8 (just anterior to anal ring) bearing setae much longer than on preceding segments, and numerous disc pores, each larger than a trilocular pore .....Dysmicoccus brevipes (Cockerell)
-- Eye not associated with disc pores; dorsum of abdominal segment 8 with setae not significantly longer than those on preceding segments; any disc pores present on segment 8 each not larger than a trilocular pore .....Dysmicoccus grassii (Leonardi)

Notes on the mealybug species

Cataenococcus ensete Williams & Matile-Ferrero is a morphologically variable species that was described by Williams & Matile-Ferrero (2000) on Ensete ventriculosum from Ethiopia. The species was found on trees infected with ensete streak virus, a badnavirus that is thought likely to be related to BSV and sugarcane streak virus. There is no direct evidence yet that this mealybug species transmits the virus. It has not been recorded from any other host plant. Williams & Matile-Ferrero (2000) illustrated C. ensete and discussed its separation from other species of Cataenococcus.

Dysmicoccus brevipes (Cockerell) is a polyphagous and tropicopolitan species that is common and widespread throughout Africa (Ben-Dov, 1994; Matile-Ferrero & Williams, 1996). It occurs on both aerial and subterranean parts of its host-plants, and shows a preference for sweetness, often causing problems on pineapple, fruit trees and sugarcane (Watson & Chandler, 2000). Dysmicoccus brevipes was recorded on plantain and Musa sp. from Eritrea, Ghana, Nigeria, Sierra Leone and Uganda by Williams & Matile-Ferrero (2000); at least some of these samples were collected from plants showing banana streak virus symptoms. The species was illustrated and discussed by Williams & Watson, 1988, and Williams & Granara de Willink (1992).

Dysmicoccus grassii (Leonardi) (synonym D. alazon Williams) is a fairly polyphagous mealybug from Central and South America that often feeds on fruit and beverage trees and on vegetables. It is injurious to bananas in the Canary Islands, where heavy infestations of the axis and fruit bunches causes premature ripening and reduced productivity. The species was recorded from Nigeria on false horn plantain, on the pseudostem under the dead leaf sheaths, by Matile-Ferrero & Williams (1966); some of the host-plants were dying of viral infection. The species was illustrated and discussed (as D. alazon) by Williams & Granara de Willink (1992). There is a risk that this damaging mealybug may spread to other parts of sub-Saharan Africa.

Ferrisia virgata (Cockerell) is one of the commonest, most tropicopolitan species and is widespread in most of Africa; it is highly polyphagous, occurring on aerial parts of mainly woody hosts. The species is quite distinctive in life, with two long, white wax pencils at the posterior end and paired dark grey, longitudinal streaks on the dorsum (Watson et al., 1995, provided a photograph). It was recorded on Musa paradisiaca from Ghana by Williams & Matile-Ferrero (2000). Williams & Watson (1988) illustrated F. virgata and the similar species F. malvastra McDaniel (under the synonym F. consobrina Williams & Watson). Ferrisia malvastra has been recorded from Ghana, Somalia and Sudan; Williams (1996) provided a key to all the known species of Ferrisia.

Geococcus coffeae Green is a small, root-feeding mealybug with distinctive, prominent anal lobes and setae, giving it a pincer-like appearance at the posterior end. The species is polyphagous and has been recorded from the roots of beverage and fruit trees (including Musa sp.), vegetables (including sweet potato), tobacco and grapes. Geococcus coffeae has been recorded from Ghana, Nigeria, Uganda, Kenya and Zanzibar (Ben-Dov, 1994), but not on Musacaeae. It was illustrated and discussed by Williams (1958), Williams & Watson (1988) and Williams & Granara de Willink (1992).

Maconellicoccus hirsutus (Green) is a highly polyphagous, damaging mealybug that originated in southern Asia. Since the early 1990s it has spread to the Caribbean, Central and northern South America, California, Florida, Hawaii and Samoa, causing concern. The saliva of this insect causes severe distortion of young growth, defoliation and even plant death in sensitive host-plants (e.g. species of Albizzia, Pithecellobium and Hibiscus). Some workers have attributed this damage to transmitted viruses (on cacao in Zanzibar (De Lotto, 1967a) and on mulberry in India (Tewari et al., 1994)), but no evidence of virus transmission has been found. Members of the genus Hibiscus are favourite hosts; Musaceae are not, but M. hirsutus has been recorded feeding on Musa sp. In Africa this mealybug has been recorded from Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Congo Republic, Gabon, Côte d'Ivoire, Kenya, Liberia, Niger, Nigeria, Senegal, Somalia, Sudan, Tanzania and Zaire, and has been intercepted from Zambia at Chicago, USA (Williams, 1996), but never on Musaceae. Maconellicoccus hirsutus was illustrated and discussed by Williams (1996a), and

characters distinguishing it from M. ugandae (known from Cameroon, Ghana, Sudan and Uganda) were given by Williams (1986).

Nipaecoccus nipae (Maskell) is a mealybug native to the Neotropical Region that feeds on fruit trees (especially guava) and palms. In life, it is a small species that feeds on leaf undersides and develops a pattern of white or pale yellow wax cushions on the dorsum, suggestive of a quilt. It has been recorded from South Africa, Tanzania (including Zanzibar) and Zimbabwe on palms but not Musaceae; however, it has been recorded feeding on Musa sp. elsewhere (Ben-Dov, 1994). Williams & Granara de Willink (1992) illustrated and discussed N. nipae.

Paracoccus burnerae (Brain) is a fairly polyphagous species that is native to South Africa, where it is one of the three most important species on citrus (Hattingh, 1993); it has also been recorded from Kenya, Angola, Zimbabwe and Zaire on a variety of hosts including Nerium oleander. The species was recorded on Ensete sp. from Kenya by Ben-Dov (1994) and Williams & Matile-Ferrero (2000), and illustrated and discussed by De Lotto (1967); Ben-Dov (1994) summarized its taxonomy.

Paraputo anomalus (Newstead) (synonyms P. ritchiei Laing and P. multispinosa James) is a morphologically variable species with the anus situated dorsally. It was recorded on the roots of Musa sp. from Uganda by Williams & Matile-Ferrero (2000) and is also known to occur in Ghana, Kenya and Tanzania on the trunks of a variety of trees, sometimes under the bark and attended by ants. Paraputo anomalus was illustrated and discussed by Williams (1958).

Phenacoccus parvus Morrison is a fairly polyphagous, neotropical mealybug that has been spreading through the Pacific islands, Australia and southern Asia over the last twenty years. It can be damaging to vegetables and herbaceous plants, especially Solanaceae, if its principal host (Lantana camara) is growing nearby (Marohasy, 1994). The species has been collected from Gabon, Senegal and Congo Republic (Williams & Granara de Willink, 1992) but not on Musaceae; however, it has been recorded on Musa sp. from elsewhere (Ben-Dov, 1994). Williams & Granara de Willink (1992) provided an illustration and discussion, and Watson et al. (1995) provided a photograph.

Planococcoides njalensis (Laing) is a highly polyphagous African species, known from Cameroon, Benin, Ghana, Guinea, Côte d'Ivoire, Liberia, Nigeria, Principe, São Tomé, Sierra Leone, Togo, Zaire and Ethiopia. The species is the most important vector of swollen shoot virus disease of cocoa (Strickland, 1951a, 1951b). There are no records of this species attacking Musaceae so far, but the species is included here because it may yet be found on these hosts. Ezzat & McConnell (1956) redescribed and illustrated P. njalensis.

Planococcus citri (Risso) is a highly polyphagous, cosmopolitan species of Old World origin; in Africa it is known to occur in Angola, Côte d'Ivoire, Eritrea, Ethiopia, Ghana, Kenya, Malawi, Nigeria, Principe, São Tomé, Senegal, Sierra Leone, South Africa, Sudan, Swaziland, Tanzania (including Zanzibar), Zaire, Zambia and Zimbabwe. It has been recorded on a wide variety of fruit, beverage and vegetable crops and ornamental plants including Musaceae. Planococcus citri was recorded on Musa paradisiaca from Ghana by Williams & Matile-Ferrero, 2000. The species was illustrated and discussed by Cox (1989); Watson et al. (1995) provided a photograph. The species is very difficult to separate from P. minor (Maskell) and P. ficus, so expert identification is recommended.

Planococcus ficus (Signoret) is a fairly polyphagous species of Old World origin, possibly from the Middle East or the Indian subcontinent. In Africa, it has been recorded from Angola, Egypt, Ethiopia, Libya, South Africa and Sudan. It was recorded on Ensete ventricosum from Ethiopia by Williams & Matile-Ferrero, 2000. The species was illustrated and discussed by Cox (1989), and is very difficult to separate from P. citri and P. minor (Maskell); expert identification is recommended.

Planococcus musae Matile-Ferrero & Williams was described from Nigeria on false horn plantain showing symptoms of banana streak virus. There was no evidence to show that this mealybug was the virus vector. The species has not been recorded from any other host-plant.

Matile-Ferrero & Williams (1996) illustrated P. musae and discussed its separation from other species of Planococcus in Africa.

Pseudococcus comstocki (Kuwana) is a polyphagous species of Palaearctic origin. In Africa it has only been identified twice, once from Ghana (on Irish potato) and once, tentatively, from Kenya (on coffee); however, it has been recorded feeding on Musa sp. elsewhere (Ben-Dov, 1994). This species is a serious pest of deciduous fruit trees in the eastern USA and Japan. Williams & Granara de Willink (1992) illustrated and discussed P. comstocki and how to distinguish it from the similar P. cryptus.

Pseudococcus cryptus Hempel (P. citriculus Green is a synonym) is a polyphagous species that is fairly widespread in the tropics. In Africa it has been recorded only from Tanzania (Zanzibar and possibly Dar es Salaam), on Citrus and Cocos nucifera; however, it has been recorded on Musa sp. elsewhere (Ben-Dov, 1994). It is often found on citrus and palms, which it can damage. In life this species resembles P. longispinus, but the wax pencils are shorter and the posterior pair are divergent and always shorter than the body (Watson et al., 1995, provided a photograph). Williams & Granara de Willink (1992) illustrated and discussed P. cryptus and how to distinguish it from the similar P. comstocki.

Pseudococcus longispinus (Targioni Tozzetti) is a highly polyphagous, cosmopolitan species of Old World origin, that feeds on a wide variety of woody and herbaceous hosts including fruit trees, vegetable crops and ornamental plants. First-instar P. longispinus on grapevines are vectors of grapevine leafroll-associated (?) closterovirus, GLRaV-3 (Peterson & Charles, 1997) and grapevine trichovirus A (GAV) (Notte et al., 1997). The species is also associated with pitting of grapevine stems (Rosciglione and Gugerli, 1986) and is an important pest on this crop in European and Mediterranean countries, Australia and New Zealand. In Africa it has been recorded from Ghana, Kenya, Malawi, Nigeria, São Tomé, South Africa, Tanzania (including Zanzibar), Togo, Zaire and Zimbabwe. There is no record of P. longispinus on Musaceae in Africa, but it has been recorded on Musa sapientum elsewhere (Ben-Dov, 1994). In life, undisturbed mature females develop parallel white wax pencils at the posterior end that are up to three times as long as the body, and shorter pencils around the rest of the body margin. The species was illustrated and discussed in Williams & Watson (1988) and Williams & Granara de Willink (1992).

Rastrococcus iceryoides (Green) is a species native to southern Asia that has been long established on the east coast of Africa (Kenya, Tanzania including Zanzibar) (Williams, 1989); it spread inland during the early 1990s to the Tanzania/Malawi boarder, where it caused serious damage to mango. In life, the species resembles rather small cottony cushion scale (Icerya purchasi Maskell, Hemiptera: Margarodidae). It is quite polyphagous and attacks the leaf mid-veins and flowers of fruit trees; it was recorded on Musa sp. from Tanzania (Zanzibar) by Williams & Matile-Ferrero (2000). Williams (1989) illustrated and discussed R. iceryoides.

Rastrococcus invadens Williams is native to southern Asia but was accidentally introduced to Ghana and Togo in 1981-2 and has since spread to Benin, Nigeria, Sierra Leone and Congo Republic. Like R. iceryoides, it feeds along the leaf veins and attacks a wide range of fruit trees; it can be very damaging to mango (Williams, 1989). In life, R. invadens develops very long, slender white wax pencils from the body margins; Williams (1989) provided a photograph, an illustration and discussion. It was recorded on Musa sp. and M. paradisiaca from Congo Republic, Ghana and Togo by Williams & Matile-Ferrero (2000).

Saccharicoccus sacchari (Cockerell) is normally found feeding on aerial parts of sugarcane, although it has been recorded moving (with the aid of ants) to the stem just below the soil surface after harvest (Williams & Granara de Willink, 1992). This species has not been collected from Musaceae in the field, but has been reported transmitting BSV between banana plants under screenhouse conditions (Lockhart and Olszewski, 1993). It is known from Ghana, Angola, Namibia, South Africa, Zimbabwe, Malawi, Tanzania, Uganda, Kenya, Somalia, Ethiopia, Sudan and Egypt. Williams (1970; 1985), Williams & Watson (1988) and Williams & Granara de Willink (1992) illustrated and discussed S. sacchari.

CONCLUSION

Mealybugs have been little studied as vectors of virus diseases, although there is evidence that they can be important vectors of viruses in plantation crops and vineyards. Studies are needed to identify the species responsible for transmission of important plant virus diseases, as once the vector's identity is known there may be good potential for vector control by host-specific natural enemies. Mealybugs have relatively host-specific hymenopteran parasitoids that have been used very successfully in classical biological control programmes, e.g. of Phenacoccus manihoti on cassava across equatorial Africa (Zeddies et al., 2001), and Rastrococcus invadens on mango and other fruit trees in West Africa (Vögele et al., 1991).

Little has been published on the identification of mealybugs in Africa in recent years; Millar (in press) provided a key to the mealybug genera of South Africa. Several mealybug species have been identified on Musa spp. in Africa (Matile-Ferrero & Williams, 1995), but the methods involved in their identification are not widely available in the region. It is important to be able to identify the mealybug vectors of BSV in order to facilitate their control.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Richard Vane-Wright, Keeper of Entomology, The Natural History Museum, for making the Museum collection and Library freely available for this work. Figure 1 is reproduced with the kind permission of the Trustees of The Natural History Museum, London, UK.
This publication is in part an output from a project funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID. (R7529/Za0365; Crop Protection Programme).

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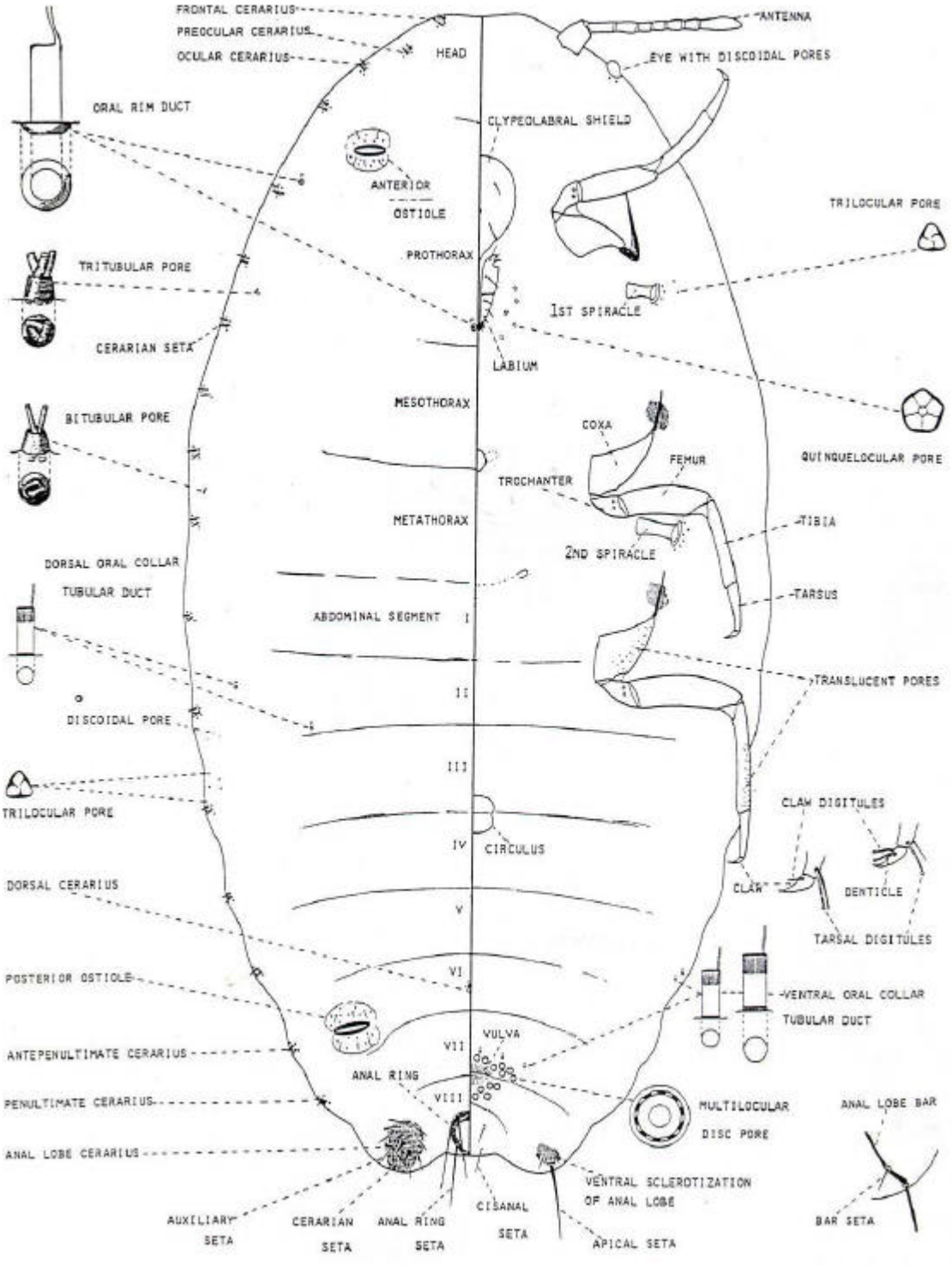

Figure 1. General morphology of an adult female mealybug (after Williams (1985), © The Natural History Museum, London).

Table 1. Recipes for reagents used in the preparation of microscope slide mounts of mealybugs, based on Watson & Chandler (2000).

Reagent	Function	Recipe and precautions
Acid Fuchsin	stains chitin and sclerotin red in acid conditions	mix 0.5 g Acid Fuchsin powder + 300 ml distilled water + 25 ml of bench hydrochloric acid; toxic, potential carcinogen
Canada balsam	Permanent mountant	thin with xylene to an easy pouring consistency; flammable, toxic fumes
Carbol xylene	dissolves fats and waxes	mix equal volumes of phenol crystals and xylene; corrosive, flammable, fumes toxic. See Histoclear phenol below for a safer alternative
Clove oil	clearing agent also dissolves fats and waxes	purchase as anhydrous clove oil; should be pale honey-coloured and transparent
Distilled water	dehydrating agent rinses out organic solvents	distilled water
Ethanol 80%	dehydrating agent rinses out stain	for preservation and partial dehydration
Ethanol 100%	dehydrating agent rinses out organic solvents	use for complete dehydration
Glacial acetic acid	acidifying and dehydrating agent	concentrated acid; corrosive, fumes toxic
Histoclear phenol	dissolves fats and waxes	mix equal volumes of Histoclear and phenol crystals; corrosive, toxic fumes
Potassium hydroxide (KOH) 10%	mascerating agent	dissolve approximately 50 g potassium hydroxide pellets in 500 ml distilled water; corrosive alkali
Xylene	organic solvent; dissolves fats and waxes	solvent for thinning Canada balsam flammable, fumes toxic

Table 2. Schedule for preparation of microscope slide mounts of mealybugs, based on Watson & Chandler (2000).

Step	Action
1.	Heat specimens in 80% ethanol at about 70°C for 5 minutes, to toughen the cuticle and dissolve some of the wax.
2.	Under a dissection microscope, puncture the dorsum of the thorax of each specimen using the tip of a very sharp needle, without damaging any other parts of the body.
3.	Use a micro-spatula to transfer specimens to 10% potassium hydroxide (KOH); heat to 80°C to macerate the body contents, until each specimen becomes translucent but not completely colourless.
4.	Using a mini-spatula, gently squeeze the body contents (including eggs and fat droplets) out through the puncture in the thorax.
5.	Rinse the specimens in distilled water for at least 10 minutes, with additional manipulation (if necessary) to expel any remaining body contents entirely. Leave the body of each specimen completely clean and dorso-ventrally flattened (as in Fig. 1).
6.	Neutralize any remaining traces of KOH by adding a drop of glacial acetic acid to the dish, as an acid environment is needed to give the stain colour.
7.	Replace the liquid in the dish with 2 drops of Acid Fuchsin stain for at least 2 minutes.
8.	Remove stain and quickly rinse excess stain away in 80% ethanol; the slower the rinse, the more colour will be removed.
9.	Quickly remove 80% ethanol and replace with 100% ethanol to stop any further colour loss and fully dehydrate the specimens; soak for a few minutes. Do not manipulate specimens in alcohol because the cuticle becomes brittle when dehydrated and will be damaged.
10.	Add a few drops of Histoclear phenol (or carbol xylene) to the dish and soak the specimens at room temperature until all fat/wax has dissolved. Very waxy specimens may require hours of soaking, or gentle heating, and several changes of dewaxing agent to remove all the wax.
11.	Remove dewaxing agent and rinse specimens for 10 minutes in 100% ethanol.
12.	Remove alcohol and clear specimens in anhydrous clove oil for at least 10 minutes (can be left overnight).
13.	Lable microscope slides before the specimens are placed on them. The best lables are made of 0.3 mm thick, white card cut in 19 mm squares, glued onto the slides with wood glue, and are written in indelible, waterproof ink or pencil. The label on one side of the slide carries the collection data (country, locality, host plant, any damage symptoms, collector's name, sample number, date of collection) and the other label carries the identification.
14.	Place a small (2 mm diameter) spot of clove oil on each microscope slide, and transfer 1-3 specimens, one at a time, into the drop using mounted needles. Working under the dissection microscope, use mounted needles to arrange specimens so that each one lies head downwards in relation to the writing on the lables.
15.	Soak up the clove oil from the specimens using the corner of a folded tissue. Place a drop of a well-liquefied mountant (Canada balsam) 6-7mm in diameter on the specimens and quickly spread it in a circular pool around them. Use the tip of a pin to lower a cover slip onto the mountant, and allow it to settle under its own weight.
16.	Dry slide mounts in a horizontal position in a dust-free environment for 3 months at about 35°C.
17.	Store slide mounts horizontally in cool, clean, dark conditions away from moisture or any risk of attack by termites.

IMPACT OF BANANA STREAK VIRUS ON THE GROWTH AND YIELD OF BANANA var. Cavendish Williams (*Musa* AAA) and var. Mbwazirume (*Musa* AAA – EA)

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Interim Technical Report for the University of Reading, UK / National Agricultural Research Organisation (NARO), Uganda component of:

Project R7529. Management strategies for banana streak virus: Epidemiology, vector studies and control of banana streak virus in East Africa highland bananas

March 2003

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CONTENTS

SUMMARY

Banana streak virus (BSV) is thought to be an important constraint to the production of East African Highland bananas in Uganda. However, knowledge of the effects of BSV on the productivity of banana crops is still sparse. We conducted an experimental programme to quantify the effects of BSV on the growth, development and yield of banana crops grown at two contrasting sites in Uganda. Of particular concern was how the effects of BSV are influenced by crop management. We aimed to improve knowledge of the effects of BSV in order to help formulate environmentally friendly and equitable management technologies that mitigate the effects of BSV on banana productivity. Results obtained for the mother and 1st ratoon crop cycles are reported here.

BSV symptoms of the local East African Highland variety Mbwazirume growing under optimal management (with mulch and fertiliser) were less frequent and less severe compared to those grown under a minimal management regime (infrequent weeding and pruning). BSV disease development was reduced by between 25 – 58% under optimal management at Kawanda and Mbarara. Optimal management increased bunch weights by 62% and 51% in the mother plant crop at Kawanda and Mbarara, respectively. In the first ratoon crop, at Kawanda, bunch weights were increased by 106%. BSV reduced bunch weights by 1.2 % under optimal management and 13.5% under minimal management, in the mother plant crop at Mbarara. At Kawanda, BSV reduced bunch yields by 16.6% under optimal management compared with 22.8% under minimal management in the mother plant crop. In the first ratoon crop, BSV reduced bunch weights under optimal management by almost the magnitude as in the mother crop. Under minimal management, however, bunch weights were reduced by 29%, a further reduction compared to the mother plant crop. This indicated that the effects of BSV on crop productivity and the difference between optimal and minimal management could increase with successive crop cycles. The effects of BSV on important growth and development processes were defined. For example, BSV reduced the rate of leaf photosynthesis, biomass, growth and the duration of development stages of East African Highland bananas. Therefore, BSV reduced the productivity of banana grown under Ugandan conditions. Moreover, the negative impact of BSV on bunch yield was greater than previously reported under well-fertilised and irrigated conditions in Australia, and tended to be greater under minimal compared with optimal management.

We conclude that improved crop management reduces both the BSV disease severity, and some of the negative effects of BSV on growth and yield of plants with symptoms. This conclusion provides a foundation for developing strategies to combat the impact of BSV. Future work needs to address three issues. First, the effect of BSV in further ratoon crops needs to be defined. It is suspected that both symptoms and their impact on bunch yield will increase in further crop cycles. Second, the impact of BSV in the presence of other pests and diseases found in Uganda can be studied now that the effects of BSV alone on banana productivity is becoming clearer. Third, strategies for farmers to mitigate the effects of BSV through the management of their plantations need to explored through farmer participation.

INTRODUCTION

Highland cooking banana (*Musa* genome AAA – EA) is an important staple in Uganda, Tanzania, Burundi, Rwanda, Eastern Congo and Western Kenya (INIBAP, 1986). It is grown mainly on smallholdings by resource poor farmers (Gold *et al*., 1998) for food although surplus is sold for cash income. However, the presence of banana streak virus (BSV) has become an important constraint to the production of the East African Highland banana in the region (Debek and Waller, 1990). Surveys conducted in Uganda in the mid 1990s showed that BSV incidence was mainly confined to the southern region of the country (Tushemereirwe *et al*., 1996). Today, BSV disease occurs throughout the country.

Banana streak virus is an economically important disease. For example, in the Ivory Coast yield losses of up to 90% have been reported on "Poyo" plants with severe symptoms (Jones and Lockhart, 1993). BSV has also been observed to cause significant yield losses when bananas are affected during the early stages of growth. Tushemereirwe *et al*. (1996) reported that in Uganda, losses of up to 100% of saleable yield occurred in bananas infected with BSV during their early stages.

Plants infected with BSV exhibit a wide range of symptoms (Lockhart, 1986; Dahal *et al*., 1998; Lockhart, 1994; Gauhl and Pasberg-Gauhl, 1995) which are normally associated with yield loss. However, there is much uncertainty concerning the magnitude of effects of BSV symptoms on banana yields. A recent study in Australia (Daniells *et al.* 2001) showed that under well-fertilised and irrigated conditions, the yield loss attributable to BSV infection was 8-11% in the first ratoon crop. BSV symptoms are thought to be influenced by climate (temperature and rainfall), and possibly by the vigour of the banana plant. Again, however, no clear relationships have been demonstrated.

This component of Project R7529 aims to gain a better understanding of the role of banana variety and crop management conditions on the expression of BSV disease symptoms and to quantify their effects on banana physiology and yield. The conclusions reached will lead to the formulation of environmentally friendly and equitable management technologies that mitigate the effects of BSV on banana productivity.

RESEARCH ACTIVITIES

The activities are numbered in accordance with the Project R7529 Project Memorandum

<u>Output 3.</u> Empirical data on the effect of climate on the disease, whether good management can alleviate the effects of the disease and restore yield to near normal and, based on these findings, formulation of a strategy for controlling, or at least managing, BSV in Uganda

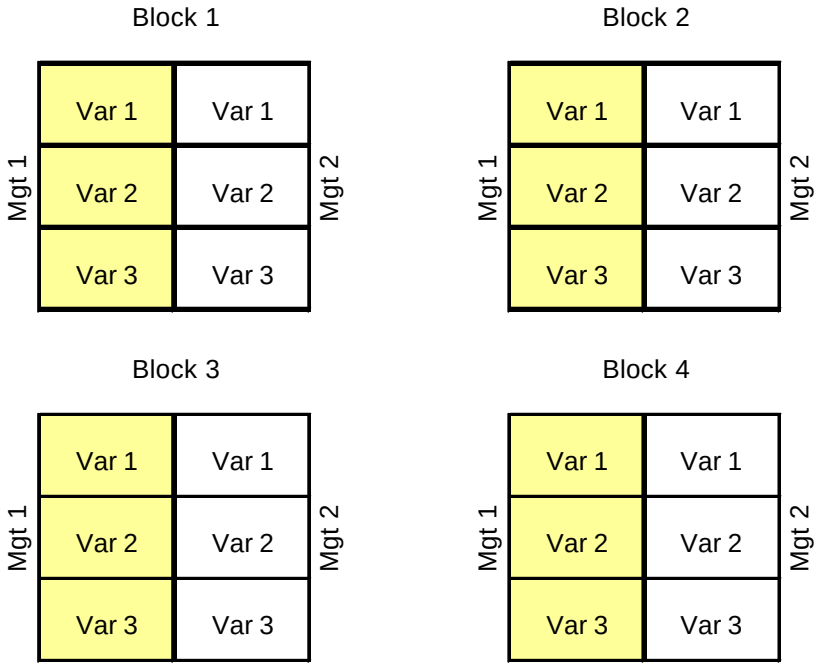
<u>Activity 3.1</u>. Trials at three contrasting sites to assess the effect of minimal and optimal crop management practices on BSV incidence and severity, and on crop productivity.

Experiments for this activity are being conducted at Kawanda Research Institute (0.42N 32.5E) and Mbarara Stock Farm (0.4S 30.4E). These are sites with contrasting weather and with local incidence of BSV. The experiments were planted in October 2001 (Kawanda) and November 2001 (Mbarara).

3.1.1 Experimental design and treatment structure

The design of these experiments was split-plot with 4 replicate blocks (Figure 1). Each block consisted of main plots of a management treatment (Mgt in Figure 1) and sub-plots of banana varieties (Var in Figure 1). The management treatment levels were randomly assigned in each block. Each of the management plots was then sub-divided into subplots to which each of the banana varieties were randomly assigned. Each subplot consisted of 20 plants.

Figure 1. Field plan of experiments at Kawanda and Mbarara for Activity 3.1. In the field the blocks are arranged in a line, and the management and variety treatments are randomly assigned.



The two management treatments were optimal and minimal management. Optimal management comprised the application of mulch (10 cm thickness) and fertilisers (150 kg N, 25 kg P and 200 kg K ha^{-1} yr^{-1}; McIntyre, per. comm.), and routine weeding, pruning and crop sanitation. Minimal management had no mulch or fertiliser, but had 2 episodes of weeding, pruning and crop sanitation during a single crop cycle.

The three banana varieties/ types treatments consisted of 2 varieties; one exotic variety and one local variety. The exotic variety was banana Cavendish “Williams”

(*Musa* AAA) and the local variety was banana Mbwazirume (*Musa* AAA-EA). For the local variety, tissue culture plants were derived from mother plants of two different BSV status, var. Mbwazirume BSV ELISA negative (ELISA -) and var. Mbwazirume BSV ELISA positive (ELISA +). Subplot size was 15 x 18m with border rows of FHIA 01 from conventional suckers. Plant density was 1111 plants ha^{-1} which gave a plant spacing of 3 x 3m.

