FINAL TECHNICAL REPORT

MOLECULAR AND BIOCHEMICAL TECHNIQUES FOR DETECTION AND IDENTIFICATION OF *RALSTONIA SOLANACEARUM* AND OTHER PLANT-PATHOGENIC BACTERIA

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Executive Summary

The project's purpose was to adapt, evaluate and use different techniques of bacterial identification for the reduction of losses caused by bacterial pathogens in vegetables.

Research activities involved:

- Participatory appraisal of farmers' perceptions of constraints to vegetable production, knowledge of and attitudes to relevant diseases and their cultivation practices in order to determine the socio-economic background to vegetable production and protection
- Field surveys and sampling of diseases of vegetables suspected to be caused by bacterial plant pathogens
- Identification and characterisation of bacterial plant pathogens in diseased plants and germplasm
- Evaluation and comparison of diagnostic techniques for their suitability in resource-poor situations
- Adaptation of diagnostic technology for bacterial disease management
- Identification of components of management strategies for bacterial diseases of vegetables

The project initially focused on Malawi (Bvumbwe Research Station) and Tanzania (Horti-Tengeru/Asian Vegetable Research and Development Centre-Africa Regional Program, Arusha) but the project had to withdraw from Malawi in the first year of the project due to transfer of the principal collaborator (Malawi) to work exclusively on coffee at another station and no other scientists were available as collaborators.

Technical outputs were the adaptation of a suite of diagnostic technologies for plant-pathogenic bacteria (semi-selective media/*BACTID*, ELISA, molecular detection (PCR and fingerprinting (RAPD) methods); evaluation of the various techniques in relation to the purpose of the diagnosis and prevailing conditions such as infrastructure and supply of electricity and water; data on distribution and incidence of target pathogens (*Ralstonia solanacearum*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas corrugata* and *Xanthomonas campestris* pv. *vesicatoria* on solanaceous crops and *X.c.* pv. *campestris* on crucifers), several pathogens being confirmed for the first time; data on contribution of infection of germplasm within cropping system; sources of variation in *R. solanacearum* and *X.c.* pv. *campestris* identified that will be important for disease control strategies; culture collection of reference isolates established; and strategies for control identified - internal quarantine, seed quality, cultivation practices. The work in Tanzania centred on PhD studies of the principal collaborator.

Dissemination outputs included 14 published papers, the manual/software on *BACTID*, manual on PCR/ELISA (final proof stage), data sheet on *X. axonopodis* pv. *citri* and other bacteria, presentations at seven conferences and seminars and use of techniques developed in teaching and training. Additional uptake pathways include possible further CPP research and dissemination in Tanzania, expansion and improvement of the *BACTID* system software into a comprehensive teaching aid and practical guide (HEFCE-funded) and additional scientific papers.

The project outputs have contributed directly to the project purpose and therefore DFID's developmental goals by developing and disseminating technology for diagnosis of bacterial diseases and determining the significance of selected bacterial plant pathogens in vegetable cropping systems.

Background

Bacterial diseases such as bacterial wilt are known to be serious in the sub-tropics and tropics but most plant pathologists in countries with such climates have had little exposure to bacteriology compared to mycology or even virology. One reason for the comparative neglect of bacteriology in developing countries is the lack of techniques for identification and detection of plant pathogenic bacteria that are appropriate for the prevailing conditions, particularly the lack of financial resources for purchase of all the different reagents and media ingredients required in "classical bacteriology". The application of modern technology like serological and molecular techniques is also problematical because of frequent power cuts and other deficiencies in the infrastructure. (Black and Sweetmore, 1995)

Various techniques for the diagnosis of bacterial wilt and the detection of the pathogen *Ralstonia solanacearum* (synonyms *Pseudomonas solanacearum, Burkholderia solanacearum*) have been developed in ODA-funded projects (I0037, X0071, X0082). These techniques were the *BACTID* system, metabolic profiling, various molecular techniques including probes and the polymerase chain reaction (PCR), and enzyme-linked immnuosorbent assays (ELISA).**]** Semi-selective media/*BACTID*, metabolic profiling and molecular techniques were further developed and adapted and field tested in Mauritius, Malaysia, Zimbabwe (Adaptive Research Initiative project (F0020). The three target countries had different levels of infrastructure and staff training; not all the techniques were equally suitable at all locations and the results provided experience to guide the choice of techniques according to the needs and the available resources.

