PROJECT COMPLETION SUMMARY SHEET

DATE Sheet Completed: 12th September 2001

Project Title:	*Management strategies for banana streak virus: variation of BSV in Uganda as an adjunct to diagnosis and epidemiology*
DFID Project Reference No:	R7478
Programme:	Crop Protection Programme
Programme Manager (Institution):	Dr Simon Eden-Green (NRIL)
Sub-Contractor (project leader's institution)	John Innes Centre
Production System:	High Potential (1.11)
Programme Purpose:	Increasing productivity and productive potential through the removal or amelioration of constraints caused by crop pests by the development, application and promotion of results of research on socially- and environmentally acceptable crop protection strategies
Commodity Base:	Musa (East African Highland banana)
Beneficiaries:	The target institutions will be IITA and NRI who are involved in project R7529. Through this project, the national programmes of Uganda and Rwanda will be strengthened and this should lead to benefits for the subsistence farmers who depend on banana production. Other relatively immediate beneficiaries will be tissue culture initiatives. There are currently three banana tissue culture laboratories in Uganda (Plant tissue culture laboratory, Kawanda; Crop Production Department, Makerere University; Tissue culture laboratory, Namulonge Research Station), four in Kenya (KARI Horticulture Research Station, Thika; Jomo Kenyatta University of Agriculture and Technology; Genetic Technologies Ltd; Kalimoni Laboratory, Juja) and four in Tanzania (HORTI Tengeru Laboratory; TANADE; University of Agriculture, Sokoine; Mikocheni Laboratory, Dar-es-Salaam); none are known in Rwanda or Burundi. Although the strain variation of BSV is likely to be different in more distant countries, in the longer term the technologies and approaches will be of benefit to other East and West African countries and to all countries where bananas (and plantains) are grown.
Target Institutions:	IITA & NRI linked to proposed project R7529. This project should provide information and diagnostics for use in this project R7529
Geographic Focus:	Uganda
Total Cost:	£64,559 + £20,750 (5 month extension) = £85,309

	Planned	**Actual**
Start Date:	1 January 2000	15 March 2000
Finish Date:	31 March 2001	31st August 2001 (including 5 month extension)

1. Project Purpose:

Programme Output for High Potential Production System 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.

The initial research objective was to assess the strain variation of BSV in Uganda (and Rwanda), to provide molecular information that will be of use for epidemiological and loss-assessment studies and to develop diagnostics for the variants. It did not prove possible to obtain samples from Rwanda and so this part of the project was removed.

The outputs of this research will be used for the epidemiological studies proposed in the sister project (R7529). It is considered important to have information on, and diagnostics for, variants of BSV in developing an understanding of the epidemiology of the virus. In the longer term, the diagnostics will be of use to Ugandan and other East African NARS for virus identification and for initiative on banana improvement by tissue culture in these countries.

2. Outputs:

On two visits to Uganda 59 samples were collected from 30 farms at 14 sites covering the major banana growing regions of that country. In addition, 92 samples were sent from the two epidemiology sites used by project R7529. A modified purification procedure was developed for BSV and virus preparations were made from 47 of the 59 farm samples and from three of the epidemiology site samples; the other samples were too small or symptomless. Serological analysis by ELISA (enzyme-linked immunosorbent assay) and ISEM (immune-specific electron microscopy) showed that few of the samples reacted strongly with a broad spectrum antiserum that detects BSV isolates from other parts of the world. DNA extraction and PCR using degenerate primers (covering a region that is considered to be highly conserved among this group of viruses) (1A-4') indicated that, at least in some cases, the poor serological reaction was not due to low virus titre. Several hundred clones were made from the PCR products of 48 of the samples and the inserts were sequenced. The sequences were compared with each other and with published and other BSV sequences. The results were:

1) If sequence differences of more than 20% differentiate between virus species, 11 BSV species could be recognised in the Ugandan samples.

2) These species fell into three supergroups that differ by more than 35% sequence. One supergroup contains BSV strains that are integrated and can be activated to give episomal infection, one supergroup contains BSV sequences that have been found integrated in a form that is not activatable ("dead") and the third supergroup contains strains that have no integrated analogue.