3.1.2 Data collection and statistical analyses

The following data/ observations are being collected at the two sites:

Weather	rainfall, air temperature, relative humidity, windspeed and solar radiation using meteorological stations. Air and soil temperature and relative humidity in each management treatment using dataloggers.
Soil	Soil gravimetric moisture content, soil N, P, K, Ca, Mg and bulk density
Plant yield	Bunch weight, number of hands per bunch
Plant development	Time of planting, sucker selection, flowering and bunch maturity. Leaf appearance
Plant growth	Light interception, rate of photosynthesis, pseudostem girth at 1m and height at the time of flowering
BSV symptoms	Presence and severity of symptoms (using the severity index of Dahal *et al.,* 1998). Symptom development as area under disease progression curve (AUDPC) derived from severity over time determined using the mid point rule of area estimation (Campbell and Madden, 1990).

Plant measurements were taken on each plant. BSV symptoms were assessed on each leaf of all plants on a monthly basis. Observations will be taken on the mother plants, and most of the first ratoon crop within the timeframe of Project R7529.

Only the major results of these experiments are reported here. By March 2003, the bunches of all mother plants at Mbarara, and the first ratoon plants in Kawanda had been harvested. The first ratoon plants at Mbarara had flowered and data collection is in progress. Hence, the following results are presented here:

Kawanda	Rainfall BSV symptom frequency and severity Bunch yields Pseudostem girth at flowering Duration from planting/sucker selection to flowering and to harvest Leaf photosynthesis
Mbarara	Rainfall BSV symptom frequency and severity Bunch yields Pseudostem girth at flowering Duration from planting/sucker selection to flowering and harvest Leaf photosynthesis

Most of the results presented here were analysed using either ANOVA in Genstat 3.2 or mixed model procedure in SAS (SAS Inst. Inc., 1997) assuming a split plot design with 4 blocks. Each site was analysed separately. Several modifications in analysis were made. For example, since var. Cavendish showed no symptoms during the entire experimental period it was excluded from analyses for BSV effects. In addition, Mbwazirume classed as either ELISA + or ELISA – had plants with and without symptoms and therefore will be considered as repeats of the same variety in some instances.

(i) Symptom expression

Descriptors of symptom expression (incidence and severity) were analysed by Genstat 3.2, in two ways: Observations of the incidence (number of plants showing symptoms) were analysed with var. Mbwazirume classed as either ELISA + or ELISA –, for the mother crop (example given in Table 1).

Table 1. General form of the ANOVA table of the split plot design with var. Mbwazirume classed as either ELISA + or ELISA –.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Block	3			
Management	1			
Main plot residual	3			
Variety	1			
Management x variety	1			
Sub-plot residual	6			
Total	15			

Second, area under disease progression curve, symptom severity index and incidence (monthly and total for plant crop) of Mbwazirume ELISA + and ELISA –, classed as a single plot of the same variety with 40 plants, were analysed using ANOVA for a randomised complete block design (RBCD) in Genstat 3.2 (Table 2).

Table 2. General form of the ANOVA table of the RCBD with Mbwazirume ELISA + or ELISA – classed together .

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Block	3			
Management	1			
plot residual	3			
Total	7			

(ii) Variety and management effects

Results of the variety and management effects were analysed using mixed model procedure in SAS (SAS Inst. Inc., 1997), where variety and management were fixed effects and the interaction between block and management was a random effect. The results presented have 2 varieties, i.e., var. Cavendish and Mbwazirume ELISA + and ELISA – classified together as var. Mbwazirme with the exception of the yield response for Mbarara which was analysed with 3 varieties, var. Cavendish, Mbwazirume ELISA + and ELISA –. The model used was:

$\mathbf{Y_{1,2,3,4,5,6,7}} = \mathbf{m} + \mathbf{block} + \mathbf{mgt} + \mathbf{error_1} + \mathbf{variety} + \mathbf{mgt.variety} + \mathbf{error_2}$ where,

Y is the response variable. $\mathbf{Y_1}$ = height; $\mathbf{Y_2}$ = pseudostem girth; $\mathbf{Y_3}$ = duration to bunch emergence (flowering); $\mathbf{Y_4}$ = duration planting/sucker selection to harvest; $\mathbf{Y_5}$ = duration bunch emergence to harvest; $\mathbf{Y_6}$ = number of hands per bunch and $\mathbf{Y_7}$ = bunch weight. $\mathbf{m}$ is an overall mean effect, **block** =block effect; Crop management (**mgt**) = optimal & minimal management. **Variety** = var. Cavendish, var. Mbwazirume classified as one variety or ELISA + and ELISA –; $\mathbf{error_1}$ refers to the main plot variation and $\mathbf{error_2}$ refers to the split plot variation.

(iii) BSV effects

Due to the presence of symptoms in both Mbwazirume ELISA + and ELISA –, BSV effects were obtained by considering these two as repeats of var. Mbwazirume, ignoring var. Cavendish (which showed no BSV symptoms), and carrying out a split unit analysis with 8 main plots as before, but taking each plant as the split unit, using mixed model procedure in SAS (SAS Inst. Inc., 1997). The symptom status and management were fixed effects and the interaction between block and management was a random effect. The following model was used in the analysis:

$\mathbf{Y_{1,2,3,4,5,6,7}} = \mu + \mathbf{block} + \mathbf{mgt} + \mathbf{error_1} + \mathbf{symp} + \mathbf{mgt.symp} + \mathbf{error_2}$ where,

Y is the response variable. $\mathbf{Y_1}$ = height; $\mathbf{Y_2}$ = pseudostem girth; $\mathbf{Y_3}$ = duration to bunch emergence (flowering); $\mathbf{Y_4}$ = duration planting/sucker selection to harvest; $\mathbf{Y_5}$ = duration bunch emergence to harvest; $\mathbf{Y_6}$ = number of hands per bunch and $\mathbf{Y_7}$ = bunch weight; μ is an overall mean effect, **block** =block effect; Crop management (**mgt**) = optimal & minimal management. Symptom status (**symp**) = with and without symptoms present during the crop cycle. Var. Mbwazirume classified as one variety or ELISA + and ELISA – ; $\mathbf{error_1}$ refers to the main plot variation and $\mathbf{error_2}$ refers to the split plot variation.

Photosynthesis measurements were made under the following controlled conditions:

- 10cm from the outside edge, halfway along the third fully-expanded leaf
- at ambient CO_2 (360-370 ppm CO_2)
- at a cuvette temperature of 27^oC
- at a cuvette relative humidity of 90%

Two parameters of leaf photosynthesis (A) were measured:

Light response curves of leaf photosynthesis (A) - Measurements of *A* were made on 3 replicate leaves of variety Mbwazirume showing BSV, and 3 not showing symptoms, at each of 1500, 1000, 800, 600, 300, 250, and 10 mols m^{-2} s^{-1} photosynthetically active radiation (PAR). A total of 42 measurements at Mbarara and at Kawanda were completed between 8/7/01 and 13/7/01. Rectangular hyperbola, a function commonly used to describe the response of photosynthesis to light, were fitted to these data. It was not intended to provide sufficient replicate leaves to allow a formal statistical comparison of these non-linear functions. Rather, these functions provided an indication of which part of the light response curve may be affected by BSV symptoms for further investigation in the remaining measurements.

Light-saturated rate of photosynthesis (A_{max}) - Measurements of A_{max} were made of 10 replicate leaves of each of 3 varieties (Cavendish Williams, Mbwazirume ELISA +, Mbwazirume ELISA –) under either optimal or minimal crop management conditions showing BSV symptoms, and 10 not showing symptoms. A total of 14 measurements at Kawanda were made between 8/7/01 and 13/7/01, and then 120 measurements at Kawanda between 27/7/01 and 31/7/01. These data were analysed in two ways: first with var. Mbwazirume classed as ELISA + and ELISA - ; and second with var. Mbwazirume classed as with symptoms and no symptoms. Analysis was in accordance with a split-plot design without equal number of plants in each block.

3.1.3 Results and Discussion

BSV symptoms

Not a single var. Cavendish plant showed symptoms of BSV at either site. Thus, there was no evidence of vector transmission of BSV into these two plantations during the mother plant and ratoon crop cycle. Since var. Cavendish showed no symptoms it was excluded from analyses of BSV effects because zero data distorts the normal

distribution and therefore makes analysis invalid. Symptoms of BSV were prevalent in both var. Mbwazirume ELISA - and ELISA + plants at both sites. There were no significant differences (P>0.05) in the number of plants showing symptoms between ELISA + and ELISA - plants in the mother plant crop at Kawanda and Mbarara (Table 3a & 3b). It is concluded that the initial classification of var. Mbwazirume on the basis of the ELISA diagnostic tests is questionable. Therefore, the rest of the

analyses involving var. Mbwazirume reports: (i) combined ELISA + and ELISA – plants as repeats of the same variety; (ii) grouped plants according to whether or not each showed symptoms.

Table 3a. Effect of management and variety on the number of plants with symptoms (per plot of 20 plants) at Kawanda with the varieties classed by ELISA

Number of plants with symptoms [1]	Mbw ELISA +	Mbw ELISA -	Mean Mgt s.e.d. = 0.13 (3 df)
Minimal management	12.75	9.25	***11.00***
Optimal management	9.25	9.00	***9.13***
Mean Var s.e.d = 1.85 (6 df)	***11.00***	***9.13***	

[1] Mgt P<0.001, Var P=0.35, Mgt x Var P=0.41

Table 3b. Effect of management and variety on the number of plants with symptoms (per plot of 20 plants) at Mbarara with the varieties classed by ELISA

Number of plants with symptoms [1]	Mbw ELISA +	Mbw ELISA -	Mean Mgt s.e.d. = 1.18 (3 df)
Minimal management	13.50	14.25	***13.88***
Optimal management	12.50	11.75	***12.13***
Mean Var s.e.d = 1.54 (6df)	***13.13***	***12.00***	

[1] Mgt P=0.23, Var P = 1.00, Mgt x Var P=0.64

In the first ratoon crop, the number of plants with BSV was higher in Mbarara than Kawanda (Table 4), similar to the mother plant crop. There were more plants with BSV in the minimal managed plots compared to the optimal managed plots at both Kawanda ($P< 0.01$) and Mbarara ($P > 0.05$). The presence or absence of BSV in this study was determined on the basis of symptoms and it is probable that optimal management reduced the expression of BSV symptoms

BSV disease development (assessed as area under disease progression curve – AUDPC, for a measure of symptom severity over time) was greater under minimal management compared to optimal management. During, the mother plant crop, BSV disease development was greater in minimally managed plots than in optimally managed plots. This difference was significant at Mbarara ($P < 0.05$) while not significantly different at Kawanda ($P > 0.05$; Table 5a). In the 1st ratoon crop, disease development was greater under minimal management compared to optimal management, but unlike the mother crop, this difference was significant at Kawanda ($p>0.01$), but not significant at Mbarara ($P > 0.05$; Table 5b). During, both crop cycles disease development was greater in Kawanda compared to Mbarara. Also, disease build up was greater in the 1st ratoon compared to the mother plant crop at both sites.

Table 4: Effect of management on the number of plants with symptoms (per plot of 20 plants) for 1st ratoon crop at Kawanda and Mbarara with var. Mbwazirume grouped together.

Number of plants with symptoms	Kawanda[1]	Mbarara[2]
Optimal management	6.12	10.50
Minimal management	11.88	12.25
s.e.d. (3 df)	1.25	1.18
Mean	***9.00***	***11.38***

[1] Mgt P=0.002, [2] Mgt P=0.180

Table 5a: Effect of management area under disease progression curve (AUDPC) for the mother plant crop at Kawanda and Mbarara with var. Mbwazirume grouped together.

Area under disease Progression curve (AUDPC)	Kawanda[1]	Mbarara[2]
Optimal management	2.71	1.27
Minimal management	3.61	2.29
s.e.d. (3 df)	0.71	0.40

Mean	***3.16***	***1.78***

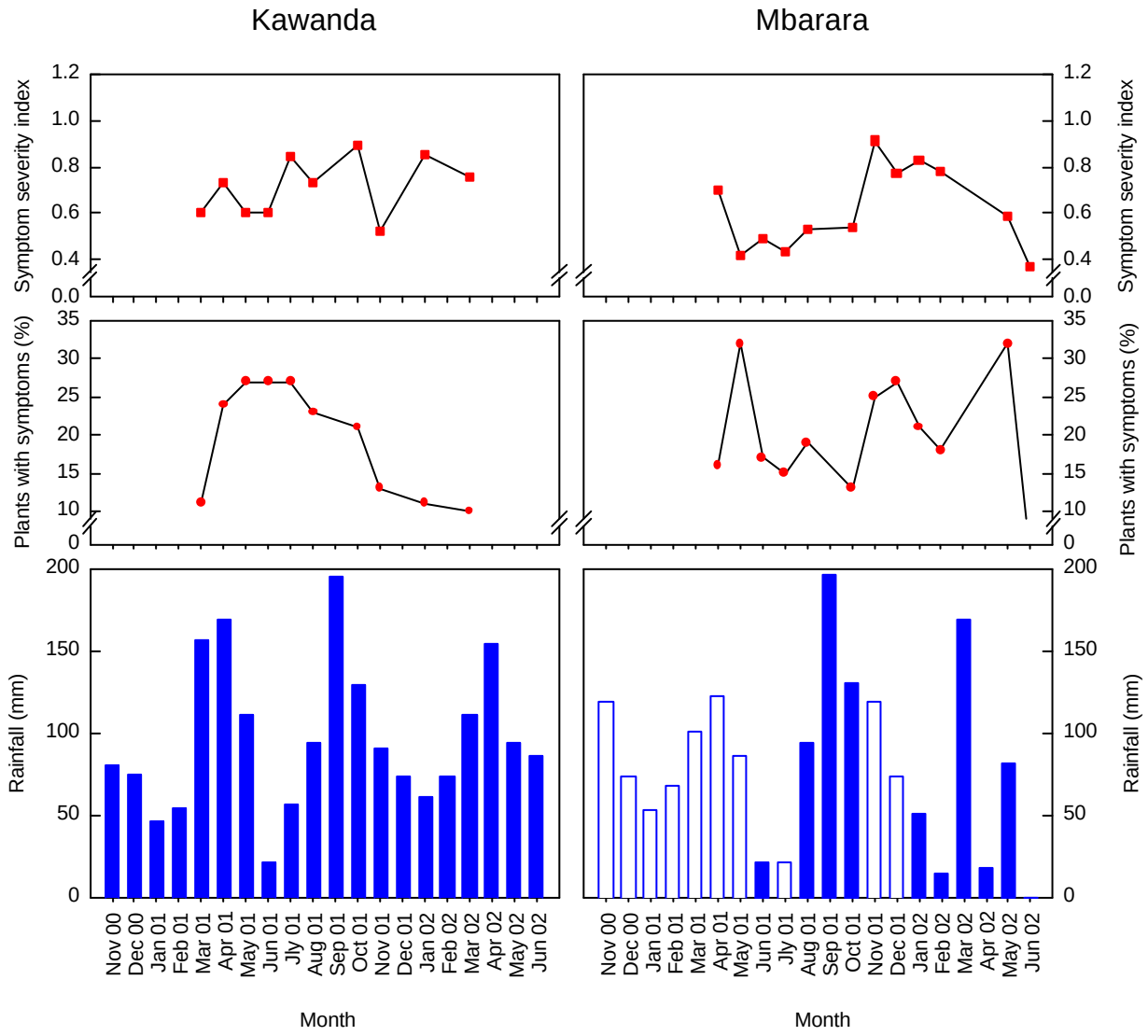
[1] Mgt P=0.257, [2] Mgt P=0.04

Table 5b: Effect of management area under disease progression curve (AUDPC) for the 1st ratoon crop at Kawanda and Mbarara with var. Mbwazirume grouped together.

Area under disease Progression curve (AUDPC)	Kawanda[1]	Mbarara[2]
Optimal management	3.05	3.04
Minimal management	7.19	4.11
s.e.d. (3 df)	1.09	0.87
Mean	***5.12***	***3.58***

[1] Mgt P=0.009, [2] Mgt P=0.257

Figure 2. Seasonal trends in rainfall, BSV symptom frequency and BSV symptom severity (on the mother plants) from planting at Kawanda and Mbarara. The rainfall data for Mbarara are either observations (solid bars) or the climatological monthly mean from the FAO ClimWat database www.fao.org/ag/agl/aglw/climwat.html (open bars).



There were seasonal trends in the incidence and severity of BSV symptoms at Kawanda and Mbarara (Figure 2 & 3). At Kawanda, about 25% of var. Mbwazirume mother plants (5 of the 20 plants per plot) showed BSV symptoms from April to August 2001 (Figure 2). Severe symptoms on plants also occurred during the months of the wet (April-May 2001 and October 2001) and dry (July 2001 and January 2002) seasons. During the 1st ratoon 30% of var. Mbwazirume (6 out of 20 per plot) showed symptoms in May 2002, the highest during the crop cycle. This month is in the first rain season. Severe symptoms were observed towards the end of cycle (October – December 2002). The onset of cool temperatures preceding and during this period could be responsible for enhancing symptom expression in the ratoon crop.

At Mbarara more symptoms were expressed in the wet months. 30% of var. Mbwazirume mother plants (6 of the 20 plants per plot) showed BSV symptoms in May 2001 and May 2002 and 25% of var. Mbwazirume plants showed symptoms in November- December 2001. Severe symptoms occurred in April, November and December 2001 as well as January 2002. During the 1st ratoon plant crop there was no apparent seasonal trend in the symptom severity (Figure 3), but a higher percentage of plants expressed BSV symptoms in May 2002 and again between October 2002 – January 2003. Here, cooler temperature again appears to be associated with the onset of the expression of symptoms.

In this study, therefore, the expression of BSV symptom was greatest during the rainy (wet) and cooler months in the mother and ratoon plant crop, respectively. This is in agreement with reports by Dahal *et al*. (1998) in West Africa where it was observed that BSV expression was more pronounced in the rainy months, and were enhanced by changes in temperature regime. More robust conclusions should emerge with successive ratoon crops as the disease progresses.

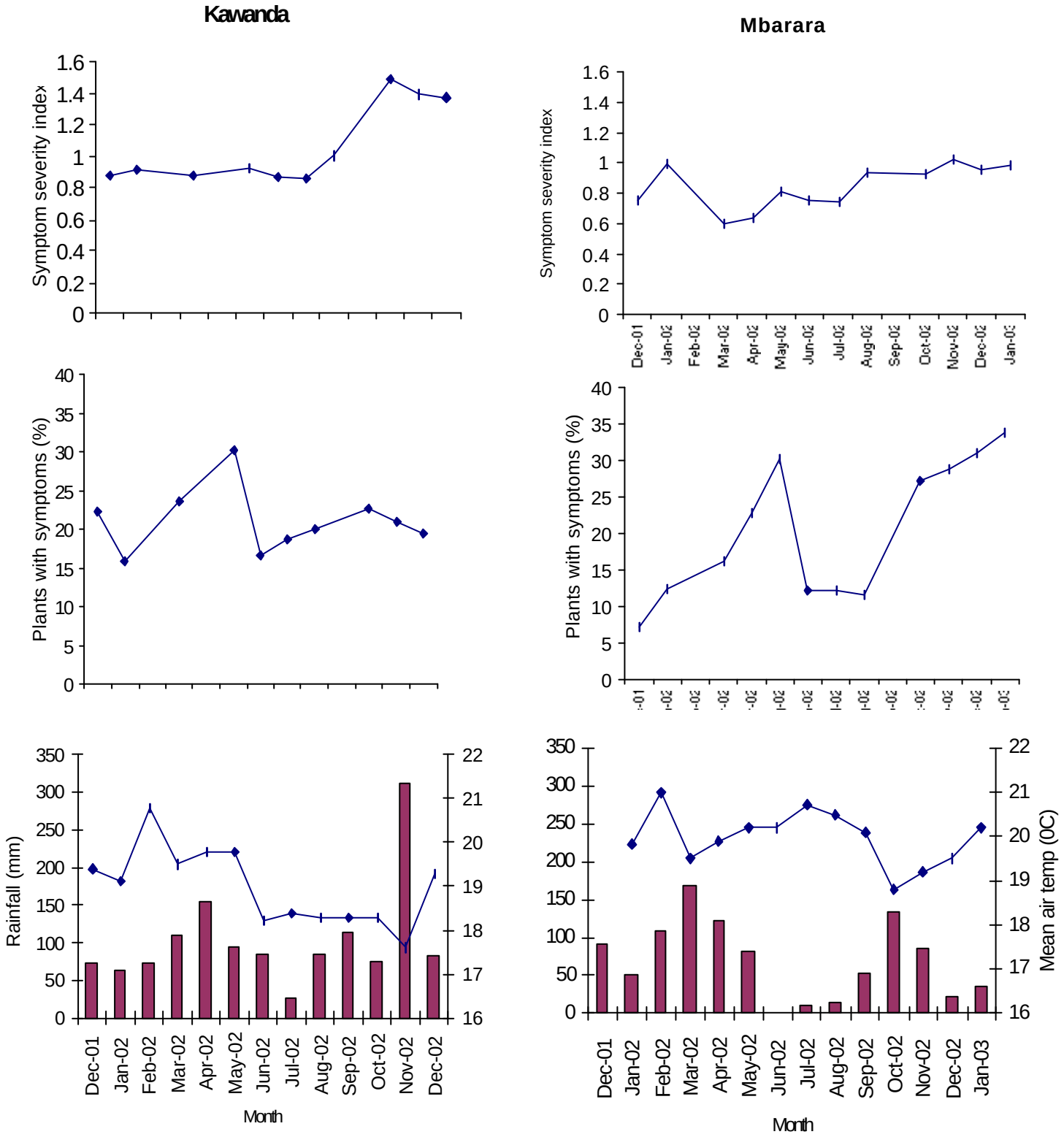


Figure 3. Seasonal trends in rainfall, air temperature, BSV, symptom frequency and BSV symptom severity (1st ratoon pants) from sucker selection at Kawanda and Mbarara. For climate solid bars represent monthly rainfall (mm) and lines represent montly mean air temperature (^{o} C).

Effect of BSV, variety and management on crop yield at Kawanda and Mbarara

At Kawanda, the mean bunch weight of var. Mbwazirume mother plants with BSV symptoms was less (by 18.9%; P<0.001) than those without symptoms. This overall difference was due to the reduction in the number of hands per bunch (P<0.05). BSV did not have a significant effect on the duration of bunch development (bunch emergence to harvest) (P>0.05). These effects of BSV symptoms were not subject to an interaction with crop management (P*>0.05)*. However, comparison of means reveal that BSV symptoms reduced bunch weight by 16.6% under optimal management and 22.8 % under minimal management (Table 6a). Similarly, BSV reduced the number of hands per bunch by 1.6% under optimal management and 6.2% under minimal management.

The effects of BSV during the 1st ratoon crop were similar to those of the mother crop at Kawanda. The mean bunch weight of var. Mbwazirume with BSV symptoms was reduced by 20.3% (P< 0.001) compared with those without symptoms which was due to the reduction in the number of hands (P < 0.001) (Table 6b). Also, BSV symptoms did not significantly affect the duration of bunch development (P > 0.05). The effects of BSV were not subject to an interaction with crop management (P > 0.05), but, BSV symptoms reduced bunch weight by 16.1% under optimal management and 28.9% under minimal management (Table 6b). Further, BSV reduced the number of hands per bunch by 4.5 and 7.6% under optimal and minimal management, respectively.

At Mbarara, mean bunch weight for var. Mbwazirume mother plants with BSV symptoms was 6.7% less but not significantly different (P>0.05) from those without symptoms. BSV also had no effect (P>0.05) on the number of hands per bunch and days from bunch emergence to harvest (Table 6c). Similar to Kawanda, there was no significant interaction effect between BSV and crop management (P>0.05). Comparison of means showed that BSV reduced bunch weight by 1.2% under optimal management and 13.5% under minimal management (Table 6c).

Therefore, we conclude that BSV reduces the yield of bananas grown under Ugandan conditions. It is possible that improved crop management in the mother crop (Kawanda and Mbarara) and 1st ratoon crop cycle (Kawanda) reduced the effects of BSV symptoms. The effects of BSV at Kawanda were greater than those reported by Daniells *et al*. (2001) for crops grown in well-fertilised and irrigated Australia. However, Daniells *et al*.(2001) studied the effects of BSV on var. Cavendish with the BSV-CAV isolate. How BSV-CAV compares to virus isolates in East African Highland bananas is not known at this point.

It is noteworthy that at Kawanda, BSV reduced bunch weight by a similar magnitude under optimal management in the mother and 1st ratoon crop cycles. In minimally managed plots, however, bunch weight was reduced more in the 1st ratoon crop compared to the mother plant crop. Therefore, we suspect that the effects of BSV on crop productivity, and the differences between the two management regimes will increase with subsequent ratoon crops. This is exemplified where bunch weights are plotted against disease development over time (Area under disease progression curve – AUDPC) for the mother and 1st ratoon crop. At Kawanda, bunch weights of diseased plants under optimal management, formed more definite clusters above the

minimally managed plants, in the 1st ratoon (Figure 4c), compared to the mother plant crop (Figure 4a). This is attributed to differences in BSV disease between the two management regimes during the crop cycles. There was only a slight higher disease development, under minimal compared to optimal management in the mother plants (Table 5a) and optimally managed mother plants exhibited the same range of disease progression as those under minimal management (Figure 4a). In contrast, in the 1st ratoon, BSV disease under minimal management was more than double that under optimal management (Table 5b). Additionally, optimally managed diseased ratoon plants exhibited a shorter range of disease development as those under minimal management (Figure 4c).

In this study the effects of BSV at Kawanda were greater than at Mbarara. Disease severity tended to be greater at Kawanda than at Mbarara (Figure 2 & 3). Environmental conditions are thought to affect disease severity (Gauhl and Pasberg-Gauhl, 1995). Kawanda was wetter than Mbarara (Figure 2 & 3). Thus, our results are in accordance with a previous study that showed that BSV symptoms were more pronounced under wetter and cooler conditions as compared to drier ones (Dahal *et al*., 1998).

Optimal crop management increased bunch weight (by 61.7%; $P < 0.001$) and the number of hands per bunch (by 20%; $P< 0.001$) compared to minimal management in the mother crop at Kawanda. Var. Cavendish produced more hands ($P< 0.001$) and had heavier bunches ($P < 0.001$) compared with var. Mbwazirume (Table 7a). Bunches of var. Cavendish took a further 44 days from bunch emergence to harvest ($P< 0.001$) compared to var. Mbwazirume.

In the first ratoon crop, optimal management increased bunch weight (by 105.8%; $P < 0.001$) and number of hands by per bunch (by 33.7%; $P < 0.001$) compared to minimal management at Kawanda. As in the mother crop, var Cavendish ratoon plants produced heavier bunches ($P < 0.001$) and more hands per bunch ($P < 0.001$) compared with var. Mbwazirume (Table 7b). Bunches of var. Cavendish took 53 more days to develop ($P < 0.001$) compared to Mbwazirume. Bunches of the 1st ratoon, particularly in the optimal managed plots, were heavier and had more hands that those in the mother plant crop (Tables 7a & b).

At Mbarara, mean bunch weight of var. Mbwazirume plants in optimal management were not significantly different from those in minimally managed plots ($P>0.05$). However, bunch weights of var. Cavendish and var. Mbwazirume ELISA+ in optimal management were heavier compared to those in minimal managed plots by 38 and 82%, respectively (Table 7c). Plants in optimal managed plots of var. Mbwazirume ELISA- registered a 3 % increase in bunch weight compared to those in minimal managed plots. The number of hands per bunch under optimal management were more (11.2%) compared to minimal management ($P<0.05$). There was a significant interaction effect between management regime and variety for duration from bunch emergence to harvest. This due to a delay in this duration of 14 days ($P<0.01$) for var. Cavendish compared to a 5 day ($P>0.05$) for var. Mbwazirume under minimal compared to optimal management (Table 7d).

Effect of BSV, variety and management on growth characteristics at Kawanda and Mbarara

At Kawanda, BSV reduced the height ($P< 0.001$) and pseudostem girth ($P< 0.001$) of var. Mbwazirume mother crop plants (Table 8a). In addition, BSV increased the duration from planting to flowering ($P< 0.001$) and harvest ($P<0.05$) by 18 and 13 days, respectively. These effects of BSV were not subject to interaction with crop management. Plant height was reduced more, and bunch emergence and maturity were further delayed, by BSV symptoms in minimal compared with optimal management (Table 8a).

In the 1st ratoon crop at Kawanda, BSV reduced plant height ($P < 0.001$), but had no significant effect ($P > 0.05$) on pseudostem girth (Table 8b). BSV increased the duration from sucker selection to flowering ($P < 0.01$) and maturity ($P < 0.001$) by 16 and 14 days, respectively. BSV symptoms were not subject to an interaction with crop management in the 1st ratoon crop, but plant height and pseudostem girth were reduce more, and bunch emergence and maturity delayed further, by BSV symptoms under minimal management compared to optimal management (Table 8b).

At Mbarara, there were no significant differences in height and girth between var. Mbwazirume mother plants with and without BSV symptoms ($P>0.05$) (Table 8c). BSV delayed bunch emergence by 12 days ($P<0.05$). The effects of BSV were not subject to an interaction with management.

In the 1st ratoon plant crop, BSV did not also reduce plant height significantly ($P > 0.05$). However, BSV significantly reduced pseudostem girth ($P < 0.05$) and delayed bunch emergence (by 22 days; $P < 0.001$). BSV symptoms were not subject to an interaction with crop management in the 1st ratoon crop, but plant height and pseudostem girth were reduce more, and bunch emergence and maturity delayed further, by BSV symptoms under minimal management compared to optimal management (Table 8d).

This indicates that improved crop management in both the mother and 1st ratoon plant crops reduced effects of BSV on banana growth and development. These effects of BSV in Uganda are greater than those reported by Dahal *et al*. (2000) whose studies in Western Africa indicated that BSV infection had no significant effect on the plant height and days to bunch emergence in plantains and bananas. It should be noted that the virus isolate (BSV-OL) in studies by Dahal *et al*. (2000), common to Western Africa, has 60% variability to the BSV isolate in Eastern Africa (John Innes Centre report, Unpublished).

Var. Cavendish plants were shorter ($P< 0.001$), and had longer durations from planting to bunch maturity ($P< 0.001$) than var. Mbwazirume in the mother plant crop at Kawanda. Pseudostem girth and duration from planting to flowering for var. Cavendish were not significantly different from those of var. Mbwazirume ($P>0.05$). In general, mother plants under optimal management were taller (by 20.1%; $P< 0.01$), had greater pseudostem girth (by 20.1%; $P< 0.001$), and shorter durations from planting to bunch maturity (by 89days; $P< 0.001$) compared with plants grown under

minimal management (Table 9a). Var. Mbwazirume and var. Cavendish plants reached bunch emergence earlier ($P < 0.001$) by 83 and 104 days, respectively, under optimal management compared to minimal management.

For the 1st ratoon crop, at Kawanda, var. Cavendish were also shorter ($P < 0.001$) and had a longer duration from sucker selection to harvest ($P < 0.001$) than var. Mbwazirume (Table 9b). Pseudostem girth and bunch emergence for var. Cavendish were not significant different from that of var. Mbwazirume ($P > 0.05$). 1st ratoon plants under optimal management were also taller compared to minimal management for var. Cavendish (by 27.5%; $P < 0.001$) and var. Mbwazirume (by 39.1%; $P < 0.001$). In addition, optimally managed plants had bigger girth (by 35.6%; $P < 0.001$) and had early bunch emergence (by 68 days; $P < 0.001$) and harvest (by 36 days; $P < 0.001$) compared to minimally managed plants (Table 9b).

