



Quick, accurate tests identify plant diseases

Validated RNRRS Output.

Easy-to-use test kits now help laboratory staff in developing countries diagnose diseases rapidly. The traditional tests are expensive and time-consuming. This means that diseases, such as bacterial wilt in potato, groundnut and tomato, and leaf spot in banana, are often not correctly detected, or not detected in time. So, crop losses from these diseases in Africa and Asia are devastating. Now, laboratories in Mauritius, Malaysia, Tanzania, Zimbabwe and Trinidad use cost-effective kits to detect these diseases quickly and accurately. The design of the kits takes into account the often poor conditions in these laboratories. The tests have great potential for certifying crops for export and import, as well for meeting food safety standards. Many plant pathology laboratories around the world have already asked for them.

Project Ref: **CPP79:**

Topic: **1. Improving Farmers Livelihoods: Better Crops, Systems & Pest Management**

Lead Organisation: **Natural Resources Institute (NRI), UK**

Source: **Crop Protection Programme**

Document Contents:

[Description](#), [Validation](#), [Current Situation](#), [Environmental Impact](#), [Annex](#).

Description

CPP79

A. Description of the research output(s)

1. Working title of output or cluster of outputs.

In addition, you are free to suggest a shorter more imaginative working title/acronym of 20 words or less.

Validated molecular diagnostic methods for important bacterial and fungal plant pathogens

2. Name of relevant RNRRS Programme(s) commissioning supporting research and also indicate other funding sources, if applicable.

Crop Protection Programme

3. Provide relevant R numbers (and/or programme development/dissemination reference numbers covering supporting research) along with the institutional partners (with individual contact persons (if appropriate)) involved in the project activities. As with the question above, this is primarily to allow for the legacy of the RNRRS to be acknowledged during the RIUP activities.

R6520: Molecular and biochemical techniques for identification and characterization of *Pseudomonas solanacearum* (01/10/1995 -31/03/1999)

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[Caribbean](#), [Europe](#), [Kenya](#),
[Malaysia](#), [Mauritius](#), [Tanzania](#),
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R6007cb and R6692: Diagnostics for Sigatoka fungal pathogens of Musa spp. (01/08/1996-31/03/1997)

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Project established linkages with Banana programmes of :

1. DFID Kenya National Agricultural Research Programme (NARP)
2. International Institute of Tropical Agriculture (IITA)
3. The Rockefeller Foundation

4. Describe the RNRRS output or cluster of outputs being proposed and when was it produced? (**max. 400 words**). This requires a clear and concise description of the output(s) and the problem the output(s) aimed to address. Please incorporate and highlight (in bold) key words that would/could be used to select your output when held in a database.

Bacterial wilt of potato, groundnut and tomato, as well as Black **Sigatoka** of **banana** cause devastating crop yield losses in many countries in Africa and Asia. These diseases are amongst the top constraints to yield improvement for banana, potato, tomato and a range of other **vegetables** in **humid** regions. A series of projects were funded by the RNRRS to innovate rapid **diagnostic methods** for these important plant pathogens, as well as general tests to improve identification of all **plant pathogenic bacteria**.

Project R6520 developed and validated a highly sensitive diagnostic test for the most important bacterial plant pathogen worldwide, namely **Ralstonia solanacearum** (synonym **Pseudomonas solanacearum**). This test was based on polymerase chain reaction (**PCR**) amplification of ribosomal **DNA**. The PCR test was able to detect latent infections in **potato tubers** enabling the **plant health** of vegetative material at **quarantine** laboratories to be assessed. Traditional methods for identification of all plant pathogenic bacteria were also modified into a cost-effective **kit** format (**BACTID**). Laboratories in developing countries have variable and often poor infrastructure, so a suite of

diagnostics were developed to suit these challenging situations. Diagnostic laboratories were set up in **Mauritius, Malaysia, Tanzania and Zimbabwe** and research staff trained. Detailed laboratory **training manuals** were produced and published by NRI to reinforce the training given. PCR diagnostics and manuals were also developed and validated under R6007cb and R6692 for the detection and discrimination of Sigatoka **leaf spot** pathogens of banana (*Mycosphaerella* spp.). These were field-tested and transferred to laboratories in **Uganda and Kenya** in the late 1990s.