In the project reported here, semi-selective media*/BACTID* were applied in Malawi (initially, see below) and the whole suite of techniques (including ELISA) in Tanzania for the study of bacterial diseases in field and pathways for their spread in the cropping systems as well as systematic evaluation of the different techniques. The intention was to focus on other diseases of vegetables in addition to bacterial wilt. Vegetable cash cropping has intensified in recent years in response to growing urban markets in Tanzania and Kenya but their bacterial diseases had not been studied in any detail. The project included a PhD training component undertaken by the principal collaborator.

PROJECT PURPOSE

To adapt, evaluate and use different techniques of bacterial identification for the reduction of losses caused by bacterial pathogens in vegetables.

RESEARCH ACTIVITIES

Overseas work in the project was originally based in Malawi and Tanzania. In Malawi, activities were based at Bvumbwe Research Station (Limbe, near Blantyre) which is responsible for research on vegetables and is the technical centre for plant quarantine. Because of the available infrastructure and because of the experience of the scientific and support staff, it was intended that the techniques to be adapted and used at Bvumbwe would come from the lower technology end of the range available, principally semi-selective media and *BACTID*. In Tanzania, the base was Horti-Tengeru (Horticultural Research Station of the Ministry of Agriculture at Tengeru, Arusha) and the adjacent Asian Vegetable Research and Development Centre - Africa Regional Programme (AVRDC-ARP) at Madiira, Arusha. There the full range of techniques would be applicable, including PCR. The field component of the split PhD studies was based there.

During the first full year of the project, the main collaborating plant pathologist at Bvumbwe was transferred to another station with duties exclusively devoted to coffee. Regrettably, the project had to withdraw from Malawi as there was no replacement counterpart. Hence the project activities varied from original plan: after activities in Malawi were curtailed, permission was obtained from the programme management to concentrate the project's financial resources on Tanzania with consequent expansion of the activities.

With the re-alignment of the project Tanzania-based activities centred around:

(a) participatory rural appraisal of vegetable production in relation to disease incidence
(b) surveys of known or suspected bacterial diseases in the field
(c) laboratory-based identification of bacterial plant pathogens

These led to achievement of all the originally scheduled outputs and additional/expanded ones made possible by devotion of project resources to the Tanzania-based work. From May until November 1996, the principal collaborator worked at NRI exploring various techniques available for appraising farmers' perceptions of diseases and crop management methods, for surveying plant diseases in the field and for detecting and identifying bacterial plants pathogens. From these a selection of techniques was made to use in Tanzania. From January 1997 until October 1998 the principal collaborator was based in Arusha, Tanzania at the Asian Vegetable Research and Development Center - Africa Regional Program in association with Horti-Tengeru (Plant Pathology Laboratory).

Participatory rural appraisal of vegetable production in relation to disease incidence

In order to determine the socio-economic background to vegetable production, to assess farmers' knowledge of, and attitudes to, relevant diseases and to probe their cultivation practices, a series of PRA events were carried out in the northern region and in the southern highlands. Field visits for PRA were run in conjunction with surveys of plant diseases during the following periods:

- Southern highlands: 11-15 March 1997 and 13-23 February 1998
- Arusha: throughout period 5 February 1997 - 17 September 1998
- Lushoto: 12-13 June 1997 and 10-11 August 1998
- Kilimanjaro: 3 June 1997 and 6 August 1998
- Zanzibar: 20-22 July 1998

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Surveys of known or suspected bacterial diseases in the field; laboratory-based identification of bacterial plant pathogens

Initial surveys and discussions supported by a visit from the project leader in late January centred on the selection of target vegetable crops and pathogens and also on whether it was appropriate for PCR to be installed by the project at Horti-Tengeru.