Similar to Kawanda, var. Cavendish plants were shorter ($P< 0.001$) than var. Mbwazirume for the mother plant crop at Mbarara. The duration to bunch emergence was less ($P<0.05$) for Cavendish than for Mbwazirume. Plants grown under optimal management were taller (by 15.5%; $P< 0.05$) and with a bigger girth (by 12.7%; $P< 0.05$) compared to those grown under minimal management (Table 9c). Bananas grown under minimal management took 1 and 8 more days ($P>0.05$) to bunch emergence and harvest, respectively, compared to optimal management.

In the 1st ratoon crop, at Mbarara, var Cavendish plants were shorter ($P < 0.001$), but pseudostem girth did not differ significantly from those of var. Mbwazirume. The duration from sucker selection to bunch emergence for both varieties was not significantly different ($P > 0.05$) when grown under optimal management. However, under minimal management, var. Cavendish plants took 24 days more ($P < 0.05$) to flower than var. Mbwazirume plants. The 1st ratoon plants grown under optimal management were also taller (by 20.6%; $P < 0.01$) and bigger (by 15.2%; $P < 0.05$) compared to minimal management. Bunch emergence was delayed ($P > 0.05$) by 14 and 19 days for var. Cavendish and var. Mbwazirume, respectively, grown under minimal management.

On average, crop management caused greater percentage increases in yield, growth and development components at Kawanda. Optimal management caused a higher percentage increase in plant height and girth at Kawanda compared to Mbarara in the mother and ratoon plant crop. Also, under optimal management, bunch emergence duration was shorter by 94 and 68 days over minimal management, on average, in the mother and 1st ratoon plant crop at Kawanda. In contrast, at Mbarara, the difference between the bunch emergence duration between the two management regimes was 1 and 3 days in the mother and 1st ratoon crop cycles. Similarly, in the mother plant crop, under minimal management, time to harvest was delayed by 89 and 8 days, at Kawanda and Mbarara, respectively. These point to the possibility that apart from BSV, crop management could be a growth limiting factor at Kawanda compared to Mbarara. Still, the yield, plant statue and vigour were better, and bunch emergence and harvest was attained much earlier at Kawanda compared to Mbarara. The fact that Mbarara is drier could be responsible for this pattern. This pattern and its interaction with BSV will become clearer with subsequent crop cycles.

Figure 4a. Bunch weights versus the area under disease progression curve for the parent crop at Kawanda

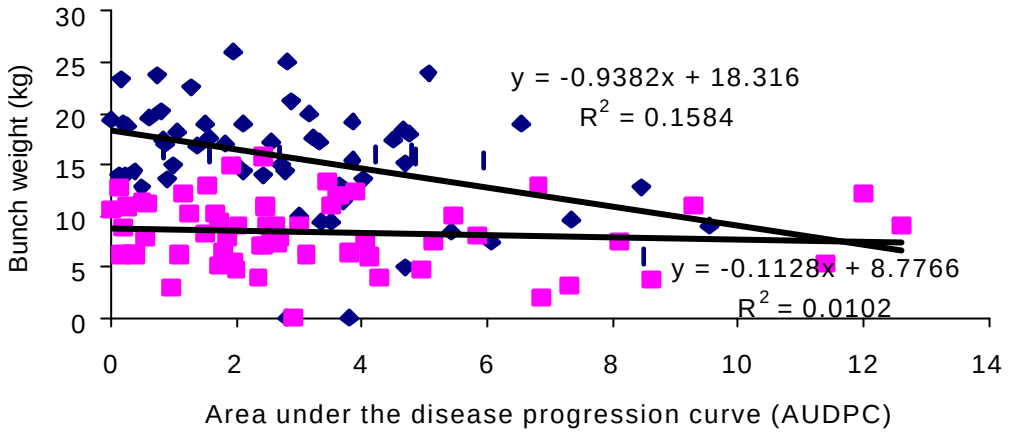


Figure 4b. Bunch weight versus area under disease progression curve (AUDPC) for the mother crop at Mbarara

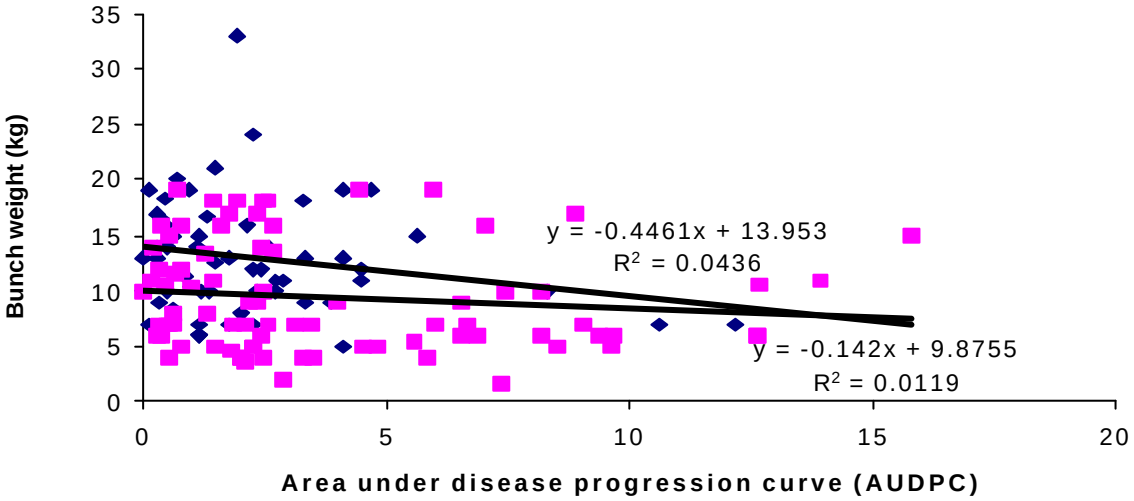


Figure 4c. Bunch weight versus area under disease progression curve for the first ratoon crop at Kawanda

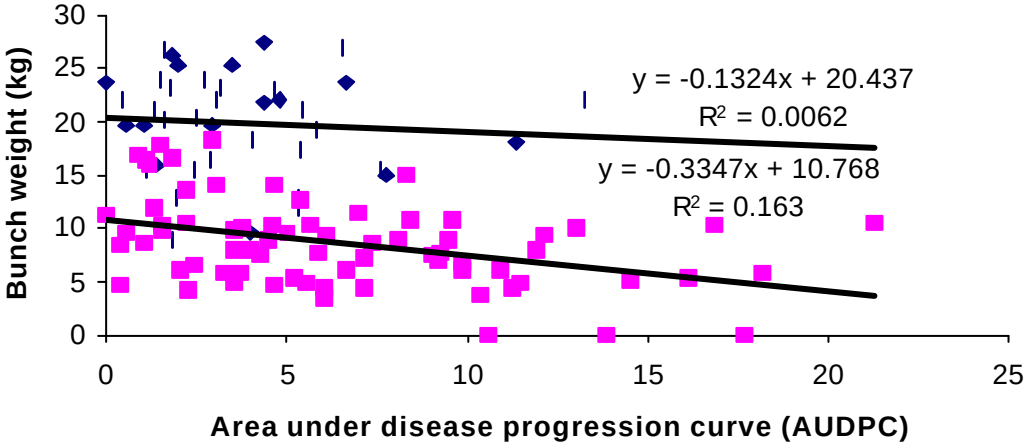


Table 6a: Effect of BSV on yield characteristics (bunch weight, number of hands and the duration from bunch emergence to harvest) of var. Mbwazirume mother plants with and without symptoms at Kawanda

Yield characteristic	**BSV symptoms**	**Management (mgt)**		**Mean symptoms**	**[b] difference in mgt**
		Optimal	**Minimal**		
Bunch weight (kg)[1]					
	without	19.33	11.39	**15.36**	7.94
	with	16.13	8.80	**12.46**	7.33
	Mean mgt	**17.73**	**10.10**		**7.63****
	[a] difference	3.20	2.6	**2.90*****	
Number of hands [2]					
	Without	7.67	6.34	**7.01**	1.33
	With	7.54	5.95	**6.75**	1.59
	Mean mgt	**7.61**	**6.15**		**1.46****
	[a] difference	0.13	0.39	**0.26***	
Days from bunch emergence to harvest [3]					
	Without	96.01	100.90	**98.46**	-4.89
	With	96.14	100.36	**98.25**	-4.22
	Mean mgt	**96.08**	**100.63**		**-4.55** ^{ns}
	[a] difference	-0.13	0.54	**0.21**^{ns}	

[1] Mgt P = 0.01, Symptoms P < 0.001, Mgt x Symptoms P = 0.55 ; [2] Mgt P < 0.01, Symptoms P < 0.05, Mgt x Symptoms P = 0.20;
[3] Mgt P = 0.07, Symptoms P = 0.84, Mgt x Symptoms P = 0.74; [a] difference in symptoms effect means and [b] difference in mgt effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 6b: Effect of BSV on yield characteristics (bunch weight, number of hands and the duration from bunch

emergence to harvest) of var. Mbwazirume 1st ratoon crop plants with and without symptoms at Kawanda

Yield characteristic	BSV symptoms	Management (mgt)		Mean symptoms	[b] difference in mgt
		Optimal	Minimal		
Bunch weight (kg)[1]					
	without	23.71	11.50	**17.60**	12.21
	with	19.89	8.18	**14.03**	11.71
	Mean mgt	**21.80**	**9.84**		**11.96*****
	[a] difference	3.82	3.32	**3.57*****	
Number of hands [2]					
	Without	8.40	6.46	**7.43**	1.94
	With	8.02	5.97	**7.00**	2.16
	Mean mgt	**8.21**	**6.21**		**2.00****
	[a] difference	0.38	0.49	**0.43*****	
Days from bunch emergence to harvest [3]	Without	98.33	96.09	**97.21**	2.25
	With	97.61	95.64	**96.62**	1.97
	Mean mgt	**97.97**	**95.86**		**2.11** *
	[a] difference	0.73	0.45	**0.59**^{ns}	

[1] Mgt P = 0.001, Symptoms P < 0.001, Mgt x Symptoms P = 0.65 ; [2] Mgt P < 0.01, Symptoms P < 0.001, Mgt x Symptoms P = 0.61;
[3] Mgt P = 0.02, Symptoms P = 0.54, Mgt x Symptoms P = 0.88; [a] difference in symptoms effect means and [b] difference in mgt effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 6c: Effect of BSV on yield characteristics (bunch weight, number of hands and duration from bunch emergence to harvest) of var. Mbwazirume mother plants with and without symptoms at Mbarara

Yield characteristic	**BSV symptoms**	**Management (mgt)**		**Mean symptoms**	**[b] difference in mgt**
		Optimal	**Minimal**		
Bunch weight (kg)[1]					
	without	12.99	10.52	**11.75**	2.47
	with	12.83	9.10	**10.96**	3.73
	Mean mgt	**12.91**	**9.81**		**3.09 [ns]**
	[a] difference	0.16	1.42	**0.79[ns]**	
Number of hands [2]					
	Without	7.70	6.86	**7.28**	0.84
	With	7.57	6.80	**7.19**	0.77
	Mean mgt	**7.63**	**6.83**		**0.80 [ns]**
	[a] difference	0.13	0.06	**0.09[ns]**	
Days from bunch emergence to harvest [3]					
	Without	119.38	127.98	**123.68**	-8.60
	With	120.90	124.40	**122.65**	-3.50
	Mean mgt	**120.14**	**126.19**		**-6.05 [*]**
	[a] difference	-1.52	3.58	**1.03[ns]**	

[1] Mgt P = 0.07, Symptoms P = 0.20, Mgt x Symptoms P = 0.31 ; [2] Mgt P = 0.07, Symptoms P = 0.45, Mgt x Symptoms P = 0.76;
[3] Mgt P = 0.05, Symptoms P = 0.66, Mgt x Symptoms P = 0.28; [a] difference in symptoms effect means and [b] difference in mgt effect means, where [ns] = not significant at 5%, [*] significant at < 5%.

Table 7a: Effect of management on yield characteristics (bunch weight, number of hands and bunch emergence to harvest duration) of mother plants of var. Cavendish and Mbwazirume plants at Kawanda

Yield characteristic	**Management (mgt)**	**Variety**		**Mean Mgt**	**[b] difference var.**
		Cavendish	**Mbwazirume**		
Bunch weight (kg)[1]					
	Optimal	21.44	17.97	**19.70**	3.47
	Minimal	14.53	9.83	**12.18**	4.70
	Mean variety	**17.99**	**13.90**		**4.09*****
	[a] difference	6.90	8.14	7.52***	
Number of hands [2]					
	Optimal	9.39	7.62	**8.50**	1.77
	Minimal	8.06	6.00	**7.03**	2.06
	Mean variety	**8.72**	**6.81**		**1.91*****
	[a] difference	1.33	1.62	1.47***	
Days from bunch emergence to harvest [3]					
	Optimal	144.18	96.09	**120.13**	48.09***
	Minimal	140.86	100.99	**120.93**	39.87***
	Mean variety	**142.5**	**98.5**		
	[a] difference	3.32^{ns}	-4.91*		

[1] Mgt P < 0.001, Variety P < 0.001, Mgt x variety P = 0.46 ; [2] Mgt P < 0.001, Variety P < 0.001, Mgt x variety P = 0.38; [3] Mgt P = 0.67, Variety P< 0.001, Mgt x variety P = 0.01; [a] difference in management effect means and [b] difference in variety effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 7b: Effect of management on yield characteristics (bunch weight, number of hands and bunch emergence

to harvest duration) of 1st ratoon plant crop of var. Cavendish and Mbwazirume plants at Kawanda

Yield characteristic	Management (mgt)	Variety		Mean Mgt	[b] difference var.
		Cavendish	Mbwazirume		
Bunch weight (kg)[1]					
	Optimal	31.41	22.52	**26.97**	8.89
	Minimal	16.62	9.58	**13.10**	7.04
	Mean variety	**24.02**	**16.05**		**7.97*****
	[a] difference	14.79	12.94	13.87***	
Number of hands [2]					
	Optimal	10.74	8.29	**9.52**	2.45
	Minimal	8.05	6.18	**7.12**	1.87
	Mean variety	**9.39**	**7.24**		**2.15*****
	[a] difference	2.69	2.11	2.40***	
Days from bunch emergence to harvest [3]					
	Optimal	152.92	98.13	**125.52**	54.79
	Minimal	146.95	95.79	**121.37**	51.15
	Mean variety	**149.93**	**96.96**		**52.97*****
	[a] difference	5.97	2.34	4.15 *	

[1] Mgt P < 0.001, Variety P < 0.001, Mgt x variety P = 0.37 ; [2] Mgt P < 0.001, Variety P < 0.001, Mgt x variety P = 0.17; [3] Mgt P = 0.02, Variety P< 0.001, Mgt x variety P = 0.26; [a] difference in management effect means and [b] difference in variety effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 7c: Effect of management on yield characteristics (bunch weight, number of hands and bunch emergence to harvest duration) of mother plants of var. Cavendish and Mbwazirume plants at Mbarara.

Bunch weight (kg) [1]	**Management (mgt)**	**Variety**			
		Cavendish	**Mbwazirume ELISA-**	**Mbwazirume ELISA+**	**Mean Mgt**
	Optimal	19.21	11.31	14.76	**15.09**
	Minimal	13.89	10.98	8.10	**10.99**
	Mean variety	**16.55**	**11.15**	11.43	
	[a] difference	5.33	0.33	6.66	4.11^{ns}

[1] Mgt P < 0.10, Variety P < 0.01, Mgt x variety P = 0.11 ; [a] difference in management effect means , where ^{ns} = not significant at 5%, * significant at < 5%.

Table 7d: Effect of management on yield characteristics (bunch weight, number of hands and bunch emergence to harvest duration) of mother plants of var. Cavendish and Mbwazirume plants at Mbarara.

Yield characteristic	**Management (mgt)**	**Variety**		**Mean Mgt**	[b] **difference var.**
		Cavendish	**Mbwazirume**		
Number of hands [1]					
	Optimal	9.42	7.64	**8.53**	1.78
	Minimal	8.50	6.84	**7.67**	1.66
	Mean variety	**8.96**	**7.24**		**1.72^{**}**
	[a] difference	0.92^{*}	0.80^{**}	0.86^{**}	
Days bunch emergence to harvest [2]					
	Optimal	164.85	120.26	**142.56**	44.59^{***}
	Minimal	150.50	125.43	**137.97**	25.07^{***}
	Mean variety	**157.68**	**122.85**		
	[a] difference	14.35^{**}	-5.17^{ns}		

[1] Mgt P < 0.001, Variety P < 0.001, Mgt x variety P = 0.78 ; [2] Mgt P = 0.11, Variety P < 0.001, Mgt x variety P < 0.01; [a] difference in management effect means and [b] difference in variety effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 8a: Effect of BSV on growth characteristics (height, girth and duration from planting to bunch emergence) of var. Mbwazirume mother plants with and without symptoms at Kawanda.

Growth characteristic	**BSV symptoms**	**Management (mgt)**		**Mean symptoms**	**[b] difference in mgt**
		Optimal	**Minimal**		
Height (cm)[1]					
	Without	297.32	247.03	**272.18**	50.29
	With	291.18	233.34	**262.26**	57.84
	Mean mgt	**294.25**	**240.19**		**54.06****
	[a] difference	6.14	13.69	**9.92*****	
Girth (cm)[2]					
	Without	61.97	50.53	**56.25**	11.44
	With	59.30	48.28	**53.79**	11.02
	Mean mgt	**60.64**	**49.41**		**11.23****
	[a] difference	2.67	2.24	**2.46*****	
Days from planting to bunch emergence [3]	Without	371.41	448.13	**409.77**	-76.72
	With	384.43	470.65	**427.54**	-86.22
	Mean mgt	**377.92**	**459.39**		**-81.47** ***
	[a] difference	-13.02	-22.51	**-17.77*****	
Days from planting to harvest[4]					
	Without	467.35	548.75	**508.05**	-81.40
	With	476.76	565.42	**521.09**	-88.66
	Mean mgt	**472.05**	**557.09**		**-85.04*****
	[a] difference	-9.42	-16.67	**-13.04***	

[1] Mgt P < 0.01, Symptoms P < 0.001, Mgt x Symptoms P = 0.10 ; [2] Mgt P < 0.01, Symptoms P < 0.001, Mgt x Symptoms P = 0.75; [3] Mgt P < 0.001, Symptoms P < 0.001, Mgt x Symptoms P = 0.31; [4] Mgt P < 0.001, Symptoms P < 0.05, Mgt x Symptoms P = 0.47; [a] difference in symptoms effect means and [b] difference in mgt effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 8b: Effect of BSV on growth characteristics (height, girth and duration from planting to bunch emergence) of var. Mbwazirume 1st ratoon plants with and without symptoms at Kawanda.

Growth characteristic	**BSV symptoms**	**Management (mgt)**		**Mean symptoms**	**[b] difference in mgt**
		Optimal	**Minimal**		
Height (cm)[1]					
	Without	343.09	251.97	**297.53**	91.12
	With	331.09	238.07	**284.58**	93.02
	Mean mgt	**337.09**	**245.02**		**92.07*****
	[a] difference	12.00	13.90	**12.95*****	
Girth (cm)[2]					
	Without	70.95	59.24	**65.09**	11.71
	With	68.58	48.94	**58.76**	19.64
	Mean mgt	**69.76**	**54.09**		**15.67****
	[a] difference	2.37	10.30	**6.33** ^{ns}	
Days from sucker selection to bunch emergence [3]	Without	272.15	319.31	**295.73**	-47.16
	With	283.69	340.48	**312.09**	-56.79
	Mean mgt	**277.92**	**329.90**		**-51.98** ***
	[a] difference	-11.54	-21.17	**-16.36** **	
Days from sucker selection to harvest [4]					
	Without	370.47	405.96	**388.21**	-35.49
	With	378.40	415.97	**402.19**	-47.59
	Mean mgt	**374.43**	**415.97**		**-41.56** **
	[a] difference	-7.93	-20.03	**-13.98** **	

[1] Mgt P < 0.001, Symptoms P < 0.001, Mgt x Symptoms P = 0.76; [2] Mgt P < 0.001, Symptoms P < 0.06, Mgt x Symptoms P = 0.23;
[3] Mgt P < 0.01, Symptoms P = 0.001, Mgt x Symptoms P = 0.33; [4] Mgt P < 0.01, Symptoms P < 0.001, Mgt x Symptoms P = 0.14;
[a] difference in symptoms effect means and [b] difference in mgt effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 8c: Effect of BSV on growth characteristics (height, girth and duration from planting to bunch emergence) of var. Mbwazirume mother plants with and without symptoms at Mbarara.

Growth characteristic	**BSV symptoms**	**Management (mgt)**		**Mean symptoms**	**[b] difference in mgt**
		Optimal	**Minimal**		
Height (cm)[1]					
	Without	292.75	245.79	**269.27**	46.96
	With	285.54	241.92	**263.73**	43.62
	Mean mgt	**289.14**	**243.85**		**45.29****
	[a] difference	7.20	3.87	**5.54**^{ns}	
Girth (cm)[2]					
	without	52.63	45.97	**49.30**	6.66
	with	52.12	46.02	**49.07**	6.10
	Mean mgt	**52.38**	**46.00**		**6.38** ^{ns}
	[a] difference	0.51	-0.05	**0.23** ^{ns}	
Days planting to bunch emergence [3]	Without	500.14	487.22	**493.68**	12.92
	With	509.95	500.92	**505.44**	9.03
	Mean mgt	**505.05**	**494.07**		**10.98** ^{ns}
	[a] difference	-9.81	-13.69	**-11.75***	
Days planting to harvest [4]					
	Without	625.05	613.87	**619.46**	11.18
	With	633.20	627.14	**630.17**	6.06
	Mean mgt	**629.13**	**620.51**		**8.62** ^{ns}
	[a] difference	-8.15	-13.26	**-10.71** ^{ns}	

[1] Mgt P < 0.01, Symptoms P < 0.05, Mgt x Symptoms P = 0.55 ; [2] Mgt P = 0.07, Symptoms P = 0.76, Mgt x Symptoms P = 0.71; [3] Mgt P = 0.26, Symptoms P < 0.05, Mgt x Symptoms P = 0.72; [4] Mgt P = 0.48, Symptoms P = 0.09, Mgt x Symptoms P = 0.68; [a] difference in symptoms effect means and [b] difference in mgt effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 8d: Effect of BSV on growth characteristics (height, girth and duration from planting to bunch emergence) of var. Mbwazirume 1st ratoon plants with and without symptoms at Mbarara.

Growth characteristic	**BSV symptoms**	**Management (mgt)**		**Mean symptoms**	**[b] difference in mgt**
		Optimal	**Minimal**		
Height (cm)[1]					
	Without	302.61	259.71	**281.16**	42.90
	With	301.67	252.29	**276.98**	43.38
	Mean mgt	**302.14**	**256.00**		**46.14***
	[a] difference	0.94	7.42	**4.18ns**	
Girth (cm)[2]					
	without	55.32	50.77	**53.05**	4.55
	with	54.34	49.28	**51.81**	5.06
	Mean mgt	**54.83**	**50.02**		**4.81 ns**
	[a] difference	0.98	1.49	**1.24***	
Days sucker selection to bunch emergence [3]	Without	376.26	354.32	**365.29**	12.92
	With	396.00	377.60	**386.80**	9.03
	Mean mgt	**386.13**	**365.96**		**10.98 ns**
	[a] difference	-19.74	-23.28	**-21.51*****	

[1] Mgt P < 0.02, Symptoms P = 0.18, Mgt x Symptoms P = 0.31 ; [2] Mgt P = 0.06, Symptoms P < 0.05, Mgt x Symptoms P = 0.68; [3] Mgt P = 0.001, Symptoms P < 0.001, Mgt x Symptoms P = 0.77; [a] difference in symptoms effect means and [b] difference in mgt effect means, where **ns** = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 9a: Effect of management on growth characteristics (height, girth and duration from planting to bunch emergence) of mother plants of var. Cavendish and Mbwazirume plants at Kawanda.

Growth characteristic	**Management (mgt)**	**Variety**			**[b] difference variety**
		Cavendish	**Mbwazirume**	**Mean Mgt**	
Height (cm)[1]					
	Optimal	226.21	294.68	**260.44**	-68.47
	Minimal	193.85	239.96	**216.91**	-46.11
	Mean variety	**210.03**	**267.32**		**57.29*****
	[a] difference	32.35	54.72	**43.64****	
Girth (cm)[2]					
	Optimal	58.81	60.79	**59.80**	-1.98
	Minimal	50.25	49.35	**49.80**	0.90
	Mean variety	**54.53**	**55.07**		**0.54**^{ns}
	[a] difference	8.56	11.44	**10.00*****	
Days from planting to bunch emergence [3]					
	Optimal	375.92	337.26	**376.59**	1.34^{ns}
	Minimal	479.73	460.11	**469.92**	19.62*
	Mean variety	**427.82**	**418.68**		
	[a] difference	-103.81***	-82.86***		
Days from planting to harvest [4]					
	Optimal	515.09	471.70	**493.40**	43.39
	Minimal	603.72	561.68	**582.70**	42.04
	Mean variety	**559.41**	**516.69**		**42.72*****
	[a] difference	-88.63	-89.97	**-89.30*****	

[1] Mgt P < 0.01, Variety P < 0.001, Mgt x variety P = 0.08 ; [2] Mgt P < 0.001, Variety P =0.64, Mgt x variety P = 0.22; [3] Mgt P < 0.001, Variety P =0.07, Mgt x variety P = 0.04; [4] Mgt P < 0.001, Variety P < 0.001, Mgt x Variety P = 0.91; [a] difference in management effect means and [b] difference in variety effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 9b: Effect of management on growth characteristics (height, girth and duration from planting to bunch emergence) of 1st ratoon plants of var. Cavendish and Mbwazirume plants at Kawanda.

Growth characteristic	**Management (mgt)**	**Variety**			**[b] difference variety**
		Cavendish	**Mbwazirume**	**Mean Mgt**	
Height (cm)[1]					
	Optimal	258.64	339.48	**299.06**	-80.84***
	Minimal	202.89	244.03	**223.46**	-41.14***
	Mean variety	**230.76**	**291.76**		
	[a] difference	55.75***	95.45***		
Girth (cm)[2]					
	Optimal	68.12	70.22	**69.17**	-2.10
	Minimal	49.16	52.85	**51.01**	-18.96
	Mean variety	**58.64**	**61.54**		**-2.90[ns]**
	[a] difference	18.96	17.37	**18.16*****	
Days from sucker selection to bunch emergence [3]					
	Optimal	262.02	275.63	**268.82**	-13.61
	Minimal	341.50	331.60	**336.55**	9.89
	Mean variety	**301.76**	**303.62**		**-1.86[ns]**
	[a] difference	-79.48	-55.97	**-67.73*****	
Days from sucker selection to harvest (days) [4]					
	Optimal	412.61	372.90	**392.76**	39.71
	Minimal	441.21	417.26	**429.23**	23.95
	Mean variety	**426.91**	**395.08**		**31.83*****
	[a] difference	-28.60	-44.35	**-36.47*****	

[1] Mgt P < 0.001, Variety P < 0.001, Mgt x variety P = 0.01 ; [2] Mgt P < 0.001, Variety P =0.35, Mgt x variety P = 0.80; [3] Mgt P < 0.001, Variety P > 0.05, Mgt x variety P > 0.05; [4] Mgt P < 0.001, Variety P < 0.001, Mgt x Variety P = 0.31; [a] difference in management effect means and [b] difference in variety effect means, where [ns] = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 9c: Effect of management on growth characteristics (height, girth and duration from planting to bunch emergence) of mother plants of var. Cavendish and Mbwazirume plants in Mbarara.

Growth characteristic	**Management (mgt)**	**Variety**			
		Cavendish	**Mbwazirume**	**Mean Mgt**	**[b] difference variety**
Height (cm)[1]					
	Optimal	213.06	288.54	**250.80**	-75.48
	Minimal	190.82	243.45	**217.14**	-52.63
	Mean variety	**201.94**	**266.00**		**-64.06*****
	[a] difference	22.24	45.09	**33.66***	
Girth (cm)[2]					
	Optimal	54.20	52.36	**53.28**	1.84
	Minimal	48.44	46.10	**47.27**	2.34
	Mean variety	**51.32**	**49.23**		**2.09**^{ns}
	[a] difference	5.76	6.26	**6.01***	
Days from planting to bunch emergence [3]					
	Optimal	473.16	505.87	**489.52**	-32.71
	Minimal	480.06	496.44	**488.25**	-16.38
	Mean variety	**476.61**	**501.16**		**-24.55***
	[a] difference	-6.89	9.43	**1.27**^{ns}	
Days from planting to harvest [4]					
	Optimal	633.75	629.50	**631.62**	8.25
	Minimal	625.51	621.90	**623.71**	3.61
	Mean variety	**629.63**	**625.70**		3.93^{ns}
	[a] difference	8.24	7.60	7.92^{ns}	

[1] Mgt P < 0.05, Variety P < 0.001, Mgt x variety P = 0.07; [2] Mgt P < 0.05, Variety P =0.11, Mgt x variety P = 0.85; [3] Mgt P = 0.91, Variety P <0.05, Mgt x variety P = 0.46; [4] Mgt P=0.46, Variety P = 0.71, Mgt x Variety P = 0.98; [a] difference in management effect means and [b] difference in variety effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Table 9d: Effect of management on growth characteristics (height, girth and duration from planting to bunch emergence) of 1st ratoon plants of var. Cavendish and Mbwazirume plants in Mbarara.

Growth characteristic	Management (mgt)	Variety			[b] difference
		Cavendish	Mbwazirume	Mean Mgt	variety
Height (cm)[1]					
	Optimal	227.87	301.98	**264.93**	-74.11
	Minimal	184.00	255.39	**219.69**	-71.40
	Mean variety	**205.94**	**278.69**		**-72.75*****
	[a] difference	43.87	46.59	**45.24****	
Girth (cm)[2]					
	Optimal	56.16	54.75	**55.46**	1.41
	Minimal	46.40	49.89	**48.14**	-3.49
	Mean variety	**51.28**	**52.32**		**-1.04**^{ns}
	[a] difference	9.76	4.86	**7.32***	
Days from sucker selection to bunch emergence [3]	Optimal	378.77	387.47	**383.12**	-8.70^{ns}
	Minimal	392.42	368.56	**380.49**	23.86*
	Mean variety	**385.59**	**378.01**		
	[a] difference	-13.65^{ns}	18.91^{ns}		

[1] Mgt P < 0.01, Variety P < 0.001, Mgt x variety P = 0.84; [2] Mgt P < 0.05, Variety P =0.48, Mgt x variety P = 0.10; [3] Mgt P = 0.79, Variety P = 0.28, Mgt x variety P < 0.05; [4] Mgt P=0.46, Variety P = 0.71, Mgt x Variety P = 0.98; [a] difference in management effect means and [b] difference in variety effect means, where ^{ns} = not significant at 5%, * significant at < 5%, ** = at < 1% and *** = at < 0.1%

Leaf photosynthesis

The light response curves of leaf photosynthesis (A) increased with an increase in PAR, until near a maximum at 1500 mols m^{-2} s^{-1} (Figure 3). Rectangular hyperbola adequately described this response. The values of Q_E (the initial slope) of these functions was similar at the two sites, but A_{max} (the light saturated rate of photosynthesis) was greatest for leaves without symptoms, at both Kawanda and Mbarara. For example, photosynthetic rate at > 800 mols m^{-2} s^{-1} was visibly greater for leaves without BSV symptoms than for those that had BSV symptoms. Therefore, it appears that A_{max} was reduced in leaves with visible symptoms of BSV. Subsequent efforts were therefore concentrated on testing the effects of interactions among variety, management and symptoms on A_{max}.