The promotion and uptake of the above diagnostic tests will assist **plant health** diagnostic research labs throughout Sub-Saharan Africa by building their capacity to make correct disease diagnoses, select the most suitable control options available as well as certifying the health status of imported/exported **vegetative plant material**. The BACTID kit tests developed also have the potential to be used for **food-borne** human pathogens and would hence have application in **food safety** testing and for food **trade** and **SPS** border controls.

5. What is the type of output(s) being described here?
Please tick one or more of the following options.

Product	Technology	Service	Process or Methodology	Policy	Other Please specify
X	X		X		

6. What is the main commodity (ies) upon which the output(s) focussed? Could this output be applied to other commodities, if so, please comment

banana, chilli, ginger, groundnut, potato, tomato, vegetables

This output provides valuable lessons on the general principles of how to detect/diagnose and control selected pathogens. As such, its findings will be applicable to the production of healthy vegetatively planting material of any crop.

7. What production system(s) does/could the output(s) focus upon?
Please tick one or more of the following options.
Leave blank if not applicable

Semi-Arid	High potential	Hillsides	Forest-Agriculture	Peri-urban	Land water	Tropical moist forest	Cross-cutting
							X

8. What farming system(s) does the output(s) focus upon?
Please tick one or more of the following options (see Annex B for definitions).
Leave blank if not applicable

Smallholder rainfed humid	Irrigated	Wetland rice based	Smallholder rainfed highland	Smallholder rainfed dry/cold	Dualistic	Coastal artisanal fishing
X	X		X			

9. How could value be added to the output or additional constraints faced by poor people addressed by clustering this output with research outputs from other sources (RNRRS and non RNRRS)? (max. 300 words).

Please specify what other outputs your output(s) could be clustered. At this point you should make reference to the circulated list of RNRRS outputs for which proformas are currently being prepared.

The following research areas will benefit from being clustered with the outputs from the research described below:

1. Research on vegetable and potato diseases in small-scale farming will benefit from using easily applied but accurate diagnostics provided by support services, either public or private. For bacterial diseases, symptoms do not generally provide a reliable guide to cause, nor does colony morphology (see B 10). Therefore, laboratory-based diagnoses are routinely necessary. Applicable RNRRS output proformas would be:

Crop Protection Programme

- Promotion quality kale seed (R8312, R8439, R7571)
- Bean root rots (R8476, R8316, R7568, R7965)
- Sustainable potato seed tuber management ((R8435, R8104, R7856)
- Increasing yield and sustainability of banana production by small-scale growers through use of improved crop management practices to control the spread and reduce the effect of banana virus diseases (R7529, R8342, R7478)

Crop Post Harvest Programme

- Indigenous vegetables (R6964/ R7487)

2. Research into appropriate diagnostic methodology for farm-based plant pathology and plant quarantine (and research into use of this technology) – aimed at improving border controls and export certification. This is in the context of implementation of the international regulatory framework provided by the Agreement on the Application of Sanitary and Phytosanitary Measures ('SPS Agreement') of the World Trade Organisation (WTO). This Agreement imposes rules that require sanitary and phytosanitary border controls (e.g. prohibition of items contaminated by certain pathogens) which must be related to actual risks to human, animal or plant life in the importing country rather than be used to disguise arbitrary and discriminatory non-tariff barriers to trade (see also 19).

3. Parallel research into diagnostics for food-borne human pathogens if BACTID were expanded to include this type of bacteria (see 16).
4. Policy-driven (socio-economic) research into SPS compliance, investigating institutional reform, competent authority creation and capacity and training needs.
5. Socio-legal research into regulatory interventions for SPS border controls.