The pathogens and crops to be studied were selected:

R. solanacearum	Tomato	Potato	(other Solanaceae)
C.m. subsp. *michiganensis*	Tomato		
X.c. pv. *vesicatoria*	Tomato	Sweet pepper	
X.c. pv. *campestris*	Crucifers		

X.a. pv. *citri* Citrus

Citrus was not within the mandate of Horti-Tengeru or AVRDC-ARP but both these institutions kindly gave assistance to the project leader to make a brief survey in the Tanga region where citrus canker was thought most likely to occur. (Citrus canker was not found and it is assumed from information available so far that this disease is absent from the mainland.)

As regards PCR it was decided that it was appropriate to set up PCR facilities at Horti-Tengeru (rather than the collaborator having to travel to the coconut research station in Dar-es-Salaam to carry out molecular analyses) and the project then purchased the necessary equipment and consumable items (see Annex 1.)

OUTPUTS

The original outputs of the project as stated in the project memorandum are given in the box below. These were all achieved.

(i) NRI manuals on BACTID (software/methods) and molecular diagnostics in uniform edition with *PlantClinic* and the *Plant Quarantine* Primer (by July 1996)

(ii) Improved capacity in overseas countries to diagnose bacterial wilt of plants, to isolate from diseased plants, to identify the causal organism and make reference cultures for future screening studies (by December 1996).

(iii) Use of improved capacity by overseas collaborators to survey the importance of bacterial diseases on crops in high potential production systems in target countries (by December 1997).

(iv) Reagents and software components of BACTID updated to allow identification at the species level of other plant pathogenic bacteria identified as being important in target countries (ongoing process with final update in May 1998).

(v) Representative culture collections of PS identified through use of the DNA probe to ensure future field studies and germplasm screening studies are performed against isolates representative of in-country variability (by May 1997).

(vi) Improved decision making in plant quarantine through improved capacity to diagnose bacterial plant disease (by March 1998).

(vii) Annual project reports and at least three scientific papers (ongoing).

However, it had already been necessary to withdraw from Malawi by the time the Tanzanian component of the project was being planned in detail. With permission to use the agreed budget exclusively for the Tanzanian component, it was possible to achieve far more than was originally planned. In particular, it was possible to evaluate and systematically compare different diagnostic techniques for use in Tanzania and to use these techniques to study the occurrence and distribution of the target pathogens and the diseases they occur. Therefore, when new objectives were agreed with CPP, the outputs were expanded as follows:

1. **Diagnostic technology for plant pathogenic bacteria adapted for use in Malawi and Tanzania** (includes original output ii, iv).
2. **The distribution of the target pathogens and their incidence within the vegetable cropping systems studied determined** (includes original output iii).
3. **The extent of infection of true seed and vegetative germplasm by the target pathogens investigated**

(additional output).

4. **The sources of variation in *R. solanacearum* and *X.c.* pv. *campestris* identified**

(includes original output v).

5. **Represented cultures of isolates of all the target pathogens retained at NRI**

(original output ii).

6. **Strategies for management of bacterial diseases of solanaceous vegetables identified**

(includes original output vi).

7. **Diagnostic techniques for their suitability in resource-poor situations evaluated and compared**

(includes original output ii).

8. **Diagnostic manuals other dissemination outputs produced**

(includes original output i and vii).
Some highlights of the results from the principal collaborators PhD thesis are included below. A copy of the thesis will be deposited with the Crop Protection Programme after the student's *viva voce* examination.

1. Diagnostic technology for plant pathogenic bacteria adapted

Malawi

Two visits to Malawi were made in 1996, 28 February - 23 March and 10 - 31 July. Bacteriological techniques were introduced to the plant pathology staff at Bvumbwe. Field collections were then made in several localities to obtain material for isolation of bacteria (using either basic media or semi-selective media as appropriate) and identification using the *BACTID* system. This work confirmed the presence of various bacterial plant pathogens suspected from symptoms: *R. solanacearum, Xanthomonas campestris* pv. *campestris* (black rot of crucifers) and *X. axonopodis* pv. *manihotis* (cassava bacterial blight). The *Xanthomonas* spp. isolates were used to test PCR primers for *Xanthomonas* in the PhD student's preliminary work at NRI.