Figure 3. Light response curves for (a) Kawanda and (b) Mbarara. Solid symbols are the average of plants without BSV symptoms and open symbols represent the average of plants showing BSV symptoms. The fitted equation was $A = A_{max} (1-\exp(-Q_E PAR/A_{max})$. The parameter estimates are given in Table 10.

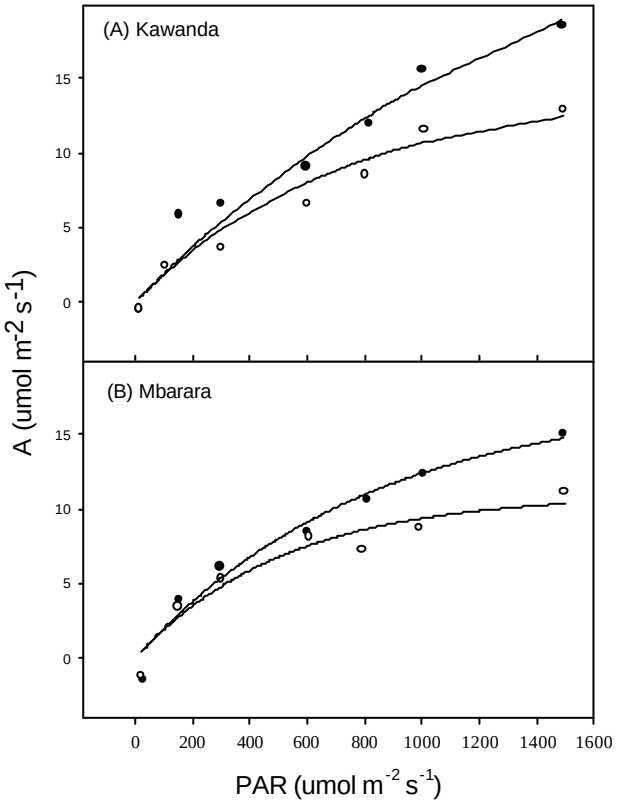


Table 10. Parameter estimates and standard errors (s.e.) for the equation fitted to the light response curves shown in Figure 3.

Location	BSV Symptoms	Amax	s.e	Q_E*	s.e
Kawanda	Present	30.75	8.90	0.020	0.003
	Absent	14.25	2.09	0.020	0.003
Mbarara	Present	17.62	2.91	0.021	0.005
	Absent	10.97	1.51	0.021	0.003

*Q_E is assumed to be same for plants with and without BSV symptoms

Light-saturated rate of photosynthesis (A_{max})

From the first series of measurements of light-saturated rate of photosynthesis (A_{max}) only 14 observations were made at Kawanda before the equipment broke. Whether or not var. Mbwazirume was classed according to ELISA or visible symptoms, leaf photosynthesis tended to be lowest for leaves with symptoms compared with those without (Table 11 and 12). The differences in A_{max} due to variety or symptoms were not significant (P>0.05) with var. Mbwazirume classed on the basis of ELISA, but the effect of symptoms in var. Mbwazirume alone was on the margins of significance (p = 0.056 for t-test). Nevertheless, no firm conclusions can be drawn with so few replicates.

Table 11. Rate of photosynthesis of Mbwazirume (Mbw) classed according to ELISA and Cavendish Williams (Cav) varieties receiving 1000 µmol m^{-2} s^{-1} PAR for plants with and without BSV symptoms.

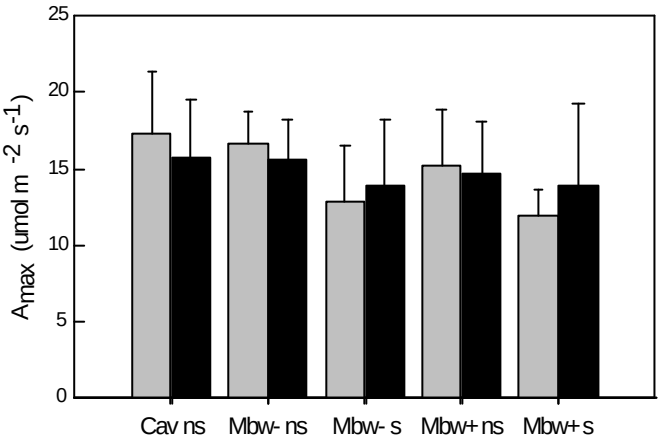
Variety	ELISA	Symptoms	Number of plants	Mean (A)	s.d. (A)
Cav		No	5	22.7	4.08
Mbw	-	No	2	23.9	4.38
Mbw	-	Yes	2	17.7	3.75
Mbw	+	No	3	21.0	4.87
Mbw	+	yes	2	15.7	1.98

Table 12. Rate of photosynthesis of Mbwazirume (Mbw) classed according to visible symptoms and Cavendish Williams (Cav) varieties receiving 1000 µmol m^{-2} s^{-1} PAR.

Variety	ELISA	Symptoms	Number of plants	Mean (A)	s.d. (A)
Cav		No	5	22.7	4.08
Mbw	Both	No	5	22.2	4.38
Mbw	Both	Yes	4	16.7	2.69

A full series of measurements at Kawanda was made on the second occasion. The trends in leaf photosynthesis with this complete dataset (Figure 4) were the same as previously (Table 11 and 12). The rate of leaf photosynthesis was greater (P=0.002) for leaves without BSV symptoms compared with those with. On average, A_{max} was 13.2 and 15.9 mols m^{-2} s^{-1} for leaves with and without symptoms, respectively. Photosynthetic rate also differed among varieties (P=0.022). Values of A_{max} for Cavendish were greater than Mbwazirume ELISA - , but Mbwazirume ELISA - and Mbwazirume ELISA + were the same. The effects of management, and all interactions among management, variety and symptom did not affect the rate of photosynthesis.

Figure 4. Photosynthetic rate (A_{max}) of var. Mbwazirume ELISA + (Mbw+), ELISA - (Mbw-) and var. Cavendish (Cav) receiving 1000 µmol m^{-2} s^{-1} PAR with (s) and



without (ns) BSV symptoms grown under optimal (solid bars) or minimal (grey bars) management.

The rate of photosynthesis was a negative linear function of the percentage visible symptoms on a leaf disc taken from where photosynthesis was measured (r^2=0.160, 38 df, Figure 5). There was no consistent effect of management or variety on this relationship. The rate of photosynthesis was a weak positive linear function of the percentage of leaf nitrogen in each leaf disc (r^2=0.079, 38 df). Again, there was no consistent effect of management and variety on this relationship (Figure 6). Further analyses confirmed that % symptoms were inversely related to % leaf nitrogen as implied by the relationships shown in Figure 5 and 6 (Figure 7).

Figure 5. Relationship between rate of photosynthesis (A_{max}) and visible BSV symptoms. Symbols are Mbwazirume ELISA + (solid), ELISA - (open) under high (circles) and low (solid) management.

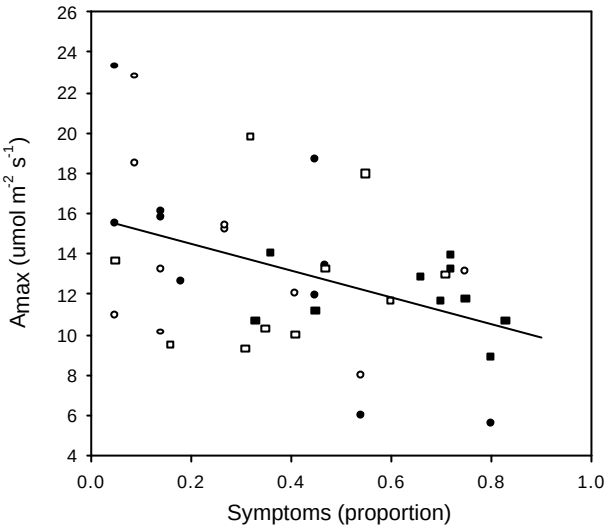
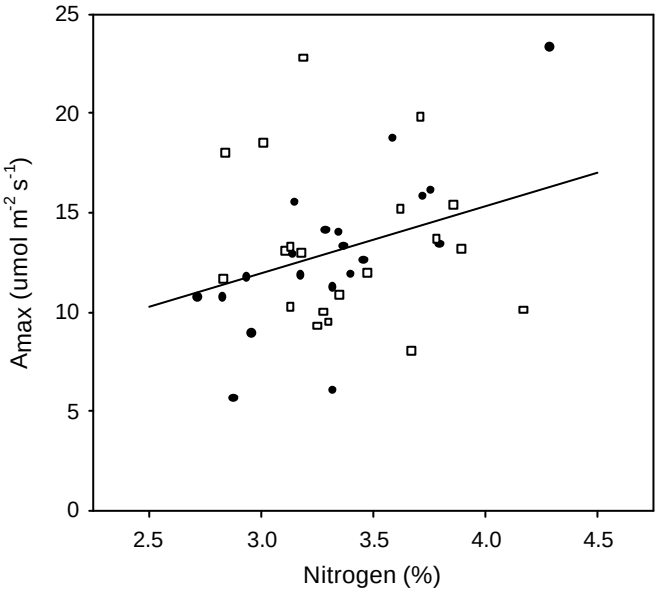


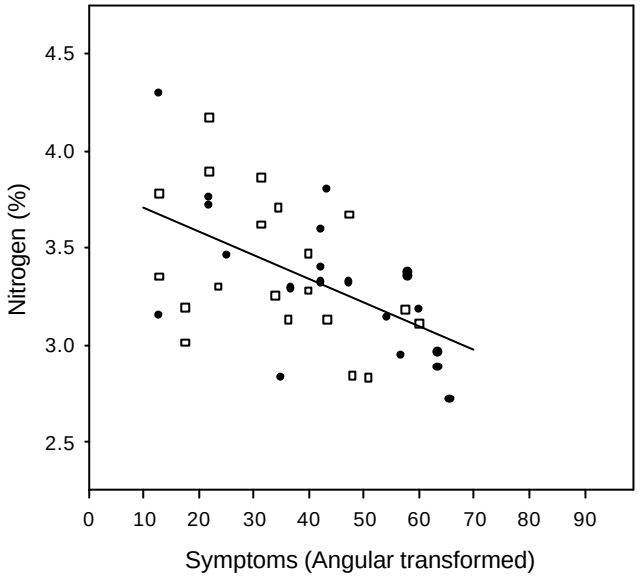
Figure 6. Relationship between rate of photosynthesis (A_{max}) and leaf N content as a percentage of leaf mass. Symbols are Mbwazirume ELISA + (●), ELISA -. (□).



Therefore, at both Kawanda and Mbarara, the rate of photosynthesis was greater in leaves without visible BSV symptoms compared to those with symptoms, and this difference increased with light intensity. Therefore, the light saturated rate of leaf photosynthesis (A_{max}) was reduced by BSV symptoms, but quantum efficiency of photosynthesis (Q_E) was not. No interactions with crop management were observed. Perhaps any effects of crop management will become detectable in the first ratoon

crop cycle and beyond. It is well known that A_{max} is a reflection of the amount of RuBISCO enzyme (the primary receptor for CO_2 in the C3 photosynthesis pathway) in the leaf, and that the largest pool of N in the leaf is in the form of RuBISCO. Thus, we conclude that BSV symptoms reduce the amount of functional RuBISCO in the leaf. This decreases the light-saturated rate of photosynthesis, and hence the potential for growth of these infected banana plants is reduced.

Figure 7. Relationship between leaf N content as percentage of leaf mass and severity of BSV symptoms on the leaf. Symbols are Mbwazirume ELISA + (●), ELISA - (□).



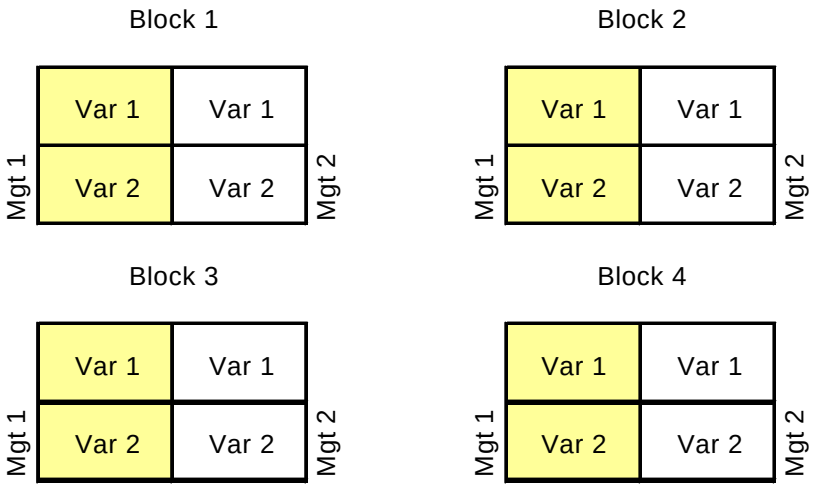
<u>Activity 3.2</u> ***Experiments to elucidate the interactions between BSV, climate and management on crop productivity using modern physiological techniques to quantify resource use by the crops.***

The experiment for this activity is being conducted at Kawanda Research Institute (0.42N 32.5E). The experiment was planted in October 2001.

3.2.1 Experimental design and treatment structure

The design of this experiment was similar to those of Activity 3.1. There was a split-plot design with 4 replicate blocks (Figure 8). Each block consisted of main plots of a management treatment (Mgt in Figure 8) and subplots of two banana varieties (Var in Figure 8). The management treatment levels were randomly assigned in each block. Each of the management plots was then sub-divided into subplots to which each of the banana varieties were randomly assigned. Each subplot consisted of 20 plants.

Figure 8. Field plan of experiments at Kawanda for Activity 3. In the field the blocks are arranged in a line, and the management and variety treatments are randomly assigned.



The two management treatments were the same as in Activity 3.1; optimal and minimal management. The two banana varieties/ types treatment were banana Cavendish "Williams" (*Musa* AAA) and banana Mbwazirume (*Musa* AAA-EA) ELISA negative. Subplot size was 15 x 18m with border rows of FHIA 01 from conventional suckers. Plant density was 1111 plants ha^{-1} which gave a plant spacing of 3 x 3m.

3.2.2 Data collection and statistical analyses

The following data/ observations are being collected:

Weather	Rainfall, air temperature, relative humidity, windspeed and solar radiation using meteorological stations. Air and soil temperature and relative humidity in each management treatment using dataloggers.
Soil	Soil gravimetric moisture content, soil N, P, K, Ca, Mg and bulk density
Plant yield	Bunch weight, number of hands per bunch
Plant development	Time of flowering and bunch maturity. Leaf appearance
Plant growth	Light interception, pseudostem girth at 1m, height from base of pseudostem to proximal end of inflorescence at the time of flowering, biomass in sequential harvests

BSV symptoms	Presence and severity of symptoms (using the severity index of Dahal *et al.* 1998)

Plant measurements were taken on each plant. BSV symptoms were assessed on each leaf of all plants on a monthly basis. Observations were taken on the mother plants, and most of the first ratoon crop within the timeframe of Project R7529.

Destructive sampling was done for two plants (one with symptoms and one without) randomly selected in each sub-plot shown in Figure 8. Each plant was separated into leaves, pseudostem, and fruit, then weighed fresh, and then dried in an oven at 70^{o}C for 48 hours to provide the dry weight.

By July 2002 the bunches of all mother plants had been harvested.
The following results are presented here:

Kawanda	Light interception by the leaf canopy
	Biomass accumulation over time
	Bunch yield

Results were analysed with a split-split unit analysis using mixed model approach in SAS (SAS Inst. Inc., 1997). Observations made at 4 time intervals in the crop cycle were combined into a single analysis in which time of destructive sampling was considered as an additional factor and treated as a split plot. Variety and management effects considered var. Cavendish and var. Mbwazirume, and BSV effects only considered var. Mbwazirume at 2 levels of BSV status, i.e., with and without symptoms. The following model was used:

(i) Effect of crop management and variety

$\mathbf{Y_{1,2,3,}} = \mu + \mathbf{block} + \mathbf{mgt} + \mathbf{error_1} + \mathbf{variety} + \mathbf{mgt.variety} + \mathbf{error_2}$ where,

(ii) Effect of crop management and symptoms status

$\mathbf{Y_{1,2,3,4,5,6,7}} = \mu + \mathbf{block} + \mathbf{mgt} + \mathbf{error_1} + \mathbf{symp} + \mathbf{mgt.symp} + \mathbf{error_2}$ where,

$\mathbf{Y_1}$ = above ground biomass; $\mathbf{Y_2}$ = Fraction of light (photosynthetic active radiation) intercepted; $\mathbf{Y_3}$ = bunch yield.

Effect of variety, crop management and symptoms on yield in this experiment were analysed similar to Section 3.1.2 (ii) and (iii).

3.2.3 Results and discussion

Effect of BSV on biomass accumulation and light interception (PAR) by var. Mbwazirume at Kawanda

BSV symptoms reduced above-ground biomass of var. Mbwazirume by 20% ($P<0.01$; Figure 9a) while BSV symptoms reduced light interception by 4.7% ($P>0.05$; Figure 9b). In this study, biomass increased ($P<0.001$) during the crop cycle. In contrast,

light intercepted by the banana at different time points of the crop cycle was not significantly different (P>0.05). The effects of BSV were not subject to interaction (P>0.05) with month of assessment in crop cycle and management for above-ground biomass and light intercepted by banana plants. The reduction in the proportion on incident light (photosynthetically active radiation) intercepted and subsequently plant biomass accumulation, due to BSV is in accordance with the reduction in the rate of leaf photosynthesis due to BSV (Figure 5).

Effect of management and variety on biomass accumulation and light interception (PAR) by var. Mbwazirume at Kawanda

The crop grown under optimal management had greater dry matter (P<0.001) and intercepted a greater fraction of incident light (photosynthetically active radiation) than those grown under minimal management (Figure 10a & b). However, the difference in light interception for the two management regimes was not significant (P=0.09). Management was subject to an interaction with time of sampling in the crop cycle for biomass accumulation (P<0.001) and light intercepted by plants (P<0.01). Biomass accumulation for the two management regimes was not significantly different in December 2001. Thereafter, biomass of plants under optimal management was consistently higher (P<0.05) than under minimal management. The fraction of light intercepted by plants was also consistently greater under optimal management compared to minimal management except in December 2001. Differences in the two management levels were only significant (P<0.01) in May 2002.

Overall var. Cavendish tended to have more biomass than var. Mbwazirume, although this was not significant (P=0.07). There was an interaction between the effect of variety and month of assessement (P<0.01). Earlier in the crop cycle (December 2001 and March 2002) var. Cavendish had less biomass than var. Mbwazirume (P>0.05). Later, var. Cavendish had more biomass than var. Mbwazirume, i.e., in May-02 (P<0.05) and July-02 (P<0.01). Therefore, var. Mbwazirume grew much faster in the early stages of development. The interaction between variety, management and month in crop cycle was not significant (P>0.05).

In this physiology experiment, the interaction between BSV and crop management was not significant (P>0.05) for bunch weight (Table 13a). Var. Cavendish gave a heavier bunch (P<0.001) than var. Mbazirume (Table 13b). Also, plants under optimal management a gave heavier bunch (P<0.001) than those in minimal management. These results are consistent with those of biomass accumulation (Figure 10a) and fraction of incident light (PAR) intercepted (Figure 10b). Data analysis is continuing in order to examine the relationship between the quantity of light intercepted (crop resource use) and biomass accumulation for plants with and without BSV symptoms under each management regime.

Figure 9a: Effect of BSV on the biomass accumulation of var. Mbwazirume under optimal (opt. mgt) and minimal (min. mgt) management at Kawanda

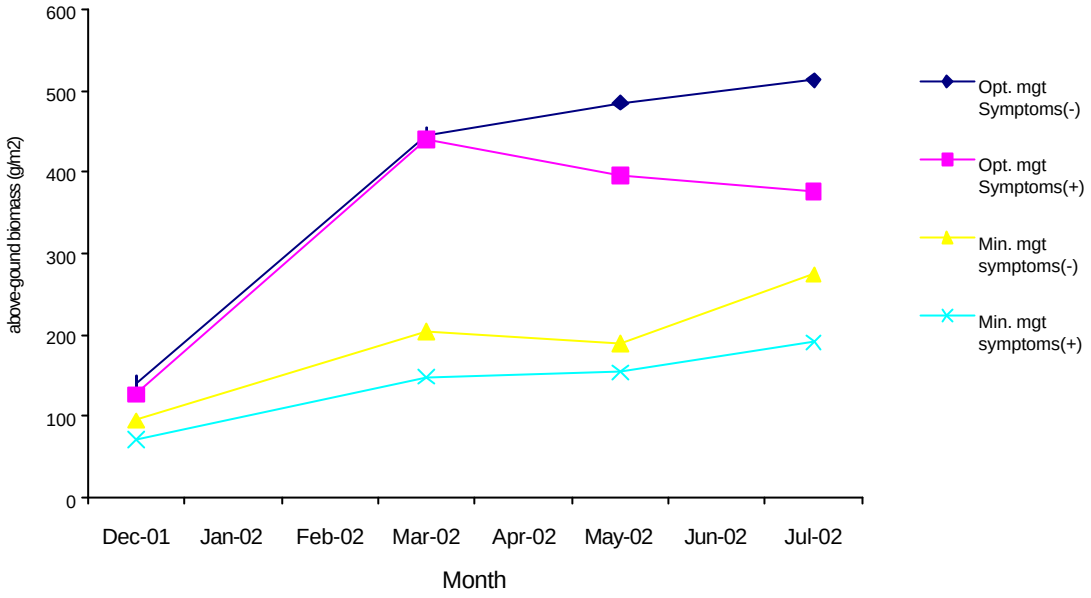


Figure 9b: Effect of BSV symptoms on fraction of light intercepted by var. Mbwazirume under optimal (opt. mgt) and minimal (min. mgt) management at Kawanda

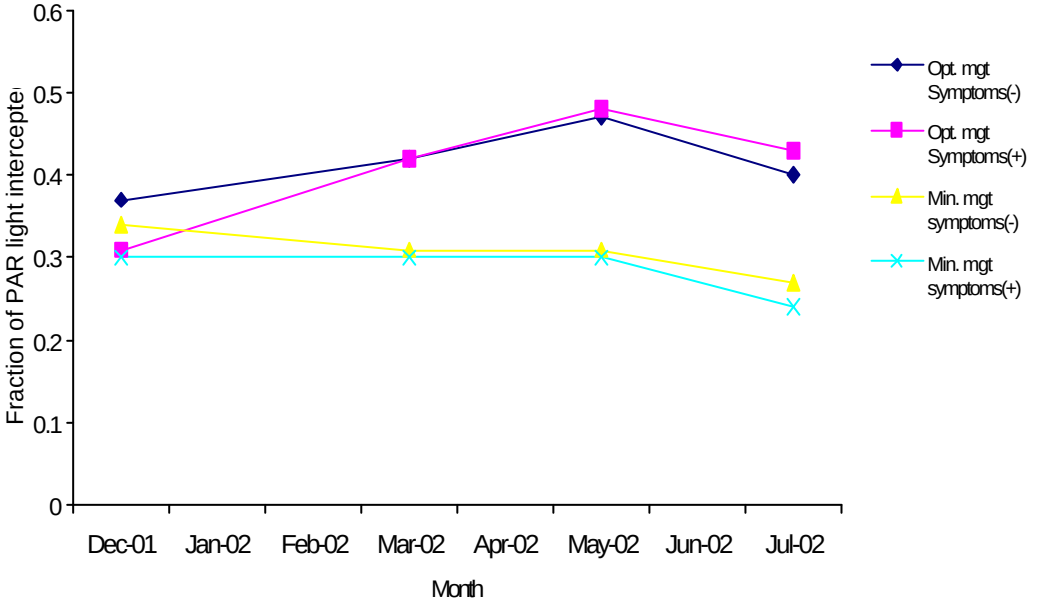


Figure 10a: Effect of manamgement on above-ground biomass of var. Cavendish and var. Mbwazirume in Kawanda

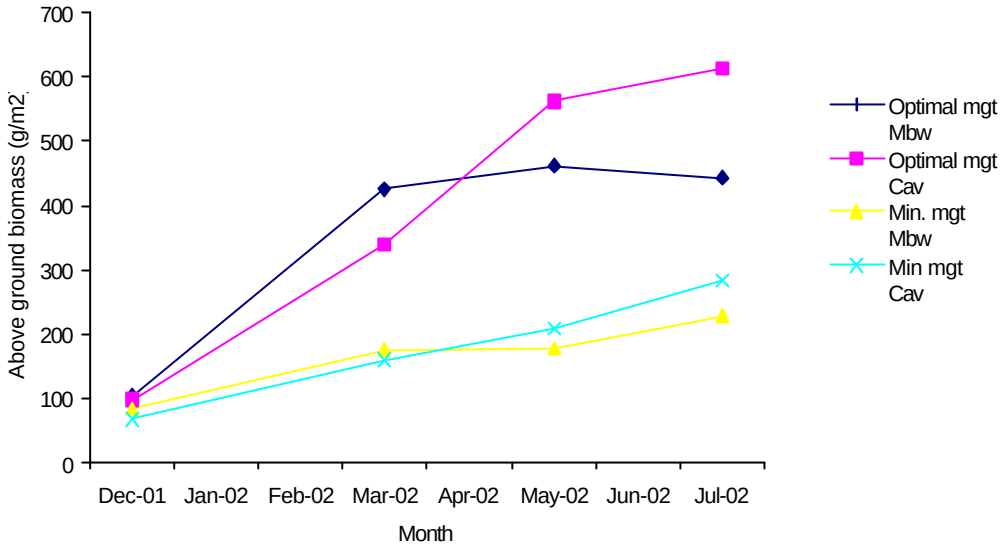


Figure 10b: Effect of management on fraction of light intercept by var. Cavendish and var. Mbwazirume at Kawanda

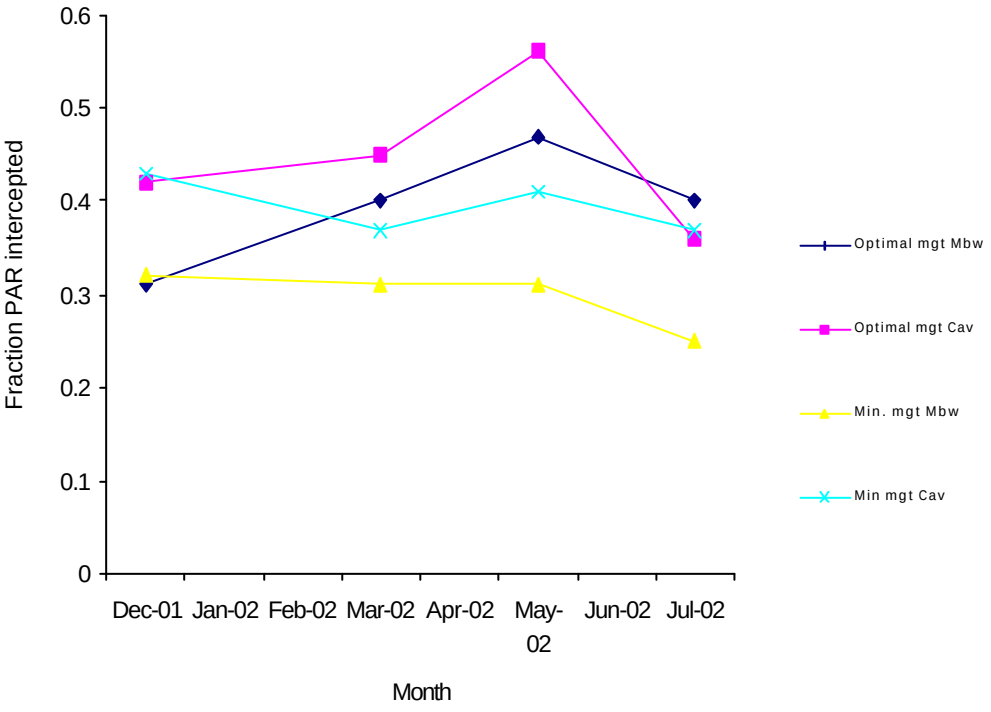


Table 13a: Effect of BSV on bunch weight of var. Mbwazirume mother plants with and without symptoms in the physiology experiment at Kawanda

Yield characteristic	BSV symptoms	Management (mgt)		Mean symptoms	[b] difference in mgt
		Optimal	Minimal		
Bunch weight (kg)[1]					
	Without	17.39	9.49	**13.44**	7.90
	With	15.80	9.11	**12.46**	6.69
	Mean mgt	**16.59**	**9.30**		**7.29****
	[a] difference	1.59	0.38	**0.98**^{ns}	

[1] Mgt P<0.01; Symptoms P = 0.17; Mgt x variety P>0.05; [a] difference = difference in symptom status means and [b] difference mgt = difference in mgt, where ^{ns} = not significant at 5%, ** = not significant at 5% .

Table 13b: Effect management on bunch weight of mother plants of var. Cavendish and Mbwazirume plants in the physiology experiment at Kawanda

Yield characteristic	Management (mgt)	Variety		Mean Mgt	[b] difference var.
		Cavendish	Mbwazirume		
Bunch weight (kg)[1]					
	Optimal	20.71	16.69	**19.70**	4.02
	Minimal	14.62	9.26	**12.18**	5.36
	Mean variety	**17.67**	**12.98**		**4.69*****
	[a] difference	6.09	7.43	**6.76*****	

[1] Mgt P<0.001; Variety P<0.001; Mgt x variety P>0.05; [a] difference = difference in crop management levels and [b] difference var. = difference in variety means *** = significant at 0.1%.

<u>Activity 6.0</u> Socio-economic and biometric study/survey to assess farmers' perceptions of BSV and strategies that might be used to control it.

This is a joint activity with the team from NRI / KARI.

An initial survey was conducted to determine farmers' perceptions and understanding of BSV and the measures they use for its control in order to provide preliminary information on bunch yields on farmer's field for further investigation beyond Project R7529. This survey was done with the help of focus group discussions and a structured questionnaire. The focus group discussions were carried out in Ntungamo and Masaka districts in Southern Uganda while the structure questionnaire was administered in Rakai in addition to the other districts. Thirty farmers were interviewed in each district. Farmers' fields were also assessed and crop management practices, BSV incidence and severity were recorded for each farm.

The survey revealed that BSV was prevalent in Masaka, Ntungamo and Rakai. Farmers described BSV symptoms accurately and perceived them to cause damage to bananas. Although, most farmers did not know the cause of BSV, some attributed the symptoms mainly to weevil infestation, infertile soils and drought. Therefore, they commonly carried out perceived weevil control (removal of corms of harvested plants, trapping and application of ash, urine and pepper) and soil and water retention related practices (mulching, manuring and application of coffee husks) to manage BSV. Rouging was considered by farmers to be the most effective measure in managing BSV. Farmers revealed that rouging was only practical in fields with low incidence of BSV. In cases, of high incidence of BSV, farmers did not consider rouging as an option because of the perception that they would lose bananas/food and is time consuming as well as labour intensive.

Some farmers used mulch, manure and coffee husks to manage BSV. This is probably because farmers believed that plants with BSV symptoms performed better when these practices were used and/or believed that BSV symptoms were soil related. Scanty information exists on the role of crop management, like mulching and manuring as well as other good crop husbandry practices in mitigating the effects of BSV. These are questions that this research initiative is addressing

CONCLUSIONS

This report provides detailed quantitative information on the effects of BSV on the growth, development and yield of banana crops growing in Ugandan conditions. Of particular importance to the question of how farmers may mitigate these effects is whether or not crop management can influence the effects of BSV. The effects of BSV, and their interaction with crop management, were already apparent in the mother and 1st ratoon crop cycle. BSV reduced growth and bunch yields at Kawanda. Reductions in yield tended to be greater under minimal compared with optimal management. Also, more plants with BSV symptoms were found under minimal compared with optimal management. The results can be summarised in terms of plantation production; the bunch yield per hectare per year for the mother and ratoon crops together. At Kawanda, production of plants without BSV was 32.9 and 27.3 t ha^{-1} per year under optimal and minimal management, respectively. In contrast,

production was 17.5 and 13.2 t ha^{-1} per year under optimal and minimal management, respectively, for plant with BSV. The average reduction in plantation production due to BSV was 21%. We expect the severity of symptoms and the effects of management to increase with each crop cycle. These responses need to be confirmed. Nevertheless, we conclude that BSV has a greater (negative) impact on banana yields in Uganda than reported previously, and that improvement in crop management may partly mitigate these yields losses.