Validation

B. Validation of the research output(s)

10. How were the output(s) validated and who validated them?

Please provide brief description of method(s) used and consider application, replication, adaptation and/or adoption in the context of any partner organisation and user groups involved. In addressing the "who" component detail which group(s) did the validation e.g. end users, intermediary organisation, government department, aid organisation, private company etc. This section should also be used to detail, if applicable, to which social group, gender, income category the validation was applied and any increases in productivity observed during validation (max. 500 words).

The outputs for this proforma are all laboratory diagnostic tests, which were initially validated by NRI scientists on collected field material and pathogen reference strains. The tests were validated through testing material and comparing the results obtained to those generated by more traditional means. Thereafter the outputs were validated in overseas laboratories by NRI scientists in collaboration with the in-country partner scientists listed in 3 (as described below).

BACTID

BACTID describes a kit for bacterial identification based on modifying traditional methods for identification of all plant pathogenic bacteria into a cost-effective kit format. The key issue in the identification of plant pathogenic bacteria is the need to distinguish a potential pathogen from contaminating non-pathogenic bacteria. Colony type and colour and cell morphology are rarely sufficient for this purpose. Hence the need for many different specialised, selective or semi-selective media and a host of reagents for confirmatory tests. Preparing these media conventionally in Petri dishes is time-consuming and the media so prepared have a limited shelf-life. The BACTID kit uses Eppendorf tubes instead of Petri dishes; the tubes can be prepared in bulk and kept for several months so the media are instantly available for use. Much smaller quantities of media ingredients and reagents are necessary for these small tubes. The BACTID kits were validated by using them for identifying plant pathogenic bacteria in working plant pathology laboratories (Plant Clinics) attached to agricultural research stations.

Ralstonia solanacearum PCR test

Project R6520 developed and validated a highly sensitive diagnostic test for one of the most important plant pathogens worldwide, namely *R. solanacearum* (Rs). This test was based on polymerase chain reaction (PCR) amplification of ribosomal DNA. The PCR test was used to diagnose this bacterium in plant material and was found to be particularly useful for screening seed potato tubers for latent infections at export/import borders. Diagnostic laboratories were set up in Mauritius, Malaysia, Tanzania and Zimbabwe and research staff trained. Detailed laboratory training manuals were produced and published by NRI to reinforce the training given.

Although no increases in productivity resulted from the laboratory tests, the PCR tests did identify infected planting material and so indirectly resulted in increased productivity for those farmers who as a result only used clean planting material (see ref: Skoglund, L., Seal, S., Elphinstone, J., Berrios, D. (1993) Study of latent infection of potato tubers by *Pseudomonas solanacearum* in Burundi. In: Bacterial Wilt (eds. G.L. Hartman & A.C. Hayward), pages 106-110. Canberra: ACIAR Proceedings no. 45).

Independently, the test was validated and adopted by over 10 of the EU quarantine laboratories as the most sensitive rapid test for Rs, when this pathogen became a particularly serious problem for the European seed potato industry.

Mycosphaerella PCR tests

PCR diagnostics and manuals were also developed and validated under R6007cb and R6692 for the detection and discrimination of Sigatoka leaf spot pathogens of banana (*Mycosphaerella* spp.). These were field-tested and validated by laboratories in Uganda and Kenya as useful for field studies of the distributions of Yellow (*Mycosphaerella musicola*- *Mm*) and Black Sigatoka (*M. fijiensis* - *Mf*) in East Africa. The PCR tests complemented surveys based on visual symptoms and showed that in Uganda, spread of the more pathogenic *Mf* had not been as rapid as anticipated. Accurate disease distribution maps were prepared using PCR data, which were of benefit to plant breeders and extension services concerned with management of Sigatoka leaf spots.

11. Where and when have the output(s) been validated?

Please indicate the places(s) and country(ies), any particular social group targeted and also indicate in which production system and farming system, using the options provided in questions 7 and 8 respectively, above (max 300 words).