Sufficient materials for several months' isolation and preliminary identification work were left at Bvumbwe. The Senior Technician in the Plant Pathology Group (which was part of the bacteriology development team) was able to use the diagnostic technology in the Farming Systems IPM Project to which she was assigned. Other personnel from Malawi received training in bacterial identification under a different project (see below).

Preliminary work in UK in preparation for Tanzania-based studies

Between May and October 1996 the principal collaborator established the basic methods that she would use in Tanzania. The work included:

- Establishing protocols for seed tests

Seed samples of a wide variety of vegetable seeds were used. Sampling and extraction methods for seeds and isolation media appropriate to the target organisms were evaluated for eventual use in Tanzania. Where there was a choice of a number of selective media for a given target organism and one contained, for example, fewer antibiotics than the other, the simpler medium would be preferred provided it gave acceptable results.

- Isolation procedures for plant tissue, especially the use of semi-selective media

The initial work concentrated on isolating various *Xanthomonas* spp. from different hosts and *C.m.* subsp. *michiganensis* from tomato seed samples from Tanzania. The pathogens extended the scope of the project from the original focus on *R. solanacearum*. *C.m.* subsp. *michiganensis* was the first Gram positive plant pathogen to be tested with the *BACTID* system.

Citrus canker bacterium (*Xanthomonas axonopodis* pv. *citri*) was isolated from lime leaves brought from Zanzibar and from leaves of *Citrus hystrix* purchased from a London supermarket specialising in oriental produce. This bacterium was originally a potential target of the project as it had been detected in Zanzibar by the predecessor project (and officially reported as a quarantine introduction) but it was not known whether it occurred in mainland Tanzania. Isolates of this bacterium, *X. axonopodis* pv. *manihotis* (from Malawi) and pathovars of *X. campestris* were used to test PCR primers for specificity.

- Procedures for metabolic profiling

Familiarity with metabolic profiling was gained using the *Biolog* system. Suitable methods for incubating inoculum for the *Biolog* plates were developed.

- Polymerase chain reaction (PCR) techniques

PCR techniques for *R. solanacearum* and *Xanthomonas* spp. were applied to the identification of isolates obtained during this work and from the NRI culture collection. These included work with primers OLI1 and Y2 used for identification of *R. solanacearum* isolates (Seal *et al.* 1993) and primers XCC and Y2 (Seal, unpublished work) and RST2, RST3, RST9, and RST10 (Leite *et al.*, 1994) for the detection of *Xanthomonas campestris* pathovars.

<u>Tanzania</u>

By the end of the project, semi-selective media, *BACTID*, ELISA and PCR had all been adapted to conditions prevailing in Arusha (Plant Pathology Laboratory of Horti-Tengeru) and used successfully to identify the target plant pathogens and other bacteria associated with the plant material and to detect the pathogens in germplasm. The results of the use of the various techniques are detailed below.

Conclusions: The project regrettably had to withdraw from Malawi but semi-selective media/*BACTID* were demonstrated there with the potential to contribute to more accurate and efficient disease diagnosis. The full range of technologies were adapted in Tanzania. Both countries now have improved capacity to diagnose bacterial plant diseases including bacterial wilt. Technologies adapted in Tanzania for detection and identifying *R. solanacearum*, *C.m.* subsp. *michiganensis* and other bacterial plant pathogens that are suitable for the prevailing laboratory conditions and the target cropping systems. *R. solanacearum* and its biovars can now be identified accurately and rapidly in Tanzania. Prior to the work already done in Tanzania, the different causes of wilting in tomato could not be distinguished by plant pathologists or extension workers. Recommendations have been made for the expansion of the *BACTID* kit that will be incorporated into the new *Windows*-compatible software being developed. The practical experience gained in preparing the *BACTID* tubes and carrying out the tests will be put to good use in compiling the encyclopaedia of media, tests and reagents that will form part of the new software package.

2. The distribution of the target pathogens and their incidence within the vegetable cropping systems studied determined.