3) The samples from the infectors in the epidemiology experiment would not amplify using the degenerate primers that gave products for the other samples but did so with other primer sets (Rdfor-Rdrev and Ghfor-Ghrev). Clones from the PCR products are currently being sequenced but the requirement for different PCR primers would indicate that these differ from the 11 species mentioned above. PCR of asymptomatic samples from epidemiology sites gave minor products that had "dead" sequences; it is possible that PCR was amplifying such sequences from the plant chromosome and that there was no episomal virus.

4. There was no correlation between the virus "species" or "supergroup" and banana cultivar or symptoms. One site, in the East of Uganda, had one virus species but all the other sites had more than one species. Eleven of the samples were infected by more than one virus "species".

In developing a robust diagnostic technique, it was shown that serological techniques such as ELISA were not reliable but that PCR using primer set 1A-4' could detect BSV in most cases; however, three cases were found in which primer set 1A-4' did not amplify a product from samples with obvious symptoms but other primer sets gave products. To distinguish between episomal virus and integrated viral sequences and to remove potential inhibitors of PCR, techniques involving capture of virus particles from sap extracts either on antiserum-coated tubes (immune-capture, IC) or directly onto the plastic tube surface (direct binding, DB) were

developed. In samples with low virus titre, IC/PCR or DB/PCR was improved by concentrating the virus by polyethylene glycol (PEG) precipitation.

3. Contribution of Outputs to Project Goal:

This project has exceeded the research goals. It has shown that there is much more variation of BSV in Uganda than was originally expected. This has implications in four areas. Specific to projects such as that on the epidemiology of BSV (R7529) it has demonstrated that the BSV epidemic in Uganda is not due to one virus strain or one introduction but indicates a large number of strains and introductions. The range of variation suggests long-standing infections with BSV. Thus, for project R7529, it is important to characterize the virus(es) in the infector plants. In such experiments, it should be possible in many cases to distinguish between spread from the infector plants and spread from outside the experimental plot. Secondly, it highlights the importance of good diagnostics for obtaining healthy material from tissue culture systems. Not only has one to be aware of the virus variability already shown but it should be recognized that there may be virus variants not detected by the current primer sets. Thirdly, this is the first example of a detailed analysis of BSV variation in an individual country and it is likely that there would be similar variation in other countries or regions where the virus has been established for a long time. Fourthly, there is the basic scientific question as to how this variation arises. There has not been enough time in the project to sequence full genomes of several of the virus species found in Uganda but it is likely that much of the variation is due to recombination. It would be interesting to know whether any recombination is between episomal variants or whether there is recombination between an episomal variant and integrated BSV sequences.

The basics of a diagnostic technique have been developed. For general diagnosis this involves extraction of sap from the test plant, concentration of the virus in that sap by PEG precipitation, binding of the virus to a plastic tube (IC or DB), PCR using primer set 1A-4' and analysis of the PCR products by gel electrophoresis. If a symptomatic sample does not give a PCR product, other primer sets should be used. For specific diagnosis of virus "species", the PCR product could be analysed by restriction enzyme cutting or by sequencing or specific PCR primers can be designed from the sequence information gained in this project.

4. Publications:

5. Internal Reports:

*Departmental seminar, July 2001

*Internal Banana Cluster meeting, Reading, April 2001

6. Other Dissemination of Results:

A poster on the work in this project entitled " A BSV epidemic in Uganda" was presented to the Association of Applied Biologists meeting in Dundee in September 2000.

7. Listing and reference to key datasets generated:

Note: for DFID's purposes, a dataset is defined as any collection of data generated by or of particular interest to the international development community. They cover from the computer-readable dataset of major surveys to small-scale data collected as part of individual projects. They include datasets used for research and analysis, the numeric machine-readable results of surveys, censuses and administrative activity, bibliographic data (but not notes from reading published texts), textual material from the transcription of historical sources or collected through qualitative research (such as diaries and fieldwork notes) and other quantitative and qualitative data including audio-visual recordings of interviews and events, photographs and artefacts.

A data set of sequences of 123 cloned PCR products and analyses of these sequences.

8. Follow-up indicated/planned:

It is anticipated that the methods and results from this project will be used and adapted for specific situation by project R7529.
As noted above, this project has raised various basic scientific questions which should be addressed to gain a further understanding of this important disease.

9. Name of author of this report:
Roger Hull **Date: 12th September 2001**