The prototype BACTID system was field tested in Zanzibar 1991-1995 (Kizimbani Research Station) under very poor laboratory conditions (virtually no electricity or running water over a two-week period). Its use under those conditions demonstrated the presence of citrus canker bacterium, not previously recorded in Tanzania. (EPPO Reporting Service 97-105, 1997). The use of BACTID alerted the authorities to this quarantine pathogen's presence and thus stimulated control and eradication

activities.

In 1997, when BACTID was adopted in northern Tanzania (Horti Tengeru, Arusha), the presence of *Clavibacter michiganensis* ssp. *michiganensis*, a serious pathogen of tomato was demonstrated, as well as several other bacterial species and strains. This similarly allowed the diseases they caused to be properly recognised and taken account of in research and extension activities. The scientific validation of the outputs is published (Black et al. 1999. Plant Disease 83: 1070).

The system was also tested in Mauritius 1992-1995, in connection with detection of another plant pathogenic bacterium that was of quarantine concern (*R. solanacearum*). Both here at MSIRI, and at AVRDC/HortiTengeru Arusha, the BACTID tests were supplemented by use of the *Rs* PCR test. PCR diagnostic laboratories in Kenya (CIP), Malaysia and Zimbabwe also validated the *Rs* PCR test between 1992-1995.

PCR diagnostic tests for *Mycosphaerella* spp. were also used routinely by Kawanda Agricultural Research Institute (Kampala, Uganda) and the National Agricultural Research Laboratories (Nairobi, Kenya) during 1996-1998 to monitor and characterise *Mm* and *Mf* in key banana producing areas of Uganda, Kenya and Tanzania. The tests were not only useful for producing disease distribution maps, but also showed which banana cultivars were particularly susceptible to the more devastating *Mf*.

The outputs are thus of cross-cutting benefit having been useful for peri-urban production systems (e.g. fruit for local markets in Zanzibar, vegetables in E. Africa), irrigated farming systems (e.g. Mauritius -multiple cropping in sugar cane plantations), high potential vegetable and banana production systems of smallholders in East African highlands.

Current Situation

C. Current situation

12. How and by whom are the outputs currently being used? Please give a brief description (max. 250 words).

The laboratories where the techniques were established under RNRRS funding still use the tests for diagnoses where project funding exists for such diagnostic tests and maintenance of equipment. However most requests about both the *Rs*- and *Mm/Mf*-PCR tests come from university research scientists from developing countries who wish to use the tests for their studies.

The PCR diagnostics have recently been used (at NRI for the Trinidad Ministry of Agriculture) to confirm the presence of *M. fijiensis* in leaf samples of French Plantain from Trinidad; this is assumed to be a new variant of *Mf* that can infect French plantain which other genotypes do not.

Requests for guidance on setting up BACTID have been received from plant pathology laboratories around the world (developed and developing countries.) The laboratories cover those engaged in routine diagnosis (Plant Clinics), research or university teaching.

13. Where are the outputs currently being used? As with Question 11 please indicate place(s) and countries where the outputs are being used (max. 250 words).

Target laboratories set up in the 1990s at MARDI and MSIRI still use the PCR tests. However the molecular technologies are not being actively used in the African laboratories due to a lack of funding to purchase reagents and carry out epidemiological studies without specific donor projects. The equipment also requires servicing to ensure it is functioning properly. The *Rs* PCR test is being used actively by European research and quarantine laboratories to determine whether potato seed tubers are suitable for export or harbour latent *Rs* infections. They are also being used for epidemiological purposes as *Rs* has been found to infect weed hosts present along European rivers, which then act as a source of inoculum for future crops. The BACTID and PCR tests are also being used at NRI for research and MSc teaching purposes.

14. What is the scale of current use? Indicating how quickly use was established and whether usage is still spreading (max 250 words).