Field surveys

Along with the PRA exercises in each region, field surveys of suspected bacterial diseases were made to determine their incidence, to be later verified by diagnostic work in the laboratory. Prior to this project in Tanzania, the different causes of wilting in tomato could not be distinguished by plant pathologists or extension workers. The techniques introduced in this project determined that in the southern highlands, *C.m.* subsp. *michiganensis* was present in 38% of wilted plants sampled. In the northern highlands, *R. solanacearum* was isolated in 43% of wilting plants, *Pseudomonas corrugata* 7% and *C.m.* subsp. *michiganensis* not at all.

The incidence of bacterial wilt varies from field to field but up to 35% of tomato or potato plants can be infected, leading to serious crop losses. *C.m.* subsp. *michiganensis* had not been positively identified prior to this project. It was detected in about 30 % of seed lots sampled. Its apparent absence in the north is highly significant (see below).

Bacterial canker and the related wilt disease causes serious losses in some areas, especially the southern highlands where it can cause almost total crop loss. The presence in farmer's saved tomato seed and commercial seed has important implications because this pathogen appears to be confined to the southern highlands (Iringa and Mbeya). On the one hand there is the possibility of confining it by application of internal plant quarantine measures. However, working against this possible control measure is the phenomenon of seed exchange between farmers in different districts and different regions and also farmers' import seed from neighbouring countries. All these sources of seed could be contaminated.

Identification and characterisation of bacterial plant pathogens in diseased plants and germplasm

Samples of plants showing appropriate symptoms were collected for isolation of bacteria. Bacteria were also isolated from the seed or tubers of these plants to study the transmission of the pathogen. Preliminary identification was achieved using semi-selective media and the *BACTID* system. ELISA and or PCR were used for confirmation.

Table 1 shows the results of isolation of *R. solanacearum* from potato and tomato fields with wilt.

Table 1. Frequency of isolation of *R. solanacearum* from potato and tomato fields with wilt.

Region	Field with Potato wilt	*R. solanacearum* isolates	Field with Tomato wilt	*R. solanacearum* isolates
Arusha	5	3	6	0
Kilimanjaro	4	0	4	4
Tanga	5	4	4	2
Iringa	3	1	13	1
Mbeya	4	3	8	1
Zanzibar	N/A	N/A	4	1
Fields positive/fields surveyed	**11/42 (23%)**		**9/117 (8%)**	

Another pathogen isolated from wilting tomato plants was *C.m.* subsp. *michiganensis* (as well as from tomato fruits with canker). All seven fields in the southern highlands yielded this pathogen which was not isolated from any of the other locations. *C.m.* subsp. *michiganensis* had been suspected from symptoms but never confirmed previously.

Finally the third wilt pathogen isolated was *Pseudomonas corrugata* (pith necrosis), but this was only found in the experimental fields of AVRDC-ARP.

X.c. pv. *vesicatoria* and *X.c.* pv. *campestris* were isolated from solanaceous and cruciferous plants respectively but the main interest with these pathogens was contamination of seed.

Conclusions: Not only was the capacity established for local plant pathologists to survey bacterial plant diseases but the Tanzanian collaborators gained valuable data on the distribution of the target pathogens with vegetable cropping systems (high potential). *C.m.* subsp. *michiganensis* and *X.c.* pv. *vesicatoria* were confirmed for the first time in Tanzania. The former is probably a relatively recent introduction and its distribution appears to be incomplete with implications for future plant health measures. Definitive means of distinguishing wilt pathogens of tomato were provided that will help avoid confusion in the future.

3. The extent of infection of true seed and vegetative germplasm by the target pathogens investigated.

Detection of R. solanacearum *in seed potato tubers*

Seed and potato tubers actually intended for use as germplasm were examined as a separate exercise. Only the results for the two major pathogens, *R. solanacearum* and *C.m.* subsp. *michiganensis* are reported here. The results for the other pathogens studied are given in Zakia Abubakar's PhD thesis.