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EFFECT OF FARMER CULTURAL PRACTICES ON BANANA STREAK VIRUS (BSV) EXPRESSION

Charles Murekezi and Jerome Kubiriba

Contents

SUMMARY

The effect of farmers' cultural practices on BSV symptom expression, mealybug abundance, and BSV effects on growth and bunch weight of East African Highland bananas (*Musa* AAA-EA) was examined in farmers' banana fields at Ntungamo, southwestern Uganda. Farmers commonly used one or more of seven distinct cultural practices. These were soil and water conservation structures (water bunds), application of organic mulch, desuckering, detrashing, application of manure, weeding and cropping pattern (banana alone or with an intercrop).

While only detrashing reduced mealybug abundance (average number of mealybug colonies per plant), possibly because dark and warm, humid conditions are favourable for the reproduction and colonisation of mealybugs, both detrashing and desuckering tended to increase the proportion of plants infested with mealybugs (mealybug incidence). Once the mealybug colonies are exposed on the pseudostem by detrashing, the chance of nymphs being blown by wind to the neighbouring plants is high. Mealybugs may also be spread to neighbouring plants when chopping and laying the chopped trash as a mulch. This, however, was not reflected in increased BSV incidence as detrashing did not influence BSV incidence and desuckering was associated with reduced BSV incidence.

Crop management practices that targeted improving soil and water conservation and soil nutrition, such as application of mulch or manure, or provision of water bunds, appeared not to affect mealybug abundance. They tended to reduce mealybug spread for unclear reasons that might have been expected to translate into reduced BSV incidence, but farms in which manure was applied had increased BSV incidence. This probably arises from the farmers' practice of applying manure to plants with BSV symptoms, perceiving the symptoms to be caused by a nutrient deficiency. BSV incidence was less in farms where several cultural practices were used, though because incidence was measured as the proportion of plants with visible symptoms, this effect may not have been on the rate of spread of the disease, but rather through an effect on symptom expression.

The effects of BSV on growth and bunch weight were reduced in farms that carried out cultural practices that reduced crop stress including frequent weeding, sole cropping, provision of soil and water conservation structures, improving plant nutrition through application of manure and appropriate desuckering and detrashing. Thus, banana growth and bunch weights were improved and the effects of BSV reduced in intensively managed farms. However it was apparent that the farmers' mulching practice did not reduce BSV effects on growth and bunch weights. This is contrary, to observations made of on-station experiments at Kawanda and Mbarara where application of mulch was one of the treatments. This disparity is probably because farmers tend to apply less mulch than recommended. This calls into question whether the applications of cultural practices in these farms were sufficient in terms of frequency and amounts.

Nonetheless, results from this study are indicative of the potential of farmers' cultural practices in improving banana productivity and mitigating BSV disease effects. Further work is required to determine the combinations of the cultural practices that are most beneficial. This study does not give an indication of at which level of BSV severity crop management ceases to be beneficial. In such a circumstance, roguing would be required. The applicability of roguing in smallholder banana cultivation, however, remains unknown.

INTRODUCTION

Banana (*Musa* sp.) is an important staple for about 7 million people in Uganda (Karamura, 1993). Areas producing bananas in Uganda have been categorised into three zones (Anonymous, 1998). These included areas of extreme production decline (most areas of Central Uganda), those of relatively high production (Mbarara and Bushenyi) and those of intermediate banana production levels (Masaka, Rakai and Ntungamo). Although the major factors behind this decline are pest/disease complex, soil fertility decline, narrow genetic base and socio-economic factors (Gold *et al*., 1993), in the intermediate production zone, *Banana streak virus* (BSV) is regarded as a prominent constraint to banana production (Kubiriba *et al*., 1997). BSV has been identified as an important constraint else where in the world (Diekmann and Putter, 1996) and studies indicate that it can reduce banana yields significantly (Dahal *et al*, 2000). It is reported that improved crop management ameliorates the effects of BSV (Dahal *et al*., 1998; Daniells *et al*., 2000). Favourable effects of improved crop management on diseases have previously been observed. For example, studies on Black Sigatoka disease showed that cultural practices mitigated the impact of this fungal disease on bananas (Holderness *et al*, 1999). Such cultural practices are commonly used by farmers in the management of banana constraints in Uganda because they require cheap and locally available on/off farm resources. As such, aspects of cultural practices could form major ingredients in the formulation of environmentally friendly and equitable practices in the management of BSV.

This study was initiated, therefore, to ascertain the potential of farmers' cultural practices in managing BSV. The specific objective of the study was to investigate the response of the East African Highland banana to BSV in relation to different farmers' cultural management practices by monitoring the effect of the practices on mealybug abundance and incidence, BSV incidence and symptom expression, and banana growth characteristics and yield.

METHODOLGY

Site

The study was conducted in Kikoni Parish, Ntungamo district in southwestern Uganda within the altitude range of 1300-1500 masl and with a bimodal (March-June and October-December) rainfall ranging from 800 mm to 1500mm. Most of the farmers had smallholdings of less than two hectares (Gold *et al*., 2002). The predominant crop at the site is the East African Highland banana (AAA-EA).

Preliminary survey and farm selection

The study was preceded by a survey conducted in February 2001 in 6 villages in Ntungamo district. In the survey, 60 farmers' fields were randomly selected across the villages of Mutanoga, Muyumbu, Kyangara, Kalegeya, Kamunyiga and Musaana. During the survey, the main farmer cultural practices were noted and the status of BSV on these farms was assessed. Thereafter, 30 farms where BSV was present were selected to participate in the study. In each of the 30 farms four quadrants measuring 15 x 15m were assigned in the banana fields. Ten plants were then selected and marked with paint (regularly renewed) in each quadrant making a total of 40 plants per farm that then comprised the data plants. Data

collection commenced in August 2001. This report covers period of August 2001 to January 2003.

Farmer cultural practices

The initial survey conducted in February 2001 noted that seven cultural practices were commonly used by farms in the management of bananas. They were (1) provision of soil and water conservation structures (water bunds), (2) application of an organic mulch, (3) regular desuckering, (4) regular detrashing, (5) manure application, (6) regular weeding and (7) cropping pattern (sole cropping of banana and intercropping banana with beans or other intercrops). Apart from cropping patterns, the other cultural practices are being carried out by farmers to manage banana plantations. For example intercropping is not a management practice, but because of the limitation of land, farmers plant other crops, mainly beans in the rainy season to obtain food protein. The 30 farmers' fields in Ntungamo District were assessed and records taken every month. Initially management practices were classified as in Table 1 by giving more weight to management practices that, based on the investigators' experience, had a direct impact on both BSV expression and mealybug population.

The total weight of the cultural practices carried out on a field was the sum of the weights of the individual practices. This total weighting was used to determine the overall management status of a farm being assessed. The overall management categories were as follows: Low 7, Moderate 8-15 and Intensive 16.

Table 1. Cultural practices and the assigned weightings

Cultural practice	**Weight**
1. Soil and water conservation structures (water bunds)	6
2. Application of mulch	5
3. Desuckering and Detrashing	4
4. Manure/soil inputs	3
5. Weeding	2
6. No Intercropping	1
(Maximum score)	**21**

However, after collecting data for one year (August 2001 to July 2002) these methods were revised to follow the criteria below:

1. Soil and water conservation structures (water bunds):
   (0) Minimum 0 –1 water bunds per plantation (gentle/no slope); 0-2 bunds (steep slope)
   (1) Adequate – more than minimal above

2. Mulch application:
   (0) Absent - no mulch evident in the banana field
   (1) Present - mulch cover of > 5 cm for a period ≥ 4 months in a year

3. Desuckering (removal of excess suckers):
   (0) Minimal – ≥ 4 plants per mat for > 5 months a year
   (1) Adequate - ≤ 3 plants per mat for > 5 months a year

4. Detrashing:
   (0) Minimal – dead leaves and sheaths present on plants for > 5months a year
   (1) Adequate – dead leaves and sheath present on plants < 5 months

5. Manure:
   (0) Minimal (little)– no/trace amounts applied in the banana field
   (1) Adequate (some) – more than trace amounts of manure applied in the field

6. Weeding:
   (0) Poor – many weeds in plots for > 5 months a year
   (1) Adequate/good – no/few weeds in banana plots for > 5 months a year

7. Cropping pattern (Use of intercrops):
   (0) Sole cropping – no systematic intercrops in the banana field
   (1) Intercropping – systematic intercrops in banana field

Data collection

Each farm was visited every month, and some times bi-monthly, to record BSV symptom expression (severity and incidence), mealybug abundance (number of colonies per plant), and proportion of plants infested with mealybugs (mealybug incidence), growth characteristics and bunch weights for mature plants. In addition, farmer cultural practices were recorded; each marked plant was assessed for noticeable foliar symptoms on individual leaves, i.e., golden yellow chlorotic streaks. BSV incidence in each farm was taken as the proportion of data plants with BSV symptoms to the total number of marked plants in the quadrants. BSV severity was determined by scoring individual leaves of the infected plants using a 0-3 scale and deriving an average symptom severity index (SSI) for each plant (Dahal *et al*., 1997). Numbers of mealybug colonies were recorded on the pseudostem of each of the marked plants from ground level to about 2 metres above ground. The proportion of plants infested with mealybugs on each farm was then derived. Plant height was measured from the base of pseudostem to the emerging inflorescence. Pseudostem circumference (girth) was measured at a height of 100 cm from the base of the plant at flowering. Bunch weights were estimated within 3 weeks of bunch maturity.

Data analysis

The effects of farmer cultural practices on mealybug abundance, proportion of plants infested with mealybugs, BSV incidence, BSV severity, growth (plant height and girth) and bunch weights were determined through data analysis using the general and generalized linear model procedures in SAS (SAS Inc., 1997). Data on mealybug abundance and BSV severity was analyzed using a repeated measure approach in general linear model procedure (GLM). Data on proportion of plants, mealybugs and BSV incidence was analyzed as composite data sets. The proportions of mealybugs and BSV incidence were analyzed as averages since they did not alter with time. Proportion of plants infested with mealybugs and BSV incidence per farm per month was analysed by Genmod procedure since the error

terms were best described by binomial distribution with logit link function for the individual practices and those for over all management by Poisson distribution with log link function.

The models used were:

$Y_{ij} = \mu$ + cultural practice$_{ik}$ + error$_{ij}$ where i=1,2 corresponding to the two levels of the cultural practice under consideration, and j=1,2,….,30 corresponds to the j^{th} farm. The model was run for each of the cultural practices k=1,2,…,7.

The Y_{ij} in this model were (in turn): Repeated measures for BSV severity, mealybug abundance (number of mealybug colonies per plant), for farm j practicing practice i. In the case of BSV severity the values of severity for the first month of assessment (August 01) was used as a covariate ($Y_{ij} = \mu$ + cultural practice$_{ik}$ + covariate + error$_{ij}$). The error terms correspond to the unexplained component of variability, assumed be normally and independently distributed with mean zero and constant variance.

For analysing BSV incidence and mealybug incidence, the model was:

Log(pij/(1-pij) = μ + cultural practice$_{ik}$ + error$_{ij}$ where pij correspond to the incidence (of mealybugs or BSV) in each farm. The error terms here follow a binomial distribution the individual practices. Log(pij) = μ + Overall management + error$_{ij}$ for poisson distribution.

The model used for analysing plant height, pseudostem girth and bunch weights was $Y_{ijk} = \mu$ + cultural practice$_{il}$ + error$_{ij}$ + symptoms$_k$ + symptoms$_k$*cultural practice$_{il}$ + error$_{ijk}$, where i=1,2 corresponding to the two levels of the cultural practice under consideration, and j=1,2,….,30 corresponds to the j^{th} farm, k=1,2 corresponding to with and without BSV. The model was run for each of the cultural practices l=1,2,…,7.

The analyses presented in the following tables are for the period August 2001 to December 2002. During this period, the numbers of farms where each management practice was being done were as presented in Table 2 below:

Table 2: Number of farms represented in each management practice level

Management Practice	**Number of Farms**	
	Practice level "0"	Practice level "1"
1. Soil and water conservation	19	11
2. Mulching	27	3
3. Desuckering	5	25
4. Detrashing	9	21
5. Manure application	24	6
6. Regular weeding	4	26
7. Intercropping	26	4

RESULTS AND DISCUSSION

Effect of farmers' management practice on mealybug abundance and proportion of plants infested with mealybugs.

The different overall management levels (derived from the sums of the weighted scores for individual practices) over the period of assessment appeared not to show any effect on mealybug abundance (average number of colonies per plant). However, detrashing significantly reduced (P = 0.0038) mealybug abundance (Figure 1). Dark and warm, humid conditions are favourable for the reproduction and colonisation of mealybugs (Watson and Chandler, 2000). Detrashing involves removal of both senescent and dry leaves and also removal of leaf sheaths from the pseudostems. Removal of leaves then opens up the canopy and detrashing exposes the mealybug colonies (usually covered by the dry sheaths) to the harsh environment and possibly to predators.

The practices aimed at improving soil and water conservation and soil nutrition such as water bunds and manuring, did not affect mealybug abundance (P>0.05) (Figure1). Improved availability of nitrogen, however, has been associated with elevated mealybug numbers in cocoa plantations in Ghana (Bigger, 1981).

The proportion of plants infested with mealybugs (incidence of mealybugs) is indicative of the rate of spread of mealybugs in the field. The management practices that influence proportion of mealybug-infested plants might also be expected to influence BSV incidence. However, farms where detrashing was done had significantly greater proportion of plants infested with mealybugs (P < 0.0001) compared to those where detrashing was not done, (Table 3) but detrashing had no significant effect (P = 0.12) on BSV incidence (Table 4). Detrashing exposes mealybug colonies under the leaf sheaths, improves illumination and consequently aeration in the banana plantation. This environment promotes movement of the mobile mealybug nymphs on the pseudostem, which then increases their chances of being blown away to neighbouring plants. If any BSV–infected plants also harbour mealybug nymphs, these may also eventually be blown to neighbouring plants increasing the chances for BSV spread. Farms where desuckering was done also had greater incidence of mealybugs (proportion of plants infested) (P = 0.0038) than where it was not done, but this was not reflected with increased BSV incidence since farms where it was done had a lesser incidence of BSV (P < 0.001). If the cut sucker is infected with mealybugs, the chances of spreading mealybugs to the neighbours are increased since it is normally chopped and laid on the ground as mulch, sometimes at some distance from the source mat. BSV incidence is dependent on success of inoculation by mealybugs, incubation period and also other factors that influence symptom expression, one or many of which may be affected by desuckering.

Good weed control appeared to have no significant effect on the average number of mealybug colonies on each banana plant (Figure 1), though the effect was highly significant on the proportion of plants infested with mealybugs (Table 3). This may be because the presence of weeds allows the mealybugs to crawl between banana plants more readily without being predated upon, and thus they are more evenly dispersed across the field. In field where weeds are not present any mealybugs remain on their original host banana and populations on individual bananas build up more than when weeds are present. Where there were water-bunds, and/or mulch or manure were applied, there were lower proportions of mealybug-infested plants than where they were absent, but it was not clear why this was the

case. Whether the bananas were grown as a monocrop or intercropped had no significant effect on mealybug abundance or incidence of mealybug infested plants.

Table 3. Management effect on proportion of plants infested with mealybugs under Farmers' conditions in Ntungamo

Practice	Parameter estimate	Chi-square (c^2)	*P*-level
1. Water bunds*	-0.32 ± 0.13	6.01	0.0143
2. Mulch application	-0.36 ± 0.14	7.10	0.0077
3. Desuckering*	0.39 ± 0.13	8.38	0.0038
4. Detrashing*	0.57 ± 0.12	22.32	<0.0001
5. Manure*	-0.43 ± 0.13	12.01	0.0005
6. Weeding	-1.11 ± 0.09	159.31	<0.0001
7. Intercropping*	-0.10 ± 0.12	0.61	0.4360
Moderate management$^+$	-0.22 ± 0.07	3.62	0.0121
High management$^+$	-0.29 ± 0.11	5.03	0.0345

$^{*+}$The error terms were assumed to be best described by binomially distribution with logit link function for the individual practices and those for over all management by Poisson distribution with log link function. In both cases, the parameter estimate for one of the levels of the management practice is fixed at zero and Genmod calculates the parameter estimate for the other level in either direction of zero. If a practice has an effect of increasing the proportion of plants infested with mealybugs, the parameter estimate is positive and if a practice has an effect of decreasing the proportion of plants infested with mealybugs, the parameter estimate is negative. In this case, the fixed level is where the individual practices are not done and low management for overall management.

Effect of farmers' cultural practice on BSV incidence and severity

BSV severity

The severity of BSV in the farms in Ntungamo appeared to increase slightly during the period of study (Figure 2). This was significant in farms that were mulched and not mulched ($P< 0.001$), practiced sole and intercropping ($P < 0.01$), detrashing and minimally detrashing ($P <0.001$), desuckering and minimally desuckering ($P < 0.01$), farms with adequate and trace waterbunds ($P < 0.01$), those with trace and adequate manure ($P < 0.001$) and weeded and poorly weeded farms ($P < 0.01$). None of these management practices affected BSV severity ($P >0.05$) and there was no interaction between farmer cultural management practices and the period of study. There was also a significant interaction between month of assessment and covariate (severity in August 01).

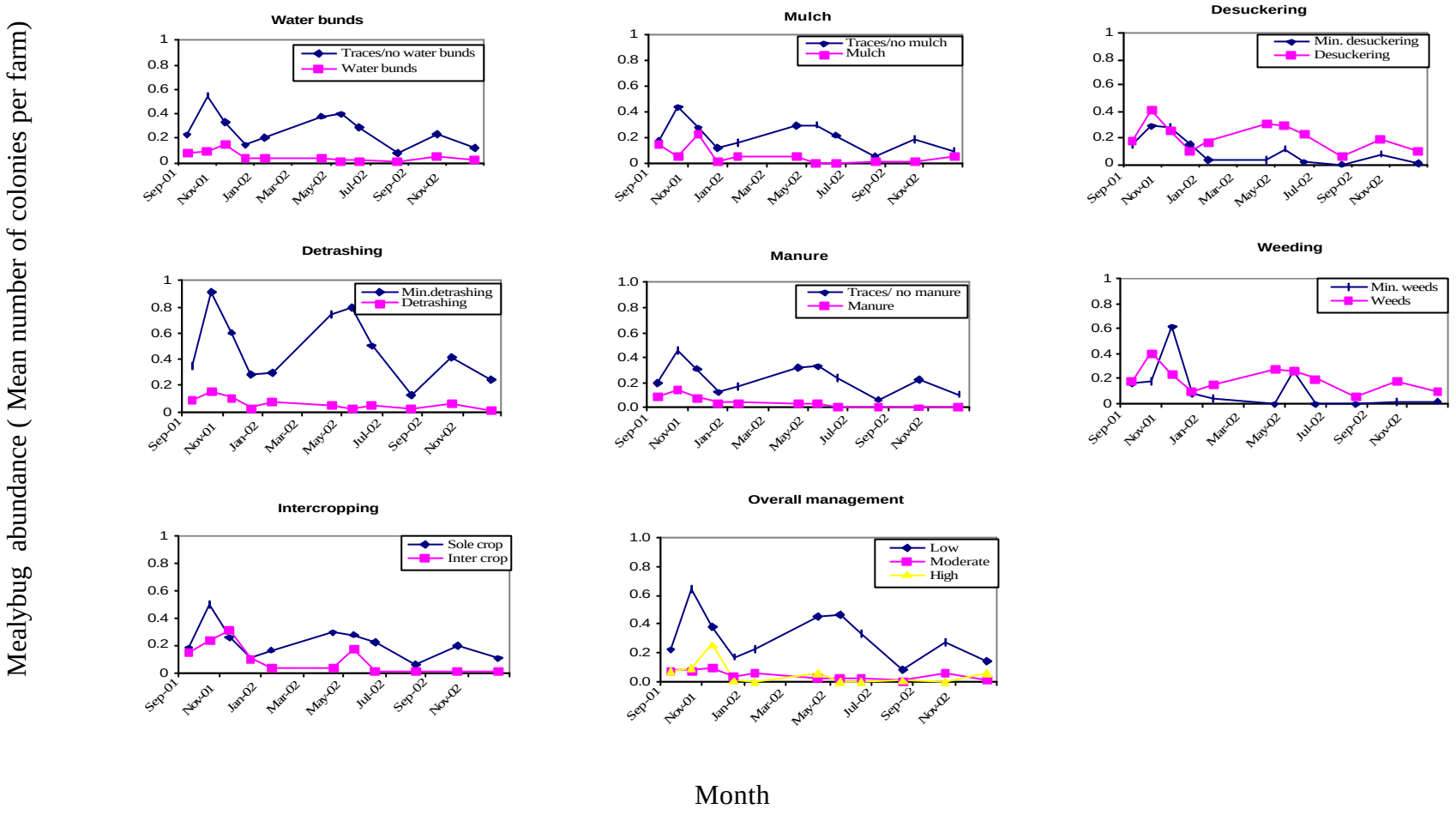

Figure 1. Management effect on mealybug abundance on farmers' fields in Ntungamo

Data was analysed individually by general linear model with a repeated measure option. Management practice adjusted to fit a year long period was used

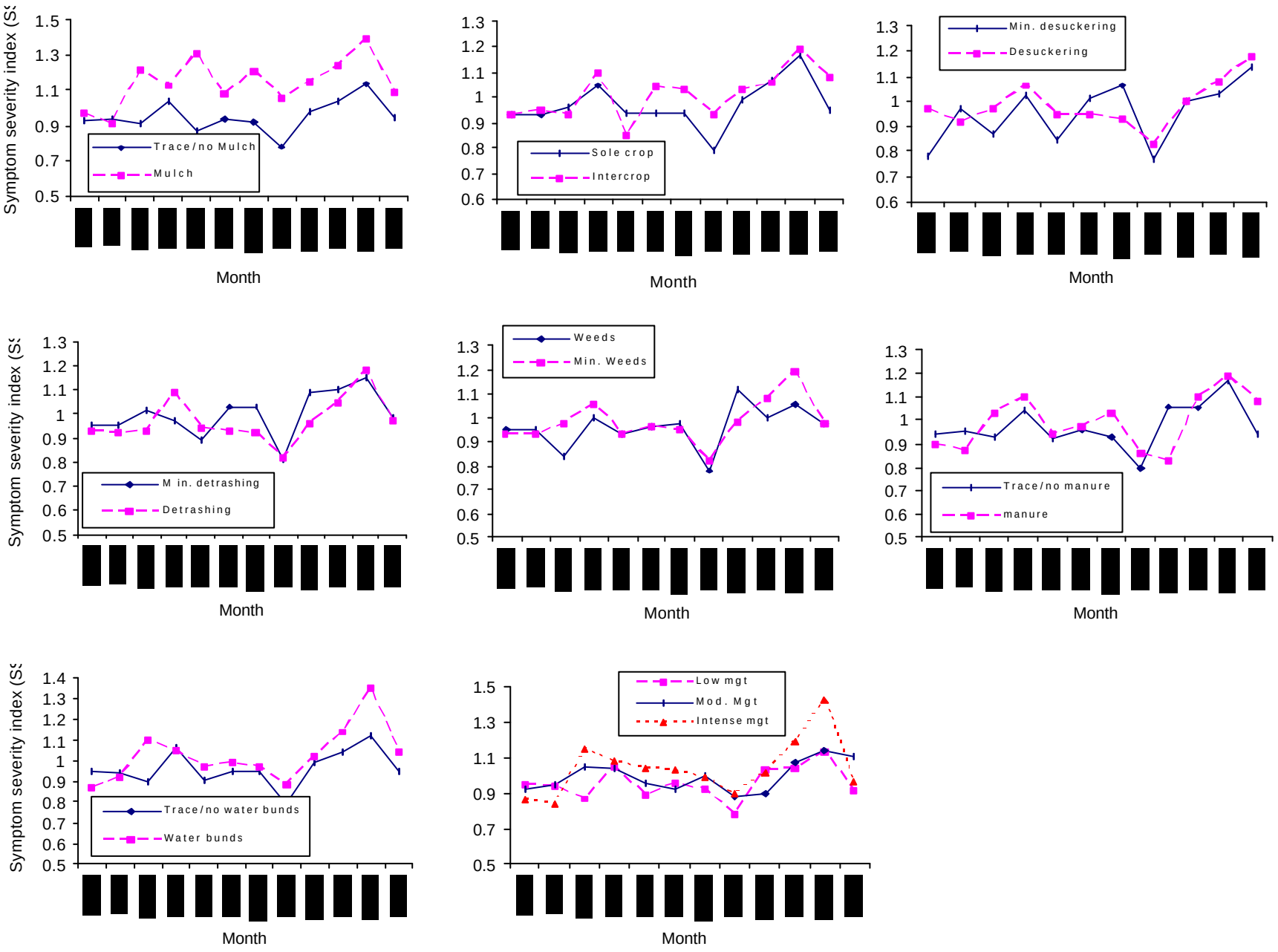

Figure 2: Effect of farmers' cultural practices on the severity of BSV disease in farmers plots in Ntungamo

BSV incidence

Farms where manure was applied had a higher incidence of BSV ($P < 0.001$) compared to those where little or no manure was applied (Table 4). Though not significant, farms where mulch was applied also appeared to have a greater incidence of BSV compared to those without mulch. However, this is not necessarily an effect of these management practices (manure and mulch) on BSV incidence, rather, when farmers encounter a problem not known to them; it is managed in accordance to its perceived cause. This observation is in agreement with results of the Survey on farmers' perceptions on BSV (R7529 Final Technical Report Annex 5&6), where farmers mentioned that they applied manure and mulch in addition to other cultural practices to address the symptoms of BSV because they believed these were nutrition-related.

Table 4. Relationship of farmer cultural practice and the average BSV incidence in farmers' fields in Ntungamo

Cultural practice	Parameter estimate	Chi-square (c^2)	p-level
1. Water conservation structures	-0.58	17.57	< 0.001
2. Mulch application	0.304	2.24	0.13
3. Desuckering	-1.01	40.16	< 0.001
4. Detrashing	-0.210	2.40	0.12
5. Manure application	1.06	51.04	< 0.001
6. Weeding	-0.200	1.23	0.27
7. Intercropping	1.417	64.56	< 0.001
8. Overall management (low-moderate)	0.085	0.58	0.45
9. Overall management (low-Intensive)	0.238	1.45	0.23
10. Overall management (moderate-Intensive)	0.153	0.55	0.46

*+The error terms were assumed to be best described by binomially distribution with logit link function for the individual practices and those for over all management by Poisson distribution with log link function. In both cases, the parameter estimate for one of the levels of the management practice is fixed at zero and Genmod calculates the parameter estimate for the other level in either direction of zero. The farms with the practice that have more BSV incidence has parameter estimate is positive and if a farms with the practice has a low BSV incidence, the parameter estimate is negative. In overall management genmod calculates estimates between all the three possible combinations of the 3 levels of overall management.

Farms where banana was grown without an intercrop (sole-cropping) had lower BSV incidence ($P < 0.001$) compared to those with intercrops (Table 4). Also, farms where desuckering was practiced had lower BSV incidence ($P < 0.001$) than those with minimal desuckering. Both intercrops and excessive suckers create crop stress by increasing plant densities in plots, and thereby competition for light, water and nutrients. Since BSV incidence was determined as the proportion of plants showing foliar symptoms, conditions creating stress that enhance the expression of disease symptoms also influence BSV incidence. Farms with soil and water conservation structures had a lower BSV incidence ($P < 0.001$) compared to farms without these structures. These structures reduce soil loss which is an important management practice in Ntungamo with slopes of > 15%, especially where ground cover is minimal under bananas. These structures also improve water penetration and retention, giving more favourable growing conditions that may result in less severe BSV symptoms and an apparently lesser incidence. Also, farms where detrashing was practiced and there was proper weed control had lower BSV incidence compared to those without these practices.

Overall, the indication was that farms that were intensively managed had lower BSV incidence compared to those moderately or poorly managed, though the difference was not significant. This is possibly because the overall management score was greatly influenced by the bigger weighting given to soil and water conservation measures (Table 1). Management practices that appeared to reduce BSV incidence probably did so through reducing symptom expression/severity, especially where there were only mild symptoms. This is supported by earlier data that these practices did not affect mealybug abundance, except with intercropping, and proportion of mealybug infested plants. The rate of spread of BSV by mealybugs (R7529 Final technical report Annex 1) is probably influenced by many different factors, and the level of incidence now is more likely related to management practices and mealybug population dynamics in earlier seasons. The perennial nature of bananas exposes them to diseases, such as BSV, that spread slowly that would not have been important in short duration/annual crops.

Growth characteristics and bunch weight

Effect of soil and water conservation structures on BSV, growth and bunch weight

BSV disease reduced plant height ($P < 0.001$), pseudostem girth ($P < 0.001$) and bunch weight ($P < 0.001$) in farms with and without soil and water conservation structures (Table 5). There was no interaction ($P > 0.05$) for the effects of BSV with soil and water structures for these growth characteristics. But, plant growth was reduced more (height by 3.8% and girth by 5.7%) by BSV in farms without compared to those with (height by 2.3 % and girth by 2.7%) soil and water structures. Also, BSV reduced bunch weight more in farms without (17.1%) compared to those with (8.5%), soil and water conservation structures. Bananas in farms with soil and water conservation structures were taller ($P<0.001$) had bigger girth ($P< 0.001$) and gave heavier bunches ($P < 0.01$) than those in farms without these structures were.

Soil and water conservation structures reduce soil loss and improve rainwater infiltration/retention, thereby improving the availability of water in soil. Consequently, the uptake of water and nutrients by plants in fields with these structures is improved resulting in the improved plant growth and bunch weight observed. The favourable growth

conditions in farms with soil and water conservation structures brought about a reduction in the negative effects of BSV on growth and bunch weight compared to the farms without these structures. Again, these results should be viewed with some caution since there may be some confounding; farmers are more likely to make water bunds on land with steeper inclination, and this type of location tends to have thinner and more nutrient-leached soils. Perhaps the average gradient of the land at each farm should be included as a co-factor.

Table 5. Effect of water bands on mean height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo

Parameter	BSV symptoms	Soil and water conservation structures (water bunds)			
		Present	Not present	Mean for symptoms	Difference
Height[1]					
	without	387.19	373.93	**380.56**	
	with	378.17	359.80	**368.99**	
	Mean water bunds	**382.68**	**366.87**		**15.81*****
	difference	(-2.33%)	(-3.78%)	**11.57***** **(-3.04%)**	
Girth[2]					
	without	64.50	61.84	**63.17**	
	with	62.78	58.31	**60.55**	
.	Mean water bunds	**63.64**	**60.08**		**3.56*****
	difference	(-2.67%)	(-5.71%)	2.62*** (-4.15%)	
Bunch weight[3]					
	without	17.56	15.07	**16.32**	
	with	16.07	12.49	**14.28**	
	Mean water bunds	**16.81**	**13.78**		**3.03*****
	difference	(-8.49%)	(-17.12%)	**2.04**** **(-12.50%)**	

[1]Water bunds $P < 0.001$, Symptoms < 0.001, Water bunds * Symptoms $P = 0.23$; [2] Water bunds $P < 0.001$, Symptoms $P < 0.001$, Water bunds * Symptoms $P = 0.10$; [3] Water bunds $P < 0.001$, Symptoms $P < 0.01$, Water bunds * Symptoms $P = 0.38$.