It has not been possible to gauge current usage of the tests accurately, which for the PCR tests is felt to be a probable indication of their low routine use. It appears that since transfer of these techniques, they are only being used actively at MSIRI and for the *Rs* test in EC quarantine labs. Nevertheless it is clear that persons trained in the molecular techniques are now familiar with such technologies and using the training and equipment received to adopt PCR techniques for pathogens for which there is project funding.

For the BACTID kit, instructions for use and its intellectual property are in the public domain as is the obsolete DOS-based software (including expert system to identify bacteria from test results and recipes and full instructions for making up all the necessary media and reagents. (Reference: Black, R., Holt, J. & Sweetmore, A (1996). BACTID - Bacteriological identification system for resource-poor plant pathology laboratories. Natural Resources Institute). Anyone with the appropriate physical resources could have set up the system and be using it.

15. In your experience what programmes, platforms, policy, institutional structures exist that have assisted with the promotion and/or adoption of the output(s) proposed here and in terms of capacity strengthening what do you see as the key facts of success? (max 350 words).

The WTO's SPS Agreement came into existence in 1995 and has started to assist adoption of

appropriate diagnostic technologies by developing countries to try to achieve compliance with this Agreement. For the first 5-6 years after the SPS Agreement came into existence, the debate in development circles was centred on whether this Agreement was equitable or if there was an in-built bias of WTO towards benefiting rich countries. However, since 2000 there has been increasing emphasis on the actual SPS issues with the realisation that the Agreement does provide an objective and non-discriminatory means of judging whether border controls imposed by an importing country are there to protect human, animal and plant health or whether they are disguised barriers to trade. The difficulty faced by developing countries now relates to the unequal distribution of resources to achieve the necessary infrastructure and capacity to comply with the provisions of the agreement, not with the agreement itself. Fortunately, development agencies are finally providing technical assistance to assist developing countries achieve the required capacity.

Meeting the import requirements in intended export markets (e.g. absence of a specified organism of quarantine status like *Rs*) is achieved through export certification and/or use of surveillance to demonstrate that certain areas are free of specified quarantine pests. Surveillance capacity is also needed to justify a country's own import requirements since organisms cannot be prohibited in imported goods if already widely present in-country or not subject to domestic controls. Greatly enhanced diagnostic capacity is therefore needed in poorer countries in order to demonstrate the (phyto)sanitary condition of their exported goods. In the case of BACTID, these lessons would apply equally to plant health as well as food safety, if BACTID were expanded to cover food-borne human pathogens as well as plant pathogens.

The banana-related diagnostics have been promoted to some extent through the activities and publications of the International Banana and Plantain Network (INIBAP) of IPGRI and collaborating organizations such as BARNESA.

A number of programmes have also been set up involving the production of healthy planting material. For seed potato tubers, vegetable seed(ling) and banana plantlet production, such programmes should in the near future be resulting in increased demand for appropriate diagnostic technologies. Examples of recent programmes in East Africa for generating healthy banana planting material are Agro Genetic Technologies Ltd, (the first commercial producer of tissue culture banana plantlets in Uganda) and the Crop Crisis Control Project (C3P), for which part of the strategy is to improve the supply of clean planting material by promoting the multiplication in disease-free nurseries.

Environmental Impact

H. Environmental impact

24. What are the direct and indirect environmental benefits related to the output(s) and their outcome(s)? (max 300 words)

The diagnostic outputs are important components of integrated pest management (IPM) strategies and the direct and indirect benefits that result are as follows:

(a) Bacterial plant diseases rarely respond to pesticides. Only copper 'fungicides' have an effect on bacterial diseases if used for prevention, but copper resistance often follows. The use of antibiotics as pesticide is now banned in most countries. Control of bacterial plant diseases therefore has to be by use of clean planting material, field sanitation and resistant varieties. However, farmers in their ignorance tend to use fungicides and other pesticides indiscriminately when confronted with a serious disease problem in their crops, particularly those with a high market value. Greater awareness of nature of bacterial diseases will lessen such ill-advised pesticide use and reduce environmental pollution.