A total of 44 potato samples were analysed comprising of 12 farmers' saved seeds lots (five undried seed and six dried seed lots), 14 field infected potato lots, four from a commercial agent in Mbeya and 14 ware potato lots. The detection of *R. solanacearum* by different techniques is summarised in Fig. 1. The presence of *R. solanacearum* in seed tubers is compared with its presence in tubers collected from wilting plants as the starting point in the history of the planting material. The difference between the levels of *R. solanacearum* detected in field-collected tubers and actual seed

tubers is dramatic. Most significantly, although the farmers' dried tubers showed nearly 30% of brown rot symptoms, *R. solanacearum* could only be isolated or detected from 1 tuber out of 57 sampled. The handling and treatment of tubers by farmers in preparation for use as planting material should therefore be investigated. Tubers without brown rot symptoms could also be infected with *R. solanacearum.* This is the phenomenon referred to as latent infection.

In general biovar 2 was detected in the above material, apart from the commercial agent's "seed tubers" (Mbeya) which yielded both biovar 2 and biovar 3.

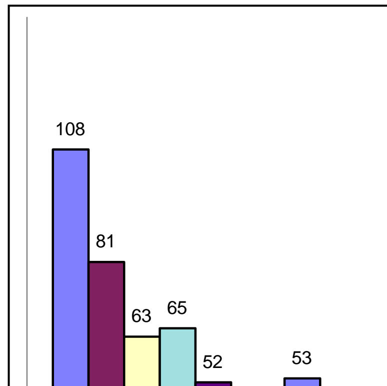

Fig. 1. Application of semi-selective media, indirect ELISA and PCR for detecting *R. solanacearum* in farmers' sources of planting material and field-infected tubers: number of tubers tested by different techniques to reveal brown rot or *R. solanacearum*.

Detection of C. m. subsp. michiganenis *in tomato seed*

A total of 61 seed lots of tomato were tested for *C.m.* subsp. *michiganensis*. These comprised commercial seed, farmers' saved seed and seed extr No. from cankered fruit by the same method as farmers use. Isolation using semi-selective media a of nfirmation by metabolic profiling showed that six of the 61 of the seed lots were contaminate tuber .m. subsp. *michiganensis*. About 33 of the 61 seed lots were tested with an ELISA kit specific s is pathogen; 12 of these samples (36%) tested positive for *C.m.* subsp. *michiganensis*.

Conclusions: The data obtained indicated that seed-borne inoculum plays an important part in the spread and development of the bacterial diseases and consequently that control of seed-borne infection would significantly reduce crop losses and improve quality.

4. The sources of variation in *R. solanacearum* and *X.c.* pv. *campestris* identified.

Assessment of phenotypic and genotypic variability in bacterial pathogens

R. solanacearum
The biovar of *R. solanacearum* isolates was determined by an adaptation of Hayward's (1964) method into the *BACTID* system (Fig. 2, Table 2). This is the first record of biovar 2 in Tanzania, and this biovar was found in all regions with highland-grown potato.