Effect of mulch application on BSV, growth and bunch weight

In both mulched and non-mulched banana fields, plants with symptoms had lesser bunch weight (by 11.6%; $P < 0.05$) compared to those without symptoms (Table 6). There was a significant interaction between BSV symptoms and farmers' cultural practice for plant height ($P<0.05$) and pseudostem girth ($P<0.01$). BSV reduced height more (by 6.1%; $P < 0.05$) in mulched plots compared to un-mulched plots (2.7%; $P < 0.05$). BSV also reduced girth more (9.3 %; $P < 0.001$) in mulched plots compared to un-mulched plots (3.7%; $P < 0.001$). Symptomless plants were taller (by 6%; $P < 0.001$) while symptomatic plants were less tall (by 2.3%; $P < 0.05$) in mulched compared to un-mulched plots and it was also observed that symptomless plants were bigger (by 8.9%; $P < 0.001$) in mulched farms compared to un-mulched farms. However, girths of symptomatic plants in mulched and un-mulched farms were not significantly different ($P > 0.05$). BSV disease reduced bunch weight by about similar margins in both mulched and un-mulched farms and bunch weights were lighter by 22% for symptomless and symptomatic plants, in mulched farms and un-mulched farms.

Farms where mulch was applied had better plant growth despite a higher BSV incidence. This was not translated in bunch weights during this study period. Despite the positive influence mulching had on growth, BSV disease reduced plant height, girth and bunch weight more in mulched plots compared to un-mulched plots. It is likely that farmers' mulch was insufficient to cause the decrease in BSV disease severity and the improvement in bunch weights as was observed for the on-station experiments at Kawanda and Mbarara (R7529 Final technical report Annex 3, Impact of BSV on Growth and Yield of Bananas) where excess mulch was applied. Indeed, farmer mulch application comprised of mulch cover of > 5cm for 4 or more months a year while the on-station mulch treatment comprised mulch cover of 5 – 10 cm through the year.

Table 6. Effect of farmers' mulch practice on height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo

Parameter	**BSV symptoms**	**Mulch**			
		Applied	**Not applied**	**Mean for symptoms**	**Difference**
Height[1]					
(cm)	without	397.96	375.38	**386.67**	22.58***
	with	373.63	365.07	**369.35**	8.56*
	Mean mulch	***385.80***	***370.22***		
	difference	24.33***	10.31***		
Girth[2]					
(cm)	without	67.44	61.91	**64.68**	5.53***
	with	61.05	59.65	**60.35**	1.40ns
.	*Mean mulch*	**64.25**	**60.78**		
	difference	6.39***	2.26***		
Bunch weight[3]					
(kg)	without	16.20	16.53	**16.37**	
	with	14.28	14.64	**14.46**	
	Mean mulch	***15.24***	***15.58***		**- 0.34 ns**
	difference			**1.91 *** **	

[1]Mulch $P < 0.001$, Symptoms $P < 0.001$, Mulch * Symptoms $P < 0.05$; [2]Mulch $P < 0.001$, Symptoms $P < 0.001$, Mulch * Symptoms $P < 0.01$; [3] Mulch $P = 0.68$, Symptoms $P < 0.05$, Mulch * Symptoms $P = 0.99$.

Effect of desuckering on BSV, growth and bunch weight

There was a significant interaction between BSV disease and farmer's desuckering practice for plant height ($P < 0.001$) and pseudostem girth ($P < 0.001$). Plant height was reduced more (by 10.3%) due to BSV infection in farms where minimal compared to routine desuckering were practiced (2.5%). Similarly, BSV infection reduced girth more (by 15.8%) in farms with excessive suckers/plants on mats compared to those with an average of 3 or less suckers per mat (3.2%). Though not significant, bunch weights were reduced (by 9.3%; $P > 0.05$) by BSV in farms irrespective of whether routine desuckering was being practiced or not (Table 7).

Desuckering did not influence ($P > 0.05$) plant height or pseudostem girth for plants without BSV symptom. However, for symptomatic plants, growth was less (height reduced by 6.0%, girth by 8.05%; $P < 0.001$) in farmers' fields having excess suckers/plants on mats compared those with 3 or less suckers/plants per mat. Bunch weights were reduced (by 35.6%; $P < 0.001$) due to excess suckers/plants compared to farms where routine desuckering was practiced regardless of the BSV status of the banana plants.

Table 7. Effect of desuckering on height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo.

Parameter	BSV symptoms	Desuckering			
		Routine	**Minimal**	**Mean for symptoms**	**Difference**
Height[1] (cm)	without	381.76	374.04	**377.90**	7.72^{ns}
	with	372.65	335.41	**364.03**	37.24^{***} (5.96%)
	Mean desuckering	**377.20**	**354.72**		
	difference	9.56^{***} (-2.50%)	38.63^{***} (-10.32%)		
Girth[2] (cm)	without	63.37	62.27	**62.82**	1.10^{ns}
	with	61.35	52.41	**56.88**	8.94^{***} (-8.05%)
`	Mean desuckering	**62.36**	**57.34**		
	difference	2.02^{***} (-3.18%)	9.86^{***} (-15.83%)	-5.94*** (-10.55%)	
Bunch weight[3] (kg)	without	17.36	10.92	**14.14**	
	with	15.45	10.21	**12.83**	
	Mean desuckering	**16.41**	**10.57**		**5.84^{***} (-35.59%)**
	difference	-1.91 (-11.00%)	-0.71 (-6.50%)	**1.31^{ns} (-9.26%)**	

1 Desuckering $P < 0.001$, Symptoms $P < 0.001$, Desuckering * Symptoms $P < 0.001$; [2] Desuckering $P < 0.001$, Symptoms $P < 0.001$, Desuckering * Symptoms $P < 0.001$; [3] Desuckering $P < 0.001$, Symptoms $P = 0.15$, Desuckering * Symptoms $P = 0.51$.

Farms where minimal desuckering is practiced normally have excess suckers/plants per mat, giving rise to high plant densities. This results in plants competing for light, water and nutrients. The growth of symptomless plants, however, was not reduced in the farms categorized as practicing minimal desuckering (≥ 4 plants per mat). The reduction in growth occurred in symptomatic plants. This implies crop stress due to excess suckers was sufficient to reduce growth for plants with BSV infection. However, crop stress arising from minimal desuckering in fields reduced bunch weights for both symptomatic and symptomless plants. Results presented here are for bunch weights per plant. Because of the greater plant density that results from minimal desuckering, the total yield per unit area is probably not reduced to the same extent in farms with minimal desuckering compared to those with optimal desuckering as is suggested by the reduction in individual bunch weights.

Effect of detrashing on BSV, growth and bunch weight

Plant height ($P < 0.001$), pseudostem girth ($P < 0.001$) and bunch weight ($P < 0.05$) were reduced by BSV disease in farms irrespective of whether they practiced routine detrashing or not (Table 8). BSV disease was not subject to an interaction ($P > 0.05$) with routine detrashing, and the practice did not have an effect ($P > 0.05$) on the plant height. However, detrashing increased plant girth (by 3.8%; $P < 0.01$) and bunch weight (by 16.2%; $P < 0.01$). Detrashing provides favourable plant growing conditions by improving illumination and plant health.

Effect of manure application on BSV, growth and bunch weight

BSV reduced pseudostem girth ($P < 0.05$) in farms with substantial and no/trace amounts of manure (Table 9). BSV was subject to an interaction with manure for plant height and bunch weight. The disease reduced plant height more (by 3.8%; $P < 0.001$) in farms that received no/trace amounts of manure compared to those receiving substantial amounts of manure. Conversely, BSV reduced bunch weight more (29.5%) in farms where substantial amounts of manure applied compared to those with no/trace amounts of manure (8.7%). Despite BSV not being subject to an interaction with manure for pseudostem girth, girth was reduced more (5.0%) in farms with trace/no manure compared to those with substantial amounts of manure applied (0.4%).

Application of manure significantly increased (by 2.03%; $P < 0.05$) pseudostem girth. Plant height of symptomatic plants was greater (by 2.9%; $P < 0.05$) in substantially manured farms compared to those in no/trace manure applied farms. There was no significant ($P > 0.05$) effect of manure on the plant height for symptomless plants. Bunch weight of symptomatic plants were marginally (but not significantly $P > 0.05$) greater in farms where no/trace amounts of were applied compared to those with substantial amounts of manure applied. But, the bunch weights of symptomless plants were significantly less (15.4%; $P < 0.05$) in farms with no/trace amounts of manure compared to those with substantial manure applied.

Table 8. Effect of detrashing on height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo

Parameter	BSV symptoms	Detrashing			
		Routine	**Minimal**	**Mean for symptoms**	**difference**
Height [1]					
	without	377.47	378.10	**377.79**	
	with	367.59	366.48	**367.04**	
	Mean	**372.53**	**372.29**		**0.24** [ns]
	detrashing difference	(-2.61%)	(-3.07%)	**10.75*** (-2.85%)**	
Girth[2]					
	without	62.85	60.99	**61.92**	
	with	60.41	57.76	**59.09**	
.	Mean	**61.63**	**59.38**		**2.25****
	detrashing difference	(-3.88%)	(-5.30%)	**2.83**[***] **(-4.57%)**	
Bunch weight[3]					
	without	16.82	13.49	**15.16**	
	with	14.66	13.09	**13.88**	
	Mean	**16.67**	**15.16**		**2.45****
	detrashing difference	(-12.84%)	(-2.97%)	**1.28**[ns] **(-8.44%)**	

[1] Detrashing $P = 0.94$, Symptoms $P < 0.001$, Detrashing * Symptoms $P = 0.73$, [2] Detrashing $P < 0.01$, Symptoms $P < 0.001$, Detrashing * Symptoms $P = 0.54$, [3] Detrashing $P < 0.01$; Symptoms $P > 0.05$, Detrashing * Symptoms $P = 0.21$.

In farms where substantial amounts of manure were applied, BSV disease incidence was greater than where no/trace amounts were applied. The greater effects of BSV on the bunch weights in the manured farms are, therefore, a direct effect of the presence of more BSV disease in these farms as compared to the no/minimal manure farms. Farmer's application of substantial amounts of manure, however, improved as well as reduced the effects of BSV on plant growth. This probably explains why farmers reported that they applied manure to plants with BSV symptoms in attempt to manage the disease (R7529 Final Technical Report Annex 5 &6, Report of survey on Farmers' perceptions of BSV, 2002). Thus, the results for manure application are probably confounded by the action of the farmers who tend to target plants with BSV symptoms for manure application rather than applying manure evenly across the plantation. Hence, it still has not been established whether the amounts of manure applied by farmers are adequate for the nutrition of the bananas in these farms as a whole, or whether heavier applications are required to minimize the negative effects of BSV and improve bunch weights.

Table 9. Effect of manure on mean height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo

Parameter	BSV symptoms	Manure			
		Applied	No/trace applied	Mean for symptoms	difference
Height[1]					
	without	373.71	378.29	**376.00**	- 4.58^{ns}
	with	374.70	364.08	**369.39**	10.63* (-2.84%)
	Mean manure	**375.66**	**371.38**		
	Difference	- 0.99^{ns} (+2.65%)	14.21*** (-3.76%)		
Girth[2]					
	without	62.49	62.70	**62.60**	
	with	62.24	59.55	**60.90**	
	Mean manure	**62.36**	**61.12**		**1.24^{ns} (2.03%)**
	difference	(-0.40%)	(-5.02%)	**1.7* (-2.72%)**	
Bunch weight [3]					
	without	18.88	15.98	**17.43**	2.90*
	with	13.31	14.59	**13.95**	-1.28^{ns}
	Mean manure	**16.10**	**15.29**		
	difference	5.57*** (-29.50%)	1.39* (-8.70%)	**3.48*** (-24.9%)**	

[1] Manure $P = 0.42$, Symptoms $P < 0.05$, Manure * Symptoms $P < 0.05$; [2] manure $P = 0.18$, Symptoms < 0.5, manure * Symptoms $P = 0.07$, [3] Manure $P = 0.38$, Symptoms $P < 0.001$, Manure * Symptoms $P < 0.05$.

Effect of weeding on BSV, growth and bunch weight

BSV caused reduction in pseudostem girth ($P < 0.001$) and bunch weights ($P < 0.001$) regardless of whether banana fields were kept free of weeds or not for the duration of the study (Table 10). BSV effects were subject to an interaction with farmers weeding practice for plant height. The presence of many weeds (poor weeding) in farms caused an overall reduction in plant height (by 6.7%; $P < 0.001$) compared to farms with no/few weeds for most of the year (2.8%; $P < 0.001$). Though not significant, BSV disease reduced pseudostem girth more (8.7%; $P > 0.05$) in farms with poor weed control compared to those where the weeding was good (3.4%; $P > 0.05$). BSV also reduced bunch weights more (15.3%; $P > 0.05$) in farms with poor weeding compared to those where weeds were well controlled (12.01%; $P > 0.05$).

Weeds compete with banana plants for soil water and nutrients, are alternative hosts for nematodes and interfere with crop growth through allelopathy. These have a negative impact on plant growth, and thereby contribute to crop stress resulting in reduced bunch

weights and yields. In this study the negative impact of BSV infection on banana growth and bunch weights was greater in farms where the weed control was poor.

Table 10. Effect of weeding on height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo

Parameter	BSV symptoms	Weeding			
		Poor	Good	Mean for symptoms	difference
Height[1] (cm)	without	371.67	379.19	**375.43**	-7.52^{ns}
	with	347.94	368.56	**358.25**	-20.62***
	Mean weeds	**359.80**	**373.88**		
	difference	23.73*** (-6.38%)	10.63*** (-2.80%)		
Girth[2] (cm)	without	60.42	62.87	**61.65**	
	with	55.19	60.73	**57.96**	
	Mean weeds	**57.80**	**61.81**		**-4.01*****
	difference	(-8.66%)	(-3.40%)	**3.69***** **(-5.99%)**	
Bunch weight[3] (kg)	without	13.51	16.98	**15.25**	
	with	11.45	14.94	**13.20**	
	Mean weeds	**12.48**	**15.96**		**-3.48*****
	difference	(-15.25%)	(-12.01%)	**2.05*** **(-13.44%)**	

[1] Weeding $P < 0.001$, Symptoms $P < 0.001$, Weeding * Symptoms $P < 0.05$; [2] Weeding $P < 0.001$, Symptoms $P < 0.001$, Weeding * Symptoms $P = 0.08$; [3] Weeding $P < 0.001$, Symptoms $P < 0.05$, Weeding * Symptoms $P = 0.99$

Effect of intercropping on BSV, growth and bunch weight

BSV disease reduced plant height ($P < 0.01$), pseudostem girth ($P < 0.01$) but not bunch weight ($P > 0.05$) whether fields were planted solely to banana or with a systematic intercrop (Table 11). The farmer cropping practice had no significant effect ($P > 0.05$) on the growth and bunch weight of bananas, numerically, height, girth and yield was greater under sole cropping. BSV disease was not subject to an interaction with farmer cropping practice ($P > 0.05$). Nonetheless, BSV reduced plant height more under intercrops (3.6%) compared to under sole crop (3.2%). BSV also reduced pseudostem girth more under intercrops (6.9%) compared to under sole crop (4.3%).

Intercrops compete with bananas for light, nutrients and water, leading to crop stress. Crop stress is probably the reason BSV disease has greater negative impact on the growth of banana plants in fields with intercrops. In this study, however, bunch weight was reduced more under sole crop. The explanation for this is that bunch weights were reduced much more by intercrops for symptomless compared symptomatic plants. The stress due to intercrops, therefore, was not sufficient to reduce bunch weights substantially.

Table 11. Effect of farmer cropping practice on height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo

Parameter	BSV symptoms	Cropping pattern			
		Intercropped	Sole crop	Mean for symptoms	Difference
Height[1] (cm)	without	381.83	378.57	**380.20**	
	with	368.45	366.54	**367.50**	
	Mean Crop prac.	**372.14**	**375.56**		**-3.42^{ns}**
	difference	(-3.59%)	(-3.18%)	**12.7** (-3.34%)**	
Girth[2] (cm)	without	62.43	62.97	**62.70**	
	with	58.12	60.24	**59.18**	
.	Mean Crop prac.	**60.27**	**61.61**		**-1.34^{ns}**
	difference	(-6.90%)	(-4.31%)	**3.52** (-5.61%)**	
Bunch weight[3] (kg)	without	15.46	16.58	**16.02**	
	with	13.83	14.64	**14.24**	
	Mean Crop prac.	**14.64**	**15.61**		**-0.97^{ns}**
	difference	(-10.54%)	(-11.70%)	**1.78^{ns} (-11.10%)**	

[1]Crop prac. $P = 0.66$, Symptoms $P < 0.01$, Crop prac. * Symptoms $P = 0.88$; [2] Crop prac. $P = 0.37$, Symptoms $P < 0.01$, Crop prac. * Symptoms $P = 0.50$; [3] Crop prac. $P = 0.41$, Symptoms $P = 0.14$, Crop prac. * Symptoms $P = 0.90$.

Effect of overall management status on BSV, growth and bunch weight

BSV disease reduced the pseudostem girth and bunch weight ($P < 0.001$) of bananas regardless of whether farms were low (poorly), moderately and intensively managed (Table 12). The effects of BSV on plant height ($P < 0.001$) were subject to an interaction with the overall management regime of the farms. Here, BSV reduced plant height by about the same amount in minimally (by 4.2%) and intensively (4.1 %) managed farms. BSV reduced plant height the least in moderately (by 0.6%) managed farms. BSV reduced pseudostem girth more in minimally (9.4%) compared to moderately (4.6%) managed farms. BSV effects were not subject to an interaction with the overall management status for girth and bunch weight, but bunch weight of diseased plants progressively declined as the farmer management regime improved. In this regard, bunch weights were reduced by BSV about the same in minimally (13.27%) and moderately (13.45%) and by less (9.0%) in intensively managed farms.

Plants in intensively managed farms compared to those that were moderately and poorly managed produced a heavier bunch ($P < 0.001$). Bunch weights appeared slightly greater in moderately, compared to lowly managed farms, although the difference was not statistically significant ($P > 0.05$). Girth in intensively compared to low and moderately managed farmers was greater ($P < 0.001$). The intensively managed farms produced the tallest plants. For symptomatic plants the growth descriptors (plant height and girth) were smallest in the poorly managed farms. Growth of symptomatic plants was improved in moderately managed farms and further so in intensively managed plots.

These results are indicative that farmers' cultural management practices, individually and collectively, potentially improve the growth and bunch weight of bananas with BSV infection in farmers' banana fields. The overall management regime levels used in this study were based on criteria that gave soil and water conservation structures the greatest weighting (Table 1). Further analysis needs to be carried out to establish which combinations of farmer practices are the most beneficial.

Reduced banana growth and the loss in bunch weight due to BSV disease in farmers' fields was also observed in the on-station experiments at Kawanda and Mbarara (R7529 Final Technical Report Annex 3). These observations are in agreement with reports that BSV infection causes stunting (Gauhl and Pasberg-Gauhl., 1995) and significant yield loss (Dahal *et al*., 2000; Daniells *et al*., 2001).

BSV infection impedes plant growth and yield through its effect on physiological processes among others. Viral infections are reported to affect photosynthetic rates and carbohydrate metabolism in plants (Tecsi *et al*., 1995). In the on-station experiments at Kawanda and Mbarara, BSV infection reduced the rate of photosynthesis of banana leaves. This was attributed to a reduction in the amount of RuBISCO enzyme (the primary receptor of CO_2) in the leaves. The effect of BSV on photosynthesis is obvious from chlorosis and necrosis seen on leaves of infected banana plants. BSV infection in farmers' fields also caused necrosis in pseudostems, and therefore affected uptake of water and nutrients and the translocation of assimilates. It is also likely that BSV infection altered carbon metabolism since viral infections are observed to lead to increased cellular respiration (Tecsi *et al*., 1995) creating a metabolic sink, and thereby diverting resources from the processes of growth and development.

Farmers' cultural practices that cause crop stress enhance the negative impact of BSV disease on growth and development. Farmers' cultural practices that caused crop stress were: poor weeding, growing systematic intercrops, minimal desuckering (excessive suckers/plants) and minimal detrashing (substantial senescent and non functional leaves on plants). These practices cause bananas to compete for light, water and nutrients affecting growth and yields. On the other hand, farmer practices that relieved crop stress reduced the impact of BSV disease. The application of substantial amounts of manure, soil and water conservation structures, sole cropping, frequent weeding, routine desuckering and detrashing were observed to reduce the effects of BSV.

Table 12. Effect of overall management on height, girth and bunch weight of banana with and without BSV symptoms in Ntungamo

Parameter	BSV symptoms	Overall management (mgt)			
		Low	**Moderate**	**High**	**Mean for symptoms**
Height[1]					
	without	375.00 [c]	377.95 [c]	413.62 [a]	**388.86**
	with	359.24 [d]	375.59 [c]	395.67 [b]	**376.83**
	Mean overall mgt	**367.12**	**376.77**	**404.64**	
Girth[2]					
	without	61.99	62.40	70.84	**65.08[a]**
	with	58.34	61.63	67.41	**62.46[b]**
.	Mean overall mgt	**60.16[c]**	**62.02[b]**	**69.12[a]**	
Bunch weight[3]					
	without	15.44	16.66	20.58	**17.56[a]**
	with	13.39	14.42	18.72	**15.51[b]**
	Mean overall mgt	**14.42[b] (-13.27%)**	**15.54[b] (-13.45%)**	**19.64[a] (-9.04%)**	

[1]Overall mgt $P < 0.001$, Symptoms $P < 0.001$, Overall mgt * Symptoms $P < 0.05$; [2] Overall mgt $P < 0.001$, Symptoms $P < 0.001$, Overall mgt * Symptoms $P > 0.05$; [3] Overall mgt $P < 0.001$, Symptoms $P < 0.01$, Overall mgt * Symptoms $P = 0.98$

CONCLUSION

Although detrashing and desuckering reduced mealybug abundance (average number of colonies), the effect was not reflected in reduced proportion of plants infested with mealybugs or BSV incidence. This is possibly because spread of BSV is relatively slow and there is a lag period such that the current incidence of BSV is related to the management practices performed in previous seasons. Growing bananas with an intercrop resulted in more mealybug colonies than when bananas were grown alone, and also had greater BSV incidence. Some of the intercrop components were perennial, like the banana, were likely to be alternative hosts for the mealybugs. Over time they could influence the amount of spread of the virus. Practices aimed at improving soil and water conservation and soil nutrition, however, did not affect mealybug abundance or proportion of plants infested with mealybugs. The apparent effects of these management practices on BSV incidence were probably as a result of their effect on reducing BSV symptom expression rather than through directly influencing rate of BSV spread.

BSV reduced plant height, girth and bunch weight. Nonetheless some management practices are beneficial in both improving plant growth and also mitigating the effects of BSV infection. The practices that appear particularly useful are sole cropping (no intercrops), soil and water conservation structures, frequent weeding, routine desuckering and detrashing, and application of manure. However, results for the latter may have been confounded by the tendency of farmers to target plants showing BSV symptoms for manure and mulch application in the mistaken belief that these symptoms are due to nutrient deficiency. In order to achieve full effect, there is probably a need for the practices to be

applied more frequently and intensively than is currently being done by farmers. The cost effectiveness of these practices against the benefit in terms of yield increase is not known and must be considered if they are to be promoted. This study does not give an indication of the yield increase achievable using the management practices for different levels of BSV incidence and severity. In instances where severity is high and management practices do not mitigate BSV effects, roguing could be the more appropriate measure in controlling the disease. Its applicability, though, is not known. Roguing may also reduce the spread of BSV. It is probable that most of these practices may not be fully beneficial individually but would be in combination.

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FARMER KNOWLEDGE AND PERCEPTIONS OF BANANA STREAK VIRUS ON EAST AFRICAN HIGHLAN BANANAS IN VILLAGES WITH RELATIVELY HIGH BSV INCIDENCE IN SOUTHWESTERN UGANDA.

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INTRODUCTION

Banana is a very important dual-purpose crop as source of food and farm cash income providing for 65% of the calorific intake for the population in Uganda (Dept. of Statistics 1998). Banana is also the leading crop in the country, occupying 31% of the total national cropped land, followed by millet (8%), cassava (8%) and sweet potatoes (5%) (Dept. of Statistics 1996). Banana, being a perennial crop, has an advantage over annual crops such as millet and maize because it does not require regular rains for germination of seed. Its canopy provides shade and permanent ground cover making the crop environmentally friendly.

In terms of food tastes and preferences, banana is the most preferred single food security crop in central and western Uganda. Banana is even referred to as "food" in the language of central Uganda. However, in the last 30 years, banana productivity has been declining due to biotic and abiotic constraints such as banana weevils, nematodes, Sigatoka diseases, exhausted soil fertility and socio-economic factors (Gold *et al*., 1993).

A baseline study of disease caused by *Banana streak virus* (BSV) using focus group discussions was undertaken in two major banana-producing districts of Masaka and Ntungamo in south-western Uganda respectively. The general objective of the baseline survey was to assess farmers' perception and knowledge of BSV, together with other biotic and abiotic constraints, which reduce banana yields, and to identify farmers' preferred control measures for BSV in the study areas.

The specific objectives of this study were:

- To assess farmers' knowledge and perceptions of symptoms and causes of BSV and other related constraints that reduce banana yields.
- To determine the management practices being used by farmers to reduce the effects of the disease on banana yields.
- To identify farmers' knowledge gaps in their interpretations of the symptoms and causes of BSV.

The research was conducted in two parts: Part one dealt with Farmers' Focus Group discussions whose results are presented in this report. Parts two dealt with individual households/group farmers' interviews, the results of which are presented in the BSV baseline socio-economic survey report.

Methodology

Systematic Purposive Sampling

The research team selected two districts, Masaka and Ntungamo that were known to have relatively high incidence of BSV (Kubiriba *et al*., 1997). The team of researchers visited and held meetings with extension officers as key informants in each of the two districts. The meetings/discussions produced lists of the most important banana producing sub counties that also had BSV. Thereafter the research team obtained a district map and marked on the map the sub counties said to have a high incidence of BSV.

Focus group discussion

The sub county informants were requested to list villages that had high incidence of BSV in Masaka and Ntungamo districts. Using the list, three and two villages were then randomly selected from Masaka and Ntungamo districts respectively. Eligible farmers' in the selected villages were those with high incidence and clear symptoms of BSV. These farmers were identified and then requested to invite at least two of their neighbours, who in turn assisted in inviting 10-15 farmers that formed the discussion groups. Care was taken to ensure that each group had a gender balance, the participants generally had farm sizes of 5.0 acres, were opinion leaders and grew banana for food and income.

The hypothesis underlying the sampling scheme of villages with relatively high incidences and severe BSV expression is that if farmers in these villages do not recognise the symptoms then it was unlikely that farmers from elsewhere would recognise them.

After assembling a village focus group at the first banana farm, the researchers explained the purpose of the visit and requested farmers to assist in describing what they considered "bad signs (symptoms)" in the banana plantation. Once the farmers exhausted the specific symptom then the group would move to the next farm and discussion would continue.

Having had a farmer describe a sign or a symptom, the group would then discuss and agree on what name the symptom was called in the local language. The researchers noted and labelled with scotch tape the symptom both in local language and in English and took a photograph of the symptom.

Table 1. The number of farmers who participated in the Focus group discussions

District	Sub-county	Village	Number of participants	Number of women
Ntungamo	Ruhama	Nyakakongi	15	7
Ntungamo	Bwongyera	Kakiika	11	3

Masaka	Kissekka	Kibaale	10	4
Masaka	Bigasa	Kyazziza	13	10
Masaka	Butenga	Butenga	10	5

- Age of participants ranged from 19 to 55 years old and education level averaged at 6 years

Results

The farmers in all the discussion groups described yellow patches on the leaves or leaves with a mixture of yellow and green as a "bad sign" on East African Highland Bananas. The condition is referred to as 'Slim w'ebitooke' literally meaning the AIDS of bananas in Bigasa sub-county (Table 3). The scientific interpretation of these signs upon examination of specimen was that they were chlorotic and necrotic streaks/blotches associated with BSV infection. According to some farmers, yellow patches on leaves normally occurred with drying of the cigar leaf (die back). Die back is, however, associated with a number of banana constraints including, weevils, nematodes, wilt disease and drought, to mention a few (Table 3). In Bwogera (Table 2), black spots in the pseudo stem were mentioned as an indication of a problem in the bananas. Farmers cut some banana plants with these symptoms, which revealed that the plants had a condition, referred to as internal pseudo stem necrosis according to the scientists' interpretation. The symptom is typical of plants with BSV and in this case the plants also had chlorotic streaks to the leaves. The farmers also said there was an association between these symptoms and black spots on the fingers of the banana bunch. In Kisekka (Table 4) and Butenga (Table 5) sub-counties, farmers reported that plants with the symptoms did not flower and if they did, they later died. These symptoms were considered to be more severe in the rainy season than in the dry season according to farmers in Kisekka. Farmers in Bigasa and Butenga sub-counties said that plants with the symptoms produced a small bunch.

Farmers attributed the symptoms, associated by the scientists to BSV infection, to weevil infestation in all the focus groups. In Kisekka, the farmers thought that yellowing of leaves was caused by failure of the plant to take up water because of drying roots caused by weevil damage. In Bwongera and Bigasa sub-counties, soil fertility was also mentioned as the cause of BSV. Some farmers in these sub-counties had no explanation of the cause of the BSV symptoms. Drought and Kaasa (black ants) were also reported as the cause of the symptoms of BSV in Butenga sub-county.

Roguing was reported to be the most commonly used method in the management of BSV symptoms. Farmers in Masaka revealed that they replanted immediately after roguing except in Kisekka where replanting was done 2 months after roguing. In addition to roguing, farmers in Bigasa and Butenga sub-counties applied biorationals (urine, pepper, tobacco), which were used primarily to control weevils. In Kisekka sub-county, corm removal was carried out to control BSV symptoms. Here too, farmers applied manure to plants with BSV symptoms and together with those in Butenga sub-county cited weeding as a means of managing the symptoms (Table 4).

Farmers in the focus group discussions described black tunnels in the lower corm (corm damage) as a sign of a banana constraint. Farmers in Masaka District observed that snapping of the banana pseudostem indicated a problem in bananas. Multiple tunnelling of the upper corm was also cited as a sign of a banana problem in Bigasa (Table 3) and Butenga (Table 5),

while rotting of the shoot was also considered as a sign of banana weevil infestation in Bigasa (Table 3). In Kisekka (Table 4), it was reported that bananas with these symptoms would not flower or if they did gave small bunches. These signs were correctly attributed to weevils. While farmers clearly differentiated between the adult and larvae stages of weevils, they had varied views on which stage caused damage to bananas. They reported that the adult weevil caused tunnelling of the corm while snapping and damage to the shoot were attributed to larvae.

Cultural management practices (pseudostem trapping, removal of the corm of harvested plants, de-trashing, applying ash, tobacco, urine and pepper) were widely used to reduce weevil infestation. In Kisekka (Table 4), farmers mentioned that they applied carbofuran (Furadan) for the control of weevils.

Yellowing and drying of leaves in Bogoya (Gros Michel), Kayinja (Pisang awak) and Ndizi (Apple banana) were mentioned as a sign of a serious constraint in Ruhama (Table 1), Bigasa (Table 3) and Kisekka (Table 4). The scientists interpreted the symptoms to be those of Fusarium wilt upon the observation of a specimen. Some farmers in the areas who described the signs associated them with a condition referred to as "Todula" but were not aware of the cause. However, some farmers attributed the symptoms to drought (Table 1) and weevils (Tables 3 and 4). Farmers generally had no idea of how to manage the disease. Those in Bigasa (Table 3), however, rogued infected plants while in Kisekka (Table 4) farmers rogued and cut infected plants.