(b) Similarly, it is recommended to destroy banana plants infected with *Rs*, *Mm* or *Mf* to remove sources of inoculum/vectors (for *Rs*). For smallholders, destruction is usually by removal of the infected plants and its suckers. If replanting with clean material is not managed in a timely manner, there is a risk of increased soil erosion. In situations where the infection is high, banana cultivation may be abandoned in favour of short-term crops which are more damaging to the soil and environment through increased soil tillage and use of pesticides.

(c) Plant quarantine that will benefit from better diagnostic capacity is essentially a preventative method of disease control – disease avoidance in formal IPM terms. With disease avoided, there will be less pesticide use with resulting benefits to the environment.

(d) Some bacterial diseases affect plants that are components of natural vegetation or plants that are important for amenity/landscape purposes (tourism). Improved plant quarantine is therefore important for habitat protection and conservation.

25. Are there any adverse environmental impacts related to the output(s) and their outcome(s)? (max 100 words)

The PCR tests can involve the use of carcinogenic/mutagenic compounds for nucleic acid isolation and PCR product detection. Such compounds have to be disposed of correctly to ensure there are no adverse environmental impacts. Certified waste disposal systems, and regulations thereof, are sometimes lacking in developing countries.

Black Sigatoka is responsible for severe yield losses in continuous banana cultivation in Africa, the Pacific and in Central America. The most effective way of controlling this disease is by systemic fungicides. Such spraying programmes are environmentally unfriendly, but this risk only relates to relatively prosperous farmers who can afford these chemicals.

26. Do the outputs increase the capacity of poor people to cope with the effects of climate change, reduce the risks of natural disasters and increase their resilience? (max 200 words)

As climates change so will disease distributions and hence the diagnostic techniques will assist in the monitoring of the spread of pathogens, particularly *Rs*, *Mm* and *Mf*. When conditions are not conducive to *Rs* it often remains present in a latent state. This presents a precarious situation as it increases the likelihood of vegetative planting material (potato tubers, banana suckers, ginger rhizomes) spreading the disease. Similarly the distribution of *Mf* (Black Sigatoka) in Uganda was determined (by PCR) to be limited to altitudes below 1350 m with a mean annual minimum temperature of 13-14°C. Global warming would change the disease distribution and may also affect host susceptibility differentially leading to unexpected yield losses for poor people.

The diagnostic outputs all assist the identification of clean planting material, which should increase crop yields and resilience for recipients thereof. This will be most marked for bananas as these can be harvested throughout the year hence providing a valuable source of food in between the harvest of other crops. Moreover, as banana is able to withstand short periods of drought or flood, the crop is more resilient than many other crops to adverse climatic conditions.

Annex

Annex 1: Effect of diagnostic outputs on various poverty groupings (modified from data produced for banana IPM proformas by Richard Lamboll, NRI)

Poverty grouping	Capital assets Human, Social, Natural, Physical. Financial	Addressing vulnerability	Outcomes
Moderate poor	Improved access to clean planting material	Shocks Drought Sigatoka Bacterial wilt Bacterial diseases	Increased productivity XX kg/ ha Y% increase Contributing to: Food security Improved income
Extreme vulnerable poor	As for moderate poor taking into account:	As for moderate poor taking into account	As for moderate poor
Assetless (or near assetless) male & female headed households in rural areas	Direct - can only benefit directly if access to land is secure for many years Indirect –processing?? Employment on farm	Little or no land Share-cropping	More emphasis on food security
Women headed households (without adult male)	Less time to engage in and more likely to be excluded from process (ca. 25% of banana growing households in Uganda and Tanzania)		
Poor people living in disaster prone or remote areas	Less likely to be engaged in process and more likely to benefit from product only		More emphasis on food security
Poor people living in urban areas			Possibly lower prices for purchase of banana, tomato and vegetables