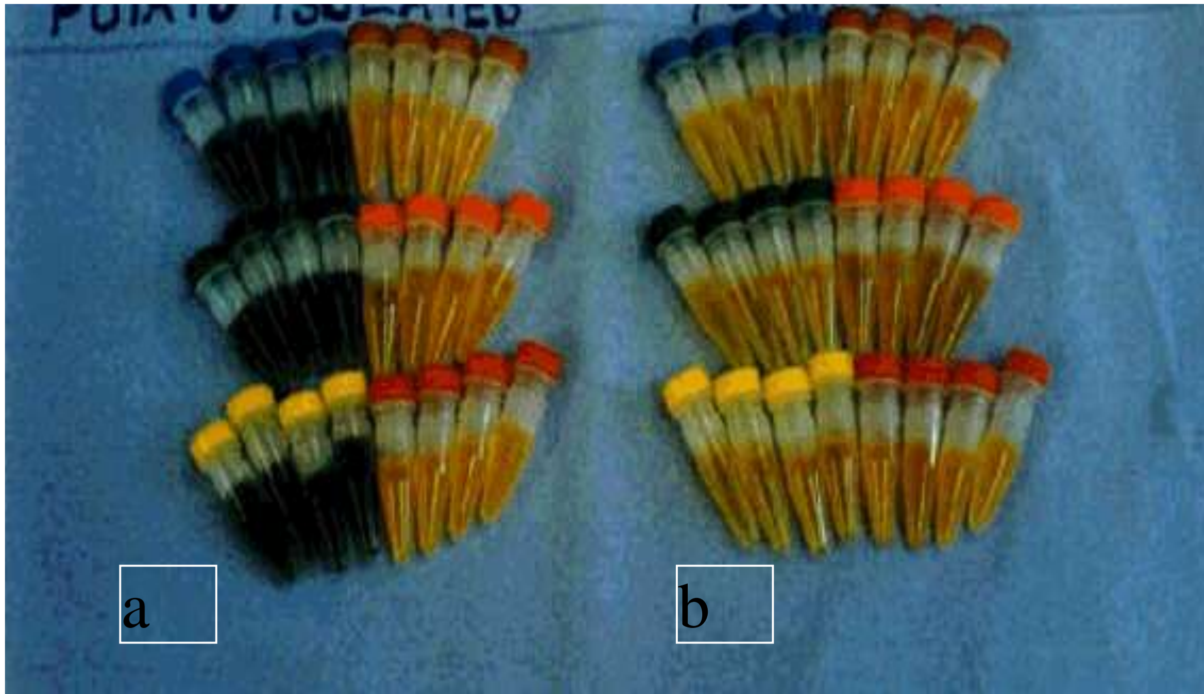

Fig. 2. *BACTID* biovar kit used to determine the biovars of isolates of *R. solanacearum* from tomato and potato: (a) biovar 2 (potato isolate); (b) biovar 3 (tomato isolate). A yellow colour indicates the metabolism of one of the differential substrates of the biovar test. Biovar 2 only metabolises disaccharides (right hand side of each set), biovar 3 metabolises disaccharides and sugar alcohols (left hand side of each set).

Table 2. Biovars of bacterial wilt pathogen, *R. solanacearum* as determined from isolates of field samples and farmers' planting material.

Region	Biovars identified per crop		
	Potato	**Tomato**	**Egg plant**
Arusha	2	-	-
Kilimanjaro	2	3	-
Tanga	2, 3	3	-
Iringa	2, 3	3	-
Mbeya	2, 3	2 (?)	-
Zanzibar	-	3	3

Metabolic profiling was also used to assess variability among 25 isolates of *R. solanacearum*. This showed that there were distinct differences between tomato and potato isolates (most of which divided into biovar 2 and biovar 3 respectively). Genetic variation of 22 isolates of *R. solanacearum* was also investigated by randomly amplified polymorphic DNA (RAPD) analysis. Genomic DNA was purified from the isolates and amplified using six 10 base oligonucleotide primers previously determined to be useful for detecting variation within *R. solanacearum*. Twenty four of the amplified bands were recorded and their presence in the different isolates used to group the isolates. Three cluster groups were revealed by cluster analysis. The similarity scale is not truly representative but the results are confirmed by principal component analysis (Fig. 3 and 4).

RAPD group 1 comprised of seven tomato isolates from the north, four of the isolates having 100% similarity, in spite of being isolated from samples collected from fields in different districts. RAPD group 2 comprised a mixture of five potato isolates, two tomato isolates, one eggplant isolate and the banana isolate (reference R207 from Belize)). Group 3 consisted of five potato isolates from the mainland and one tomato isolate from Zanzibar. This analysis has shown that there is considerable genetic variation of the pathogen present, which may be associated with hosts and geographical location. The presence of pathogen variation should be taken into account in selecting or breeding lines for resistance. The RAPD results also support speculation that *R. solanacearum* is transmitted by water flowing freely from one field to another in irrigation furrows:- four bacterial wilt isolates from different and spatially separated tomato crops in Kilimanjaro had a very high degree of similarity and it is suggested that they could originally have come from a common source in irrigation water before secondary transmission by human agency.

Xanthomonas campestris *pathovars*

Metabolic profiling of isolates of *X. campestris* pv. *campestris* revealed differences related to pathogenicity of the strains. Isolates identified as *X. campestris* pv. *armoraciae* were less aggressive on crucifer plants than isolates identified as *X. campestris* pv. *campestris*. For the tomato and *Capsicum* pathogen *X. c.* pv. *vesicatoria* the results indicated the existence of more than one strain, although this work did not try to establish the predominant strains present. Such an assumption is suggested from the observations made following the process of colony purification on YDC medium. Two different colony types were produced, the bright yellow mucoid colonies very similar to those of the reference strain (NCPPB422, type strain from New Zealand) were produced by both tomato and sweet pepper isolates (collected from market fruits). Yellow colonies not as bright as those

described above and non-mucoid were also recovered from the commercial sweet pepper seed. However, all isolates appeared similar on Tween B medium, matching colony characteristics of *X. c.* pv. *vesicatoria* described by McGuire *et al*. (1986). Similarly, all these isolates produced brown lesions when inoculated onto tomato seedlings.

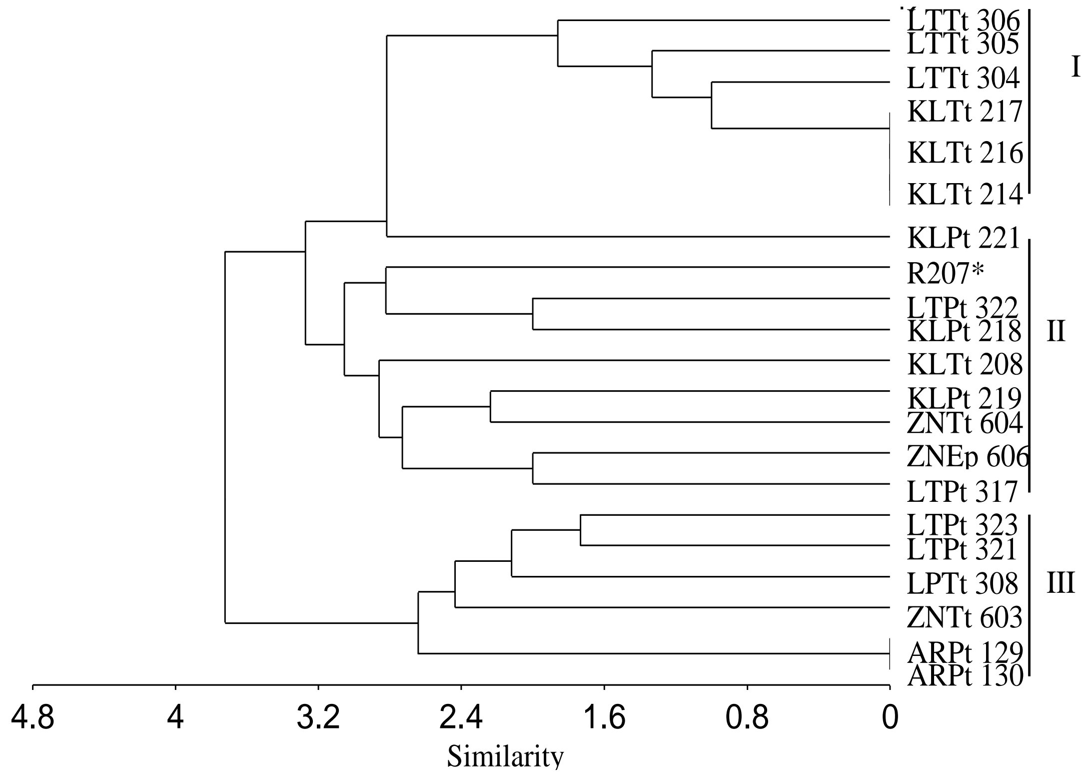

Fig. 3. Clustering of isolates of *R. solanacearum* from potato and tomato on the basis of random amplified polymorphic DNA (RAPD) using unweighted pair- group method with arithmetic means (UPGMA). R207 is a banana isolate included for comparison.