Some farmers also described rotting of roots (Tables 3 and 4), toppling and drying resulting in small bunches (Table 3) as signs of a constraint in bananas. The examination of the roots revealed that the symptoms were a result of nematode infestation by the scientists. The farmers reported that weevils, black ants, and high mat were the primary causes of these symptoms. Pseudostem trapping, de-trashing and earthing-up were identified as ways of managing the constraint.

White insects found on banana, pineapples and sugarcane were referred to as constraints to banana only in Butenga, Masaka (Table 5). But the farmers did not know the cause of these constraints nor were control measures for these insects known. The insects were identified as mealybugs on examination.

Several signs, namely, failure to fruit and death (Table 1), die-back (Tables 3 and 5) and small bunches were considered as those of constraints in bananas. Weevils, high mat, dry roots and soil infertility were mentioned as causes of these signs. The symptoms could be due to any one or combination of, nematodes, weevils, poor nutrition, moisture stress and disease from a scientific point of view. Management options known to farmers to counter the constraints were weed control, loosening of soil, application of ash and manure and removal of dry sheath to the base of the pseudo stem.

Discussion

Farmers could describe signs associated with BSV but attributed the symptoms to other constraints. Notably, weevil infestation was commonly associated with symptoms of BSV by farmers in all the focus group discussions held. This was especially so where bananas with BSV symptoms were also infested with weevils. Weevils are the most noticeable pests in bananas; therefore, it is logical for farmers to associate BSV symptoms with weevils

In addition to weevils, farmers associated signs/symptoms attributed to BSV with soil infertility and moisture stress. This is probably due to the fact that plants with BSV infection show either one or a combination of the following symptoms: poor growth, leaf yellowing, cigar leaf necrosis (die-back) and small bunches. These are typical moisture stress and nutrient deficiency symptoms. The perception is reinforced by observations that the symptoms are more pronounced in marginal soils and in drought conditions. Farmers in Butenga sub-county mentioned drought as a cause of the symptoms typical of BSV infection. In contrast, those in Kisekka sub-county observed that BSV symptoms were more severe in the rainy season as compared to the dry season. Daniells *et al.,* (1999) and Offei (1997) corroborate the latter, but an earlier study by Lockhart (1986) reported that BSV symptoms are more severe in the dry season. These variations in symptom expression could be attributed to differences in environmental conditions and/or virus strains (Walker, 1990). Recent work has shown that there are at least 11 different 'species' or strains of BSV in Uganda (Harper *et al*., 2002).

Farmers with access to extension services, for example in Kisekka sub-county, reported ignorance about BSV and related its symptoms to other constraints. This suggests that even the field extension personnel do not know about BSV and also associate the symptoms of BSV with other constraints. Nonetheless, farmers often claimed to be managing plants with BSV infection in a unique manner. Among the practices that they reported they were adopting in the management of plants with BSV symptoms were roguing and the application of urine, ash and manure. The latter basically provides plant nutrients. These practices are indigenous and have evolved from their experiences. The application of nutrients potentially has the effect of mitigating the effect of BSV on bananas (Dahal *et al*., 1998). Roguing of infected materials and replanting with symptomless plants is one of the recommended practices for management of BSV. Farmers, however, do not have a mechanism of screening planting material for replanting since they have no knowledge about BSV especially when the virus is at the initial stages of development. This is possibly, why in Bwongyera where roguing and replanting is practised, farmers observed that there is no remedy for the BSV symptoms.

All the focus group discussion sessions held described signs/symptoms attributed to weevil infestation (tunnelling of corm and snapping) and accurately associated them with weevils. However, they viewed the adult and larvae stages of the weevil as different pests. The farmer's lack of knowledge about the life cycle of weevils is reflected in the management options that they use to control weevils. For example, in Bigasa sub-county, tunnelling of the corm is perceived to be due to the adult weevil so farmers use pseudostem trapping while corm removal of harvested plants is employed when bananas snapped. This is because farmers attribute the latter to weevil larvae. To effectively control weevil infestation knowledge of the life cycle of the weevil is important. Practices that reduce weevil populations like trapping and those that deter weevils from laying eggs like pseudo stem splitting, as well as those that denying larvae feeding grounds such as removal of corms of harvested plants, must be combined. Therefore,

dissemination of the cultural control practices, popular with farmers due to their low cost, could be more beneficial to farmers if it includes other information such as on the life cycle of the weevil; more so if this information relates to the control measures. To manage weevils, farmers commonly use a concoction of animal urine, ash, pepper and tobacco. It is only in Kisekka sub-county that farmers mentioned that they used carbofuran (Furadan) to control weevils. This is probably because they have access to information through the extension service. However, use of insecticide is limited due to its prohibitively high cost.

Focus group discussions held in Masaka revealed that farmers associate signs of nematode damage (root necrosis and toppling) with those attributed to weevils. Nematode infestation has been reported as a major constraint of bananas in Masaka (Gold *et al*., 1993) and farmers consistently mentioned the signs/symptoms that are generally attributed to nematodes. In addition to weevils, farmers in Kisekka sub-county associated nematode infestation with earthworms that they have adopted for nematodes. They are unable to visualise the microscopic nature of nematodes and therefore perceive nematodes as macroscopic worms found in the rhizosphere of the banana plant. As such, farmers use practices for the management of weevils to target nematodes as well. In addition, farmers in Bigasa sub-county reported that earthing-up was a measure used to manage signs due to nematodes because "high mat" was perceived as a cause of the problem. Not only do farmers carry out ineffective measures to control nematodes, they also carry out practices such as intercropping bananas with beans, which is known to favour nematode build-up in bananas. Beans are an alternative host for some nematodes and a bean intercrop will increase nematode populations in bananas (Namaganda *et al*. 2000). The absence of reports about signs of nematode infestation in Ntungamo is attributed to the fact that the district generally has a low incidence of nematode damage. Studies done in the district have reported that it has low populations of burrowing nematodes that are responsible for nematode damage (Okech *et al.*, 2000).

Signs of Fusarium (Panama) wilt were accurately described but were also associated with weevil damage in Masaka and drought in Ntungamo. Several farmers in both districts termed it as an unknown disease of Pisang awak, Gros Michel and Apple bananas. The association of Fusarium wilt to drought is due its similarity to moisture stress symptoms. It is related to weevils because plants with Fusarium wilt are usually also infested with weevils. However, Fusarium wilt is the only constraint that farmers do not manage with weevil control measures despite the fact that it is attributed to weevils. The management option of choice for the farmers is roguing. This is due to the devastating nature of the disease. Through experience farmers have learnt that it takes only one infected plant to destroy a whole plantation and therefore infected plants are best rogued quickly. Pisang awak is used for brewing banana wine or gin and Gros Michel and Apple bananas are dessert bananas. These bananas are a cash crop for the farmers and their livelihoods depends on them. The devastating nature of Fusarium wilt has earned it the name "Todula", literally meaning do not brag. This is in reference to the fact that once the disease attacks a farmer's plantation he is left without a source of income and a livelihood. Fortunately, several cultivars that are resistant to Fusarium have been tested (Tushemereirwe, *et al*. 2000) and are available at Research Stations. However, the focus group discussions revealed that little information about Fusarium and its control is available to the farmers.

Conclusion

Generally, farmers were aware of the signs of constraints limiting banana production. However, they found it hard to clearly associate causes to signs/symptoms. Farmers described symptoms of BSV, Fusarium wilt and nematodes as causing crop loss, but their symptoms were attributed to other constraints. The only farmers' descriptive symptoms that were in agreement with scientists' interpretations are those of weevil infestation.

Farmers said symptoms attributed to BSV by scientists caused poor growth and small bunches. According to the farmers the symptoms were mainly caused by weevils and also by drought and soil infertility. As a result most of the practices used by farmers to manage BSV symptoms instead targeted weevils and soil infertility. Although these management practices do not target BSV, they may mitigate the symptoms since the plants become less stressed by other pests/diseases or become vigorous as a result of improved nutrition.

Misidentification or management of one constraint for another is understandable since they have to be associated with what can be visualised to the problems encountered. BSV that is caused by virus, nematodes and Fusarium wilt that cannot easily visualised by farmers seem to be less important and yet could be the major constraint to banana production. There is need for more dissemination of information regarding the symptoms/signs and management practices for BSV (and the other diseases/nematodes) for it to be managed more effectively.

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Table 1: Responses from the focus group discussion held in Nyakakongi village, Kashari parish, Ruhama sub-county, Ntungamo district

Farmers' description of Sign/ symptom of a banana constraint	Farmers' perceived cause of symptom	Management options known to farmer for constraint	Scientific interpretation of sign/symptom
• Yellowing of leaves	Weevils (adult and larvae)	Rogue affected plant	Golden yellow streaks/blotches on leaves due to BSV
• Drying or rotting of tongue (cigar) leaf;	Weevils (adult and larvae)	Rogue affected plant	Drying of cigar leaf as a result of internal necrosis due to BSV.
• Flowering plant does not give bunch and dies	Weevils (adult and larvae)	Removal of corms of harvested plants	Nematode infestation Weevil infestation Moisture stress Soil infertility Wilt diseases
• Drying of leaves of Musa, Pisang Awak, Gros Michele and Apple bananas	Drought Unknown disease	None	Fusarium wilt
• Bunch fingers have brown covering	Unknown	Remove male buds to reduce moving from sick plant to healthy plant	Thrips
• Weevils and black tunnels in corm	Weevils	Pseudostem trapping, applying rabbit urine and ash and corm removal	Weevil infestation

Comment is that the farmers lack technical information because there is no extension staff.

Table 2: Responses from the focus group discussion held in Kakiika village, Kashaha parish, Bwongera sub-county, Ntungamo district.

Farmers' description of Sign/ symptom of a banana constraint	Farmers' perceived cause of symptom	Management options known to farmer for constraint	Scientific interpretation of sign/symptom
• Yellowing of leaves	Weevils (larvae) and insect with teeth (adult)	Rogue affected plant	Golden yellow streaks due to BSV
• Pseudostem has black spots • Heart (meristem) is black • Tongue (cigar) leaf dry and fails to emerge • Fingers develop black spots	Unknown Caused by soil-infertility	Rogue affected plant Symptoms have no remedy.	Black spots in pseudostem and black heart is internal necrosis due to BSV. Drying of cigar leaf as a result of internal necrosis.
• Black tunnels in corm	Weevils (adult)	Removal of corm of harvested plants	Weevil infestation

Table 3: Responses from the focus group discussion held in Kyazziza village, Bigasa sub-county Masaka district.

Farmers' description of Sign/ symptom of a banana constraint	Farmers' perceived cause of symptom	Management options known to farmer for constraint	Scientific interpretation of sign/symptom
• Multiple tunneling of the upper corm • Black tunnels in lower corm • Snapping • Rotting of the heart of the pseudostem (meristem)	Adult weevils Weevil larvae	Pseudostem trapping Applying ash, urine, pepper, tobacco Corm removal De-trashing.	Weevil infestation
• Cigar leaf drying, • Poor plant growth	Don't know Weevil Soil infertility	Rogue the affected plant	BSV Nematode Weevils Moisture stress Wilt disease
• Leaves that are a mixture of yellow and green termed as AIDS of bananas. Results in small bunches	Weevils(Adult) Weevil Larvae Don't Know Soil infertility	Rogue plant and replant Apply ash, urine, pepper	BSV
• Yellowing and drying of leaves affecting Musa, Pisang l'Awak, Gros Michele, Apple banana	Weevils Don't know	Rogue affected plant	Fusarium wilt
• Drying of roots • Rotting of roots • Toppling of bananas • Resultant in small bunches	Black ants Weevils High mat	Earthing-up bananas De-trashing Trapping	Nematode infestation

Table 4: Responses from the focus group discussion held in Kibaale village, Nakalembe parish, Kisekka sub-county Masaka district.

Farmers' description of Sign/ symptom of a banana constraint	Farmers' perceived cause of symptom	Management options known to farmer for constraint	Scientific interpretation of sign/symptom
• Rotting of roots	Weevils Earthworms	Trapping weevils using leaves and pseudostem and killing them. Splitting of pseudostem (sanitation)	Nematode infestation
• Black tunnels in corm • Snapping • Bananas may not flower and if they do, give small bunches	Tunnelling by black ants Weevils	Removing dry sheath Application of carbofuran (Furadan) Mulch away from the mat Removal of corms of harvested plants	Weevil infestation
• Yellow patches of the leaves, turn brown and dry the leaf completely including drying of cigar leaf • Plant may not flower or if bears fruit finally dies • Symptoms more sever in the rain season as compared to the dry season	Drying of roots damaged by weevils above resulting in the failure of water uptake	Rogue plants showing these symptoms and replant 2 months later Applying manure Corm removal Regular weeding	BSV infection
• Poor plant growth affecting Musa, Gros Michele and Apple banana • Result in browning (patches) of the middle section of corm • Gives a smelly odour and core of corm (meristem) rots	Not known Weevils	**Cut down the diseased plants** Rogue plant	**Fusarium wilt**

Table 5: Responses from the focus group discussion held in Butenga village, Kawoko parish,Butenga sub-county Masaka district

Farmers' description of Sign/ symptom of a banana constraint	Farmers' perceived cause of symptom	Management options known to farmer for constraint	Scientific interpretation of sign/symptom
Disease/condition affecting the cigar leaf (Drying of cigar leaf)	Weevil (adult) Weevil larvae bores through the heart (meristem) of the plants Poor plantation management Soil infertility Disease/condition existed in suckers	Rogue and replant Replant health plants Apply ash and urine	Nematodes Weevils Drought Wilt Disease BSV
Yellow patches on the leaves Drying of cigar leaf Plants don't flower or give small bunch	Weevil infestation Black insects Drought	Loosen soil Weed Apply ash, pepper, tobacco & urine Rogue plants with symptoms and replant Split corms of plants with rotten cores (necrosis)	BSV
White insects found on bananas, pineapples and sugarcanes	None	None	Mealybugs
Small bunches with curling leaf	Weevils Soil infertility, Soil loss High mat Dry roots	Weed control Loosen soil Remove dry sheath to base of pseudostem Apply ash and urine	Nematodes Weevils Poor nutrition Moisture stress diseases
Snapping of plants Rotting base of the pseudostem	Weevil	Trap weevils using pseudostem and pick the following day – not effective. Apply ash and urine Remove corm	Weevils

Table 6: Summary of responses from the focus group discussion

Farmers' description of Sign/ symptom of a banana constraint	Farmers' perceived cause of symptom	Management options known to farmer for constraint	Scientific interpretation of sign/symptom
- Yellowing of leaves - Leaves have a mixture of yellow and green termed as Slim w'ebitoke (AIDS of bananas) dies back - Yellow patches on leaves turn brown and dry leaf completely, plant may give small bunch, not flower or bear bunch. - Symptoms more severe in rain season as compared to dry season - Pseudostem has black spots - Fingers develop black spots	- Weevils (adult and larvae) - Soil infertility - Drying of roots damaged by weevils resulting in the failure of water uptake - Black ants - Drought - Do not know	- Rogue affected plant - Roguing plants showing symptoms and replanting two months after. - Apply manure, ash, urine and pepper - Symptoms have no remedy - Corm removal - Regular weeding - Loosen soil - Split corms of rotten cores (internal necrosis of pseudostem)	BSV infection
- Rotting of the heart of pseudostem (meristem) - Poor plant growth - Tongue (cigar) leaf dries - Flowering plant does not give bunch and dies - Small bunches with curling leaf	- Weevil larvae - Weevil adult - Soil infertility - Poor plantation management - Disease/condition existed in suckers - High mat - Dry roots	- Rogue affected plants and replant with healthy plants - Apply ash and urine - Weed control - Loosen soil - Remove dry sheaths to base of pseudostem	- BSV infection - Nematode infestation - Weevil infestation - Soil infertility - Drought/moisture stress - Wilt disease

Table 6 (continued): Summary of responses from the focus group discussion

Farmers' description of Sign/ symptom of a banana constraint	Farmers' perceived cause of symptom	Management options known to farmer for constraint	Scientific interpretation of sign/symptom
- Weevils and black tunnels in corm - Multiple tunnelling in upper corm - Snapping - Black tunnels in lower corm - Bananas do not flower, if they do give small bunches - Rotting of base of pseudostem	- Weevils (Adult and larvae) - Tunnelling by black ants and weevils	- Pseudostem trapping - Applying rabbit urine, ash, tobacco, pepper - Removal of dry sheath - Corm removal - Removal of corm of harvested plants - Application of carbofuran (chemical) - Mulch away from mat - Trap weevils with pseudostem and pick the following day – not effective	Weevil infestation
- Yellowing and drying of leaves of Kayinja (Pisang awak), Bogoya (Gros Michel) Ndizi (Apple bananas) - Poor plant growth affecting Kayinja (Pisang awak), Bogoya (Gros Michel) Ndizi (Apple bananas. Result in the browning of the middle section of corm	- Drought - Weevils - Unknown disease	- Cut down disease plants - Rogue affected plants - Not known	Fusarium wilt of bananas
- Drying of roots - Rotting of roots - Toppling of bananas resulting in small bunches	- Black ants - Weevils - High mat - Earthworms	- Earthing-up bananas - Removal of dry sheaths - Weevil trapping - Splitting pseudostems (sanitation)	Nematode infestation
- White insects found on bananas, pineapples and sugar canes	- Not known	- Not known	Mealybugs
- Bunch fingers have brown covering	- Unknown	- Removal of male buds to reduce spread	Thrips

BASELINE SURVEY REPORT: BANANA STREAK VIRUS ON EAST AFRICAN HIGHLAND BANANAS IN SOUTH AND WESTERN UGANDA

INTRODUCTION

Banana is the most important single food security crop grown by the majority of small scale /resource poor farmers in central, eastern and western regions of Uganda (MAAIF, 1999). Uganda is the leading banana producer in Africa. In 1989, Uganda produced 44% of the total production in East Africa and 24% of the total production in Africa (FAO, 1990). Studies from a diagnostic survey (Rubaihayo, 1993) indicated that most (65%) of the farmers in the banana producing districts of Uganda ranked bananas as their number one or two preferred food crops. Other studies have shown that 72% of the farmers in central, eastern and western regions of Uganda allocated 40% of their farmland to growing bananas (Ngambeki *et al.,* 1992).

However, in Uganda most bananas are consumed locally, with per capita consumption of 300-500kg/year of edible portion (Ngambeki *et al*., 1992, Adupa *et al.*, 1993). At the national level, it has been shown that cooking banana (matoke) is the leading staple food consumed on an average making 8.67% of the staple consumed per household, per month (integrated household survey 1993). This level of banana consumption is followed by cassava at 6.22%, sweet potatoes 5.64%, maize 4.79% and millet 3.7%.

The point price elasticities of demand for bananas in Uganda were estimated to be –0.11 to –0.13 for urban consumers (Adupa, 1993). In addition, income demand elasticities of major staple foods were estimated for millet 0.10, cassava 0.13, bananas 0.16 and maize 0.2 (Vanegas, 1990). Both price elasticities of demand and income demand elasticities of bananas suggest a) that 10% increase in price of bananas will reduce the demand of bananas by a very small margin of only 1 to 1.3%. b) That 10% change in the levels of income in urban centres will change the demand for bananas by 1.6%. This implies that bananas is regarded an essential consumer food and that quantities demanded remains stable irrespective of fluctuations in market prices and/ or changes in the levels of income.

Although bananas are very important and have been grown in Uganda for over 100 years (Simmonds, 1996), yields have steadily declined since the 1970s, while the area under production increased (MAAIF, 1999). The yield decline has been attributed to a number of factors including pests, diseases, declining soil fertility and socio-economic problems, among others (Gold *et al*., 1993). *Banana streak virus* is part of the disease complex that limits banana production but its importance has only been recognised recently (Tushemereirwe *et al*., 1996). It is therefore important to find out the perceptions of farmers about the disease in terms of cause, symptom description and farmers' preferred management practices. This then could be the basis for the development of management practices that form part and parcel of the farmers' social set up and therefore be more acceptable to them.

Objectives of the study

The study aimed at:

(a) Assessing farmers knowledge or/and perception of BSV in relation to banana production

(b) Determine the management practices being used by farmers to manage the disease.

The area of study

The research team selected three districts (Masaka, Ntungamo and Rakai) in southern and western Uganda that were identified as having high *Banana streak virus* incidence (Kubiriba *et al*., 1997). Masaka district is located in the Lake Victoria crescent agro-ecological zone, within longitude 31 0 E to 32 0 E and latitude 0 0 S to 0.35 0 S. it has an altitude of about 1200m.a.s.l. with moderate temperatures and a bimodal rainfall pattern (March-May and September-November). The main economic activities are agricultural, focussing on growing perennial crops such as bananas and coffee followed by annual crops including sweet potatoes beans and maize. It has a population of about one million people.

Rakai district is adjacent to Masaka, located in the southern-dry-land agro-ecological zone within longitude 31^0E to 31^0 40'E and latitude 0^0 00'S and 1^0 00'S. It has an altitude of about 1200m.a.s.l. with high temperatures and bimodal rainfall similar to Masaka. Its main economic

activities are agriculture focussing on bananas, coffee, finger millet, maize, beans and sweet potatoes. It has a population of about 0.5 million people.

Ntungamo district is located in the southern dry land agro-ecological zone within longitude $30^0$00'E and $30^0$32'E and latitude $0^0$30'S and $1^0$30'S with an altitude of about 1500 m.a.s.l. It has moderate temperatures and a bimodal rainfall similar to Masaka and Rakai. Its main economic activities are agriculture focussing on growing bananas, sweet potatoes, finger millet and livestock keeping.

SAMPLING METHODOLOGY

The research team visited and held meetings with the extension officers as key informants in each of the three districts. The team was informed on the general banana farming over the districts and possible distribution of BSV within the sub counties. A multistage random sampling scheme was used to sample farmers for interview and sampling for BSV. Stratification was based on administrative units. Using the sub-county as a main basis for stratification, each district was divided into areas or sub counties having predominantly banana based farming systems. The sub counties were then sub-divided into parishes and then parishes into villages. The sub counties that had high and low incidence of *Banana streak virus* were identified. Using stratified, systematic and purposive sampling procedures, about 36, 39 and 69% of sub-counties of Rakai, Masaka and Ntungamo districts respectively were selected for the study.

At the selected sub county headquarters, the team was informed of parishes with bananas and those without. Similarly, an indication of BSV level was communicated to the team. On this basis two parishes were systematically selected such that they do not border each other. Again on similar basis two villages were selected in each of the selected parishes. In each village candidate farmers were those who had more than one acre of banana plantation with BSV symptoms. Lists of such farms were prepared with the help of extension staff and the key informant in the selected villages. A farmer was then randomly selected to represent each village for the baseline survey.

In all a sample size of 90 farmers were selected from 88 villages, 45 parishes, and 26 sub-counties (Table 1). A structured questionnaire was used to collect data relevant to management of banana crop and in particular BSV from household heads on each farm. In each case, the interviews were conducted in the field and additional to this the team visited the farmers' fields and scored BSV severity, incidence and management (reported elsewhere).

Table 1. Sampled sub- counties, Parishes and Villages in the study area

Site	Total No. of sub-counties	Total No. of sub-counties sampled	No. of Parishes sampled	No. of Villages sample
Rakai	22	8	15	28
Masaka	23	9	15	30
Ntungamo	13	9	15	30

Data analysis

The data was coded and entered on spreadsheets. The analyses were carried out on SAS (SAS Institute Inc, 1990). Cross-tabulation and descriptive statistics was used to summarize the data.

RESULTS AND DISCUSSION

Demographic characteristics of the farmers in the study area

The characteristics of banana farmers {household heads (HH)} sampled for this study are partly described by their demographic variables such as age, education and sex (Table 2). Of the HH sampled, 35 were female and the other 55 were male. Overall, the average age of the HH in the study area was 46.9 years, with those in Rakai being relatively younger (44.6 years) than those in Masaka (46.7 years) and Ntungamo (49.7 years). Forty percent (40%) of the farmers were aged between 19 and 40 years, 34% aged between 41 and 59 years and 26% were over 60 years old. Male farmers averaged 47.8 years old and females 45.6 years old. Apart from in Ntungamo, most of the female farmers were relatively younger than the males.

Male HH had on average received more schooling (6.1 years) than the females (4.6 years). Family size ranged from 2 to 21 persons with an overall average of 7.5 persons per family.

Table 2. Demographic characteristics of the farmers in the study area

Variable	Average per district (± Standard error)			
	Rakai	Masaka	Ntungamo	Overall
Age of farmers (yrs	44.6 ± 3.05	46.7 ± 2.76	49.7 ± 3.39	46.9 ± 1.76
Age of male farmers (yrs)	47.2 ± 3.91	48.5 ± 3.65	47.9 ± 4.67	47.8 ± 2.35
Age of female farmers (yrs)	39.6 ± 4.70	44.9 ± 4.21	53.2 ± 4.11	45.6 ± 2.64
Education of farmers (yrs)	7.8 ± 0.68	4.9 ± 0.44	3.7 ± 0.62	5.5 ± 0.40
Education of male farmers (yrs)	8.4 ± 0.98	5.1 ± 0.62	4.6 ± 0.62	6.1 ± 0.51
Education of female farmers (yrs)	6.6 ± 0.67	4.7 ± 0.66	0.8 ± 0.80	4.6 ± 0.58
Family size (persons)	8.3 ± 0.69	6.8 ± 0.71	7.2 ± 0.75	7.5 ± 0.41

Sources of agricultural information

Availability of technical information is one the major factors that may influence the effective management of any banana disease. Of the farmers responding, 33 indicated that their main source of agricultural knowledge was from immediate family/relations (= indigenous knowledge), while 20 said extension services, and 14 said fellow farmers (Table 3). However, the distribution of main sources of information was very different in Rakai compared to in Ntungamo and Masaka.

Table 3. Primary sources of banana production information

Source of information	Number of farmers			
	Rakai	Masaka	Ntungamo	Combined
Indigenous knowledge	0	16	17	33.0
Extension	8	6	6	20.0
Fellow farmers	10	1	3	14.0
Seminars	6	1	1	8.0
Radio	2	3	1	6.0
NGOs	0	1	1	2.0
Tours / visits	0	1	0	1.0

Farmers identified constraints to banana production, and management targeting those constraints

Overall, most of the HH (87/90) ranked weevils (both larvae and adult) as either the first or second most important constraint to banana production (Table 4). Twenty six farmers ranked yellowing of leaves/black spots on fruits/ leaves of plants rotting in Matoke (AAA-EA) as first or second most important constraint. The scientists in the team interpreted these symptoms to be those of BSV. Other important constraints that were ranked highest were: weeds (20), drying of bananas (12), nematodes (12), exhausted soils (9), yellowing of leaves on Kayinja (Pisang awak) and Ndizi (apple bananas) (3), drought (4) and drying cigar of leaf (5) (Table 4). These constraints were interpreted as those of weed infestation, Black Sigatoka, nematode infestation, soil infertility, Fusarium wilt and die-back. Die-back could be attributed to various causes,

including, moisture stress, BSV infection, Fusarium wilt, weevil and nematode infestation according to the Scientists' view.

Table 4. Major banana production constraints faced by farmers in these districts of Uganda

Constraint as defined by the farmers	Scientist's interpretation of constraints	Farmers ranking the constraints as first or second most important in each district			
		Rakai	Masaka	Ntungamo	Overall
Weevils (adult/larvae)	Weevil infestation	19	40	28	87
Yellowing of leaves/black spots on fruit/ heart of plant rotting in matooke	BSV disease	10	7	9	26
Weeds	Weed infestation	6	8	6	20
Toppling of plants/drying roots	Nematode infestation	3	5	4	12
Drying of bananas	Black Sigatoka	7	2	3	12
Exhausted soils	Soil fertility	3	6	-	9
Yellowing of leaves and rotting of inner corm on Kayinja and apple bananas	Fusarium wilt	-	1	2	3
Dry spell/drought	Moisture stress	1	3	-	4
Cigar leaf dries	Die-back**	5	-	-	5
Others		9	5	1	15

** Die-back may be caused by BSV disease, moisture stress, weevil and nematode infestation.

The farmers described using various practices to try to overcome the constraints (Table 5). Most of the practices were used to target weevils and soil infertility, but were largely appropriate. Corm removal, pseudostem trapping, detrashing/ desheathing and splitting of pseudostem were the main practices reported as used to manage weevil infestation. The main practices reported to be used to manage soil infertility were the application of mulch, coffee husks and/or manure. In contrast, practices reported to target green yellow mixture on leaves in matoke (BSV), Pisang awak or apple bananas (= Fusarium wilt) and toppling and drying of roots (= nematode infestation) were varied and often inappropriate. This is probably because farmers do not perceive these constraints as individual problems and commonly associate them with other easily visualized constraints such as weevils and soil infertility. For example, farmers reportedly used

corm removal, appropriate in weevil management, to control BSV symptoms. Additionally, a few farmers (a total of only 9 in all sites) responded that they considered managing BSV related symptoms (Yellow green mixture on leaves in matooke) despite the fact that these symptoms/constraints were ranked second after weevils.

Most of farmers had modest formal education (5.5 years) and used indigenous knowledge in their farming activities. As a result, it appears they are more familiar with farming practices passed on from generation to generation. Therefore, it is likely these farmers were not aware of new problems and practices targeting them. This could explain why farmers ranked weevils highest as compared to BSV and Fusarium wilt symptoms. Similarly, management practices targeting constraints were mainly cultural and often inappropriate because they targeted perceived causes of constraints.

Table 5. Farmers' identified constraints of banana production and practices targeted to manage the constraints

Constraints identified by farmers	Scientific interpretation of constraint	Management Practice	Number of farmers using the management to target a given constraint per district			
			Rakai	Masaka	Ntungamo	Overall
Weevils	Weevil infestation	Corm removal	13	8	12	33
		Pseudostem Trapping	9	6	3	18
		Detrashing/ desheathing	7	-	2	9
		Pruning /desuckering	2	-	1	3
		Splitting pseudostems	1	3	2	6
		Apply mulch, coffee husks, manure	1	-	1	2
		Weeding	-	1	-	1
		Application of ash/urine and pepper	5	4	2	11
		Roguing affected plant	-	2	-	2
Yellow green mixture on leaves of cooking bananas	BSV disease	Corm removal	1	-	1	1
		Pruning/ desuckering	-	-	5	5
		Roguing affected plants	1	-	1	2

Table 5: continued: Farmers' identified constraints of banana production and practices targeted to manage the constraints

Constraints identified by farmers	Scientific interpretation of constraint	Management Practice	Number of farmers using the management to target a given constraint per district			
			Rakai	Masaka	Ntungamo	Overall
Yellowing of leaves of Pisang awak, Gros Michel	Fusarium wilt	Apply mulch, coffee husks, manure	-	-	1	1
		Detrashing/ desheathing	-	-	1	1
		Pruning/ desuckering	-	-	3	3
		Splitting pseudostems	-	-	1	1
		Roguing affected plants	2	-	1	3
Toppling and drying of roots	Nematode infestation	Pruning/ desuckering	-	-	1	1
		Splitting pseudostems	-	1	1	2
Dry spell/drought	Moisture stress	Apply mulch, coffee husks, manure	5	5	-	10
		Corm removal	1	1	1	3
		Detrashing/ desheathing	2	2	-	4
		Pruning/ desuckering	-	-	1	1
		Splitting pseudostems	-	-	1	1
Drying of cigar leaf	Die back	Apply mulch, coffee husks, manure	1	-	-	1
		Pruning/ desuckering	-	-	1	1
		Application of ash/urine and pepper	2	-	-	2
		Roguing affected plants	2	1	-	3

Table 5: continued: Farmers' identified constraints of banana production and practices targeted to manage the constraints

Constraints identified by farmers	Scientific interpretation of constraint	Management Practice	Number of farmers using the management to target a given constraint per district			
			Rakai	Masaka	Ntungamo	Overall
Unproductive soils (lunyu)	Soil infertility	Corm removal	1	1	1	3
		Apply mulch, coffee husks, manure	10	7	2	19
		Weeding	1	1	-	2
		Application of ash/urine and pepper	-	1	-	1
Small bunches	Low yields	Corm removal	-	-	1	1
		Weeding	-	1	-	1
		Detrashing/ desheathing	-	-	2	2
		Pruning/ desuckering	-	1	1	2
		Roguing affected plants	-	-	1	1

Farmers' description of related symptoms in addition those identified by scientists as those of BSV

After the scientists showed the farmers plants with BSV symptoms (chlorotic streaks), most farmers across the sites (districts), described three main symptoms associated with the condition, namely, leaves turning green to yellow (26 farmers), yellow streaks on leaves (10 farmers) and cigar leaf drying/rotting of core of pseudostem (30 farmers) (Table 6). These symptoms were identified as chlorotic streaks/blotches and die back /pseudostem necrosis by scientists upon examination. There was variation in the major symptom expressed by bananas with BSV in the districts. Most farmers described die back /pseudostem necrosis as the dominant symptom expressed by plants in Rakai (10) and Masaka (14) as compared to Ntungamo (6). This could probably be attributed to difference in environmental conditions. Rakai and Masaka districts neighbour each other and have similar environmental conditions compared to Ntungamo. Thus, plants with BSV signs in Rakai and Masaka expressed similar symptoms which were different from those expressed in Ntungamo.

Three farmers also reported BSV symptoms to be the cause of toppling and snapping of bananas. This could possibly be due to the fact that plants with BSV symptoms are often also infested with nematodes/weevils, and therefore, the farmers' perception that BSV symptoms are linked to nematodes/weevils. Similarly, die back is attributed to several other factors.

Table 6. Farmers' description of disease symptoms identified by scientists as *Banana streak virus* (BSV)

Farmers' Responses	Number of farmers by district			
	Rakai	Masaka	Ntungamo	Overall
Leaves turn green to yellow	5	8	13	26
Yellow streaks on leaves	3	3	4	10
Cigar/tongue leaf dries/ rotten heart of plant	10	14	6	30
Toppling	2	-	1	3
Do not known	-	1	2	3
Others	-	3	-	3

Farmers' perceived causes of BSV symptoms identified by scientists as those of BSV and management practices targeting them

Most farmers (58) in the study areas were not aware of the cause of the BSV symptoms (Table 7). A few farmers attributed the symptoms identified as those of BSV to weevils (13), drought (7), poor management (5) and exhausted soils (3). The dominant practice targeting BSV symptoms was roguing infected plants (43) (Table 8). Roguing was used by farmers as the last resort after they had attempted to use other practices to manage BSV symptoms and failed.

Most of the farmers in Masaka (18) and Ntungamo (19) did not replace infected plants that were rogued. However, in contrast, most farmers in Rakai did replace infected plants that had been rogued (16). Roguing infected plants and replanting is one of the practices recommended by the extension services to manage BSV. Farmers in Rakai were more aware of roguing infected plants and replanting as a management practice targeting BSV symptoms. This is probably because of the interaction with extension services. In Table 3, it is shown that the main sources of agricultural information for farmers in Rakai were extension agents (8) and seminars (6) that are mainly conducted by the extension service providers. In Ntungamo and Masaka, however, the main source of agricultural information was immediate relatives (indigenous) and hence they rogued infected plants but did not replace them.

However, it should be noted that while farmers in Rakai had access to extension services, most of the farmers (15) were not aware of the cause of BSV symptoms (Table 7). This suggests that even the extension service providers in contact with these farmers may not have sufficient knowledge about BSV. This has implications for the effectiveness of roguing infected plants and replanting as a measure to manage BSV since farmers are probably more likely to replace rogued plants with other infected suckers.

From a broad perspective of the banana cropping system, farmers associated BSV symptoms with those of other constraints. BSV symptoms were isolated from other constraints and farmers could

not tell the cause of BSV symptoms. This suggests that farmers are not aware of the causes and appropriate management practices for the disease.

Table 7. Farmers' response on what causes disease symptoms shown to them and identified by scientists as Banana Streak virus (BSV)

Farmers' Responses	Number of farmers by district			
	Rakai	Masaka	Ntungamo	Overall
Banana weevils	3	6	4	13
Exhausted soils	1	1	1	3
Bad crop management	1	-	4	5
Drought/lack of rains	6	1	-	7
Do not know	15	22	21	58

Table 8. Farmers practices used to manage symptoms shown to them and identified by scientists as *Banana streak virus* (BSV)

Management practice	Percentage of farmers using the practice (%)			
	Rakai	Masaka	Ntungamo	Overall
Rogue all affected plants	6	18	19	43.0
Rogue and Replace Infected plants	16	-	1	17.0
Cut down affected plants	1	3	1	5.0
Remove affected plants	2	1	-	3.0
Corm removal	1	3	2	6.0
Trapping	-	-	1	1.0
Apply ash and urine	-	2	-	2.0

Conclusion

In conclusion plants with symptoms of BSV infection were found in the three districts included in the study, Rakai, Masaka and Ntungamo. Generally, farmers in the three districts recognised the symptoms of BSV infection (as identified and described by the scientists in the study team) as constraints to banana production, but did not know the cause and they tended to attribute them to weevil infestation and declining soil fertility. Because of this lack of knowledge and understanding of banana streak virus disease, and the difficulty in visualising the cause, the farmers did not rank BSV as a high priority constraint. The lack of knowledge and understanding of the causes of the symptoms also meant that the farmers tended to use management practices that targeted poor soils and weevils to try to control the BSV symptoms. However, the farmers claimed that they would rogue and replant as a final resort if the management practices against weevils or declining soil fertility did not reduce the severity of symptoms sufficiently. The lack of knowledge concerning BSV by the farmers is probably a reflection of the relatively recent nature of the disease in Uganda and the lack of awareness of the disease by extension workers. Since the farmers appeared willing to take on board the advice offered by the extension services (at least in Rakai), it would seem appropriate to target the extension services and key informant farmers for any new or revised recommendations of management practices to control BSV symptoms.

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Detection of *Banana streak virus* in banana plants from Uganda

Jerome Kubiriba, Nawshad Joomun and Lawrence Kenyon.

Background

When CPP-funded project R7529 (Epidemiology, vector studies and control of banana streak virus in East Africa highland bananas) was being formulated, a good deal of information was emerging on how BSV sequences can be integrated in certain *Musa* genomes and how these might be activated to give rise to episomal (true) infections and disease. However, nothing was known about what was driving the BSV epidemic in Uganda, or what effect BSV infection has on the East African highland bananas that predominate there. Antisera had recently been developed at IITA (Nigeria) that appeared to work reliably in detecting the virus in the Nigerian *Musa* breeding lines and hybrids (Dahal *et al*, 1998), and since it was anticipated that these would also work in Uganda, IITA were contracted to provide sufficient antiserum and reagents for project R7529. However, since it was known that in other areas BSV (and other badnaviruses) are very variable serologically, a sister project (R7478) was established with the John Innes Centre to assess the molecular variability of the virus in Uganda. If needed, a diagnostic method for use in the epidemiology or transmission studies in Uganda would also be developed.

The final report for R7478 was circulated in mid September 2001 (Hull and Harper, 2001). During two missions to Uganda, leaf samples and suckers had been collected from about 60 plants showing BSV symptoms from a west - east transect across Uganda covering most of the banana growing areas. Virus particles were purified from each sample. When these were tested for affinity with a cocktail of 24 antisera (Lockhart) using ELISA and ISEM, a number gave very weak readings indicating that many were not reacting strongly with the antiserum mixture. Polymerase chain reaction (PCR) was then used to amplify a region of the virus genome including parts of the reverse transcriptase (RT) and RNase H (RH) genes. The PCR amplification products were cloned and then sequenced. The sequences derived from the different samples were aligned with similar sequences from other badnaviruses. Phylogenetic analysis showed that there were probably at least 11 different "pseudo species" (<80% sequence homology between species) of *Badnavirus* present in the bananas of Uganda. These species are divided between three super clusters of sequences, and one cluster of these is apparently more similar to *Sugarcane bacilliform virus* (ScBV) than to any of the other badnaviruses included in the analysis (Harper et al. 2002).

Once the field trials (Activities 1, 2 and 3 of project R7529) were planted, attempts were made to use the antisera provided by IITA for routine indexing of the plants and to determine if symptom severity is proportional to virus titre in infected plants. Unfortunately, the results were inconsistent and could not be relied upon. This apparent variable reaction with the antiserum is probably due to serological variability as reflected by the wide genetic diversity of strains/species of BSV present in Uganda. Because of this, the JIC group set about trying to develop a diagnostic method for detecting all non-integrated forms of BSV. They arrived at two procedures (Hull and Harper 2001):-

1. Direct binding of the virus to the walls of PCR tubes followed by amplification of the viral DNA by PCR using degenerate universal *Badnavirus* oligonucleotide primers (BADNA-1a + BADNA 4').
2. Immunocapture PCR where the virus particles are first trapped on the PCR tube wall using antiserum and then the *Badnavirus* DNA is amplified by PCR using the same universal primers as above.

Since it was important to many components of project R7529 to have BSV diagnostics available in Uganda, it was agreed that it would be appropriate to transfer funding, originally intended for antiserum production by IITA-Nigeria, to enhancing the plant virology capacity

in Uganda (Dr Legg's lab at Namulonge/Sendusu and The biotechnology lab at Kawanda) so that BSV molecular diagnostic tests could be conducted there. This report summarises the activities and results of the work to undertaken to build on the outputs of R7478 to establish reliable BSV diagnostic facilities in Uganda.

Activities

In September 2001, Kenyon and Kubiriba tried to use the procedures described in the final report for R7478 to detect BSV in banana leaf samples from Uganda using the facilities in Dr Legg's laboratory at Namulonge.

Between February and October 2002, Nawshad Joomun undertook a short research project as part of his MSc (Natural Resources) studies at University of Greenwich-NRI. He used a set of BSV-infected banana plants originally collected from Uganda for project R7478 and subsequently transferred to the quarantine glasshouse at NRI, and built on the diagnostics developed by R7478 by testing IC-PCR with different *Badnavirus* primers and different *Badnavirus* antisera and mixtures of antisera. He also spent two weeks at the John Innes Centre, Norwich, under the supervision of Dr Harper testing different methods for concentrating *Badnavirus* particles from crude sap preparations prior to IC-PCR (Joomun 2002).

Table 1. Universal *Badnavirus* PCR primers used in this study

Name	Sequence	Source
BADNA 1a	CTN TAY GAR TGG YTN GTN ATG CCN TTY GG	Harper
BADNA 4'	TCC AYT TRC ANA YNS CNC CCC ANC C	Harper
BADNA-FP	ATG CCI TTY GGI ITI AAR AAY GCI CC	Yang *et al* 2003
BADNA-RP	CCA YTT RCA IAC ISC ICC CCA ICC	Yang *et al* 2003

In June 2002, Kenyon carried a small thermocycler (PCR-machine - Omn-E), micro-pipettes and reagents to Uganda, and with Kubiriba, optimised an IC-PCR test using concentrated plant sap at the IITA Sendusu (food science) laboratory.

Results

Initial attempts in 2001 to transfer the procedures developed during R7478 to the laboratory at Namulonge, Uganda, were unsuccessful, and even PCR using the BADNA-1a + BADNA-4' primers (Harper *et al.*, 1999) and total banana DNA as template gave inconsistent results.

Subsequently, a different pair of universal *Badnavirus* PCR primers, BADNA-FP + BADNA-RP, (Yang *et al.*, 2003) designed by G. Hafner (then at the Queensland University of Technology) were tested and found to give more consistent amplification of *Badnavirus* sequences. Since these primers were designed to the most conserved regions of the reverse transcriptase (RT) gene and RNAse-H (RH) gene of badnaviruses, they will prime the amplification of a stretch of about 579bp of both integrated and episomal *Badnavirus* DNA. For this reason, these primers cannot be used directly for detecting BSV in banana plant total DNA extracts since an amplicon of about 579bp is produced with DNA from all samples whether infected with BSV or not (Figure 1). These primers also detected contaminating genomic DNA when direct binding of virions to the PCR tube walls was tested (Figure 2).

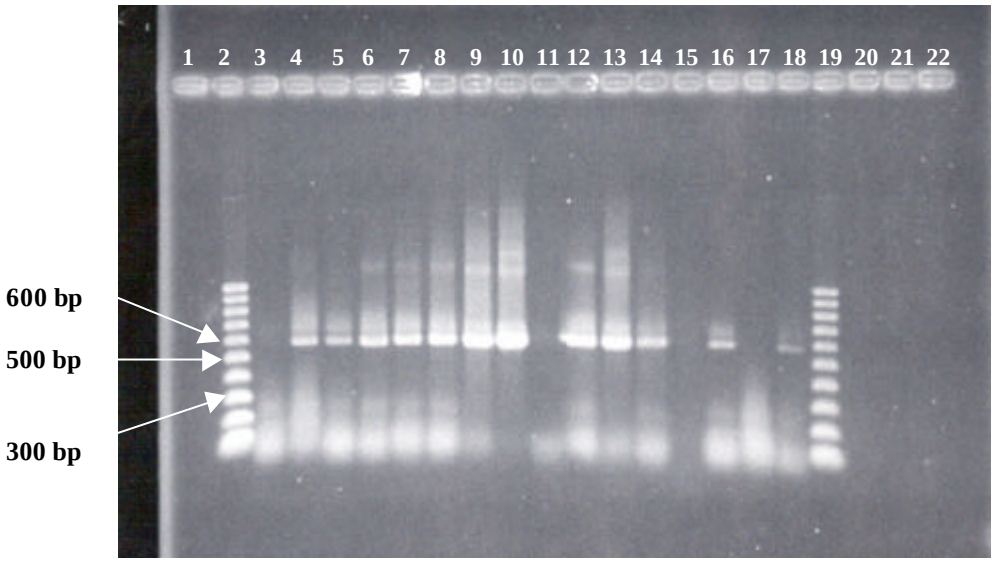

Figure 1. Agarose gel electrophoresis of products from PCR using primers BADNA-FP/BADNA-RP on total DNA extracts from banana leaf material of different Musa cultivars. Lanes contain: (2 and 19) are 100 bp DNA ladder (ABgene), (3 and 17) negative [water] control, (4 and 10) healthy cv. Williams and (5 to 9) are cvs. UG23, UG24, UG46, UG08 and UG45 respectively and (11-15) are corresponding 1:10 diluted samples and (18) positive control (diluted suspension of purified BSV virions).

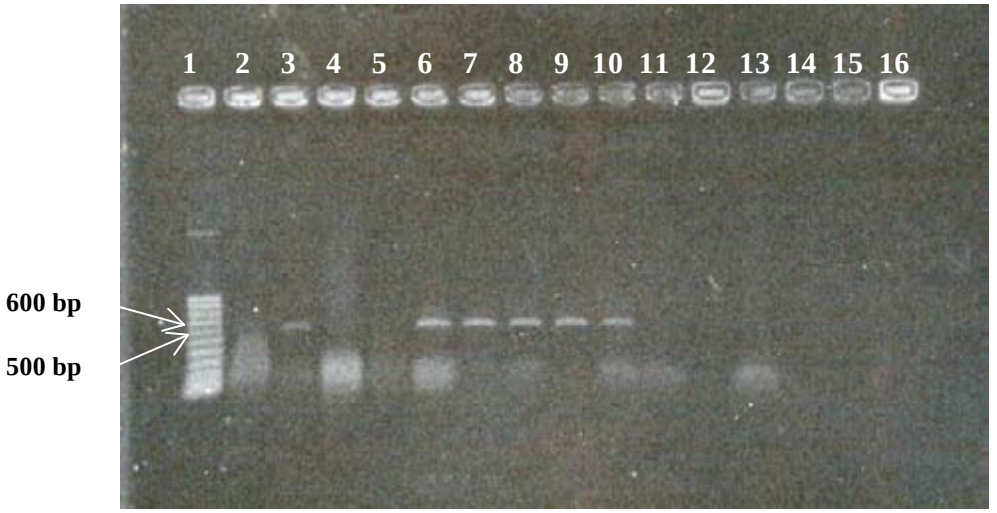

Figure 2. Agarose gel electrophoresis of products from PCR using primers BADNA-FP/BADNA-RP following direct binding [DB] of crude banana leaf sap extracts. Lanes contain: (1) 100 bp DNA ladder (Promega) (2 to 10) crude sap from UG03, UG23, UG24, UG45, P004, UG41, UG45, UG51, and UG10 respectively.

In order to overcome the problem that the BADNA-FP/BADNA-RP primer pair will detect both integrated and episomal BSV sequences, Nawshad Joomun adopted an immuno-capture-PCR procedure. Using the Agdia BSV antiserum (an early cocktail of badnavirus antisera from Dr Ben Lockhart) or the Agdia ScBV antiserum to capture virus particles from sap he was able to consistently get a negative PCR with sap from non-infected plants, and slightly less consistently get a positive PCR with sap from certain infected plants (Figure 3). However, the PCR product was not usually very abundant (feint band on a gel) suggesting that there was a low titre of template in the infected plant sap samples or only few particles were being captured to the tube walls by the antiserum.

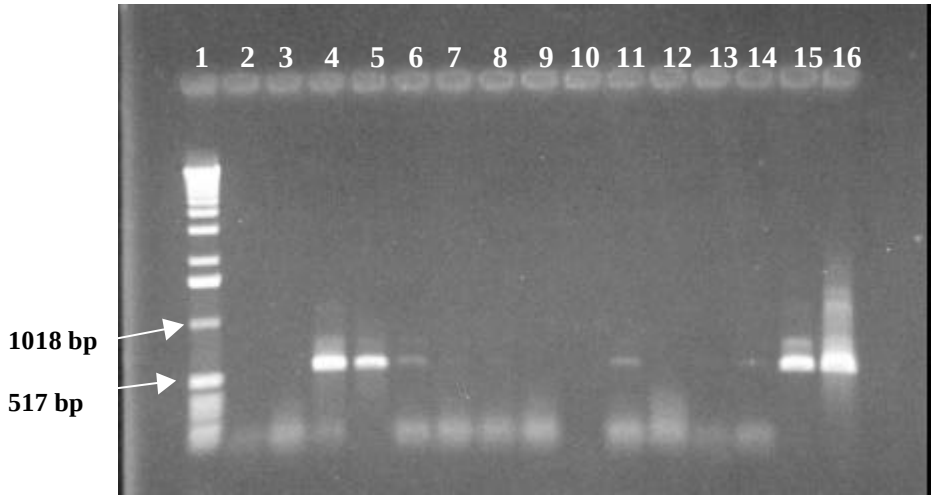

Figure 3. Agarose gel electrophoresis of products from IC-PCR using primers BADNA-FP/BADNA-RP and trapping virions from crude banana leaf extracts using Agdia BSV and Agdia SCBV antisera separately. Lanes contain: (1) 1 kb DNA ladder (Invitrogen), (2, 4, 6, 8) samples UG24, UG23, UG41, UG45 respectively (using anti BSV) and (3, 5, 7, 9) corresponding samples with anti SCBV, (10) control healthy cv. Williams, (11 to 14) stored samples from a previous experiment, (14) water control and (15 and 16) positive control (Bright BSV).

To increase the amount of PCR product produced in positive reactions (indicated by more intense fluorescence of the 579bp band on the agarose gel), a sap concentration procedure based on precipitation using 4% polyethylene-glycol (PEG) and 0.1M NaCl (developed by G. Harper, JIC, [R7478]) was tested. The virions in the sap were concentrated between 6 and 12-fold before being added to the antiserum coated tubes resulting in a greater titre of virus particles being available for capture by the antisera in each tube. This system produced clear positive reactions from some, but not all leaf samples showing BSV symptoms, dependent on which antiserum was used for capturing (Figure 4).

Because it was suspected that the lack of reaction (detection) of some apparently BSV infected samples was because of the serological variability (heterogeneity) of the BSV samples (Lockhart and Olszewski, 1993), the Agdia SCBV antiserum, the Agdia BSV antiserum, and a more recent cocktail of BSV antisera (PMx R2-2C; kindly provided by B. Lockhart, University of Minnesota) were compared in the immuno-capture stage. Because different samples tested positive when different antisera were used, to make the test less strain-specific a mixture of all the available antisera was used to capture the virus particles before PCR. This procedure produces positive reactions with many Ugandan banana leaf samples (Figure 5), but still needs further evaluation to determine what proportion of strains of virus it misses and how sensitive it is (it is not particularly useful if it only produces positive reactions with samples that are showing moderate to strong symptoms of infection).

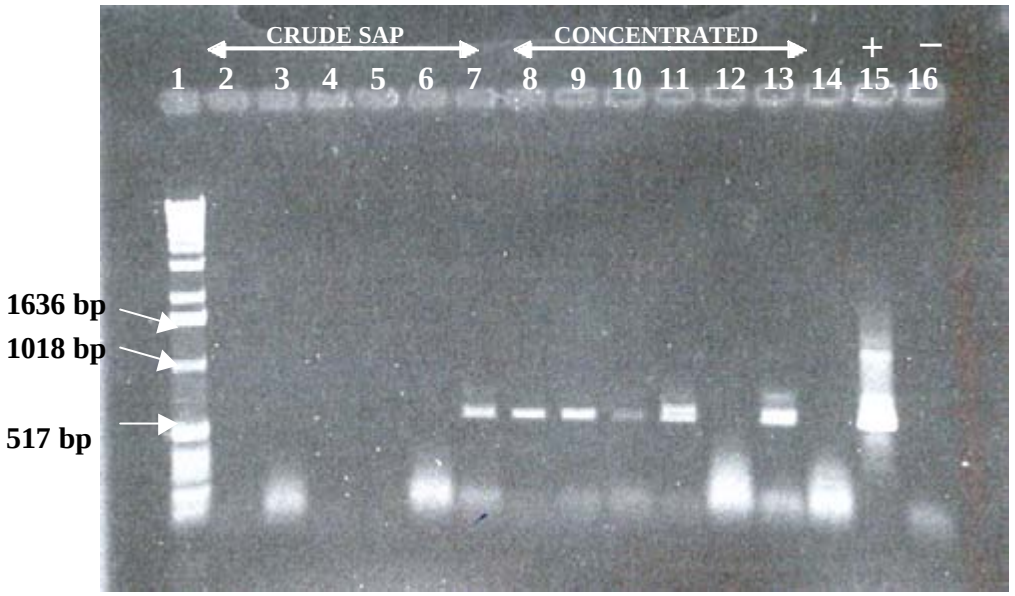

Figure 4. Agarose gel electrophoresis of products from IC-PCR on crude and concentrated leaf samples trapped with Agdia BSV and Agdia ScBV antisera separately. Lanes contain: (1) 1 kb DNA ladder (Invitrogen), (2, 4, and 6) crude extracts from samples UG03, UG10 and UG46 respectively (using anti BSV) and (3, 5 and 7) corresponding samples with anti SCBV, (8-13) concentrated extracts, (14) P006 (crude) using Agdia BSV, (15) positive control (Bright BSV) and (16) negative control.

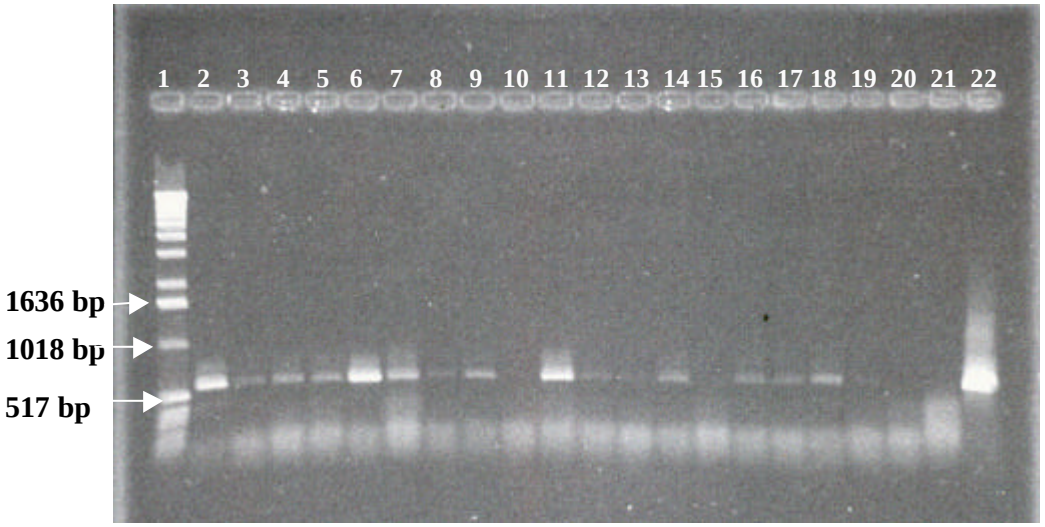

Figure 5. Agarose gel electrophoresis of products from IC-PCR on concentrated leaf samples trapped with a mixture of Agdia ScBV, Agdia BSV and PMxR2-2C antisera. Lanes contain: (1) 1 KB DNA ladder (Invitrogen), (2-20) samples UG41, UG45, UG 46, UG51, UG60, POO4, UG03, UG12, UG04, UG24, UG 53, UG07, UG06, UG08, UG14, UG23, UG28, UG64 and UG01 respectively, (21) negative control and (22) positive control (Bright BSV).

Discussion and Conclusions

Reliable detection of the virus(es) causing banana streak disease is made difficult by the presence of *Badnavirus* DNA sequences integrated into the host genome, the great serological and genetic heterogeneity of the causal badnaviruses, and the often low titre of virus particles presented in infected plants. The detection system arrived at through these studies (presented in Box 1) tries to overcome these problems by:

- Using the sensitivity of the polymerase chain reaction,
- Using the universal *Badnavirus* PCR primers BADNA-FP and BADNA-RP to prime amplification of part of the reverse transcriptase and RNase-H genes of all known Badnaviruses,
- Using immuno-capture of virus particles (virions) to reduce the risk of false positives caused by amplification of host-integrated *Badnavirus* sequences
- Using a mixture of several polyclonal antisera in the immuno-capture stage to make the test less virus strain /species specific
- Using a sap concentration procedure to increase the likelihood of sufficient virions being presented to the antiserum mixture for binding for PCR amplification to occur when virus titres in the samples are low.

The procedure appears to be giving consistent and reliable results with samples from Uganda that exhibit some BSV symptoms. However, further work is required to determine the sensitivity of the test on infected samples with very mild or no symptoms. The procedure should also be tested on a greater range of BSV isolates/species since it is not known whether (and it seems unlikely that) the antiserum mixture used will trap all variants of the virus. Since from the phylogenetic analysis it appears that some isolates from banana cluster more closely to the *Dioscorea* bacilliform viruses or the Cocoa swollen shoot viruses, it may be that the antisera produced against these, and other badnaviruses should be included in the immuno-capture mixture.

The use of direct binding of virions to the PCR tube walls was an attempt to overcome the problem of serological heterogeneity of the virus strains and antiserum specificity, but was discarded as a method because host genomic DNA, including integrated *Badnavirus* sequences, were also bound to the tube walls giving occasional false positive results. Nawshad Joomun tried to use ion exchange chromatography to both concentrate virions from plant sap and to exclude host genomic DNA. However, he found that with Q-sepharose ion exchange resin, genomic DNA was eluted under similar conditions to the virus particles resulting in false positives from contaminating integrated *Badnavirus* DNA. It may still be worth testing other ion-exchange resins and elution conditions to find a combination where DNA is eluted under sufficiently different conditions such that contamination is avoided. Incorporation of a DNase treatment to the sap may also help in this regard.

For routine diagnosis of BSV a sensitive system that reliably detects all variants of the virus is required. However, the observation that a plant may be infected with several different variants and the possibility that these variants might have different biological/virulence and vector specificity characteristics means that under some circumstances it may be desirable to be able to differentiate between virus strains/species (genotypes). For example, in Ntungamo the BSV symptoms are typically chlorotic streaking along the leaf veins, whereas in Rakai there is usually a more general chlorosis and mottling of the leaves often accompanied by distortion and necrosis of the candle leaf. To start to try to answer the question as to whether these differences are caused by the presence of different strains of virus in the two districts, Kubiriba has started to investigate whether restriction fragment profiling of the IC-PCR products can be used to identify (crudely fingerprint) different strains. So far, only the 4-base cutters *Taq I* and *Alu I* have been shown to cut the PCR product from some of the Ugandan

strains, but more work is required to determine if these are sufficient to discriminate between the different strain groups.

With the findings presented here, and the transfer of materials and information to Uganda, the facilities and capability are now in place for detection of many BSV strains to be carried out in Uganda in support of epidemiology and virus management research. However, the high cost of the test, both in terms of reagents and skilled technicians time, means that for now the test is only likely to be used for research purposes and will not be used for routine screening of germplasm or as part of a disease control strategy.

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Acknowledgements*:*

We gratefully acknowledge the help and guidance provided by Dr Glyn Harper and Prof. Roger Hull (John Innes Centre, Project R7478), and for their hosting of Nawshad Joomun at the John Innes Centre during part of his MSc research project. We also thank Dr James Legg and his team for allowing us to use the facilities of the plant virology laboratory at Namulonge and Sendusu, Uganda, and Dr Greg Hafner (QUT, Brisbane) for revealing the sequences of the BADNA-FP and BADNA-RP PCR primers to us before publication.

Box 1. Procedure for detection of BSV in banana leaf samples

Sap concentration:

1. Macerate 1g fresh leaf sample in a "Bioreba Universal" bag using a seam roller, adding 4ml sample buffer (PBS + 0.1% $NaSO_3$)
2. Transfer sap samples to microcentrifuge tubes and centrifuge 10 min (13,000 rpm)
3. Take off supernatant 'sap'
4. Kept back some sap as 'normal' sample
5. To 1200µl of sap, add 150µl of 40% PEG and 150µl of 1.0 M NaCl
6. Shake and roll tubes over occasionally for 2 hr at RT
7. Centrifuge at 13,000rpm for 20 min.
8. Resuspended pellet in 100µl sample buffer (easiest by using micropipette tip) = 'concentrated' sap.

Immuno capture PCR

- Coat 0.5 l thin-wall PCR tubes with 100 l of 1 g/ml antiserum (formed from equal concentrations of Agdia CAB-81800[anti BSV], Agdia CAB-72200[anti ScBV] and PMxR2-2C) in carbonate coating buffer
- Incubate tubes at room temperature for ca 3 hr.
- Wash tubes x 3 with PBS-T
- Add 50µl "concentrated" sap /tube and incubate in fridge (4ºC) overnight
- Wash tubes x 3 with PBS-T

Add 50 l PCR mix/ tube

	50µl	**Concentrations**	**For 30 reactions**
10x PCR buffer	5	1x	150
$MgCl_2$ (25mM)	6	3mM	180
'dNTPs (10mM)	2	0.4mM	60
BADNA-FP (10µM)	5	1µM	150
BADNA-RP (10µM)	5	1µM	150
Promega Taq	0.25	1.25U	7.5
Template	2		0
H_2O	24.75		802.5

Cycling conditions

94ºC	4 min	
94ºC 55ºC 72ºC	30 sec 1 min 2 min	40 cycles
72ºC	10 min	

Electrophoresis:
Mix 16 l of PCR product with 4 l of 5x loading buffer
Load in to 1.5% agarose gel in TAE
Electrophorese at ca. 7V/cm for about 1 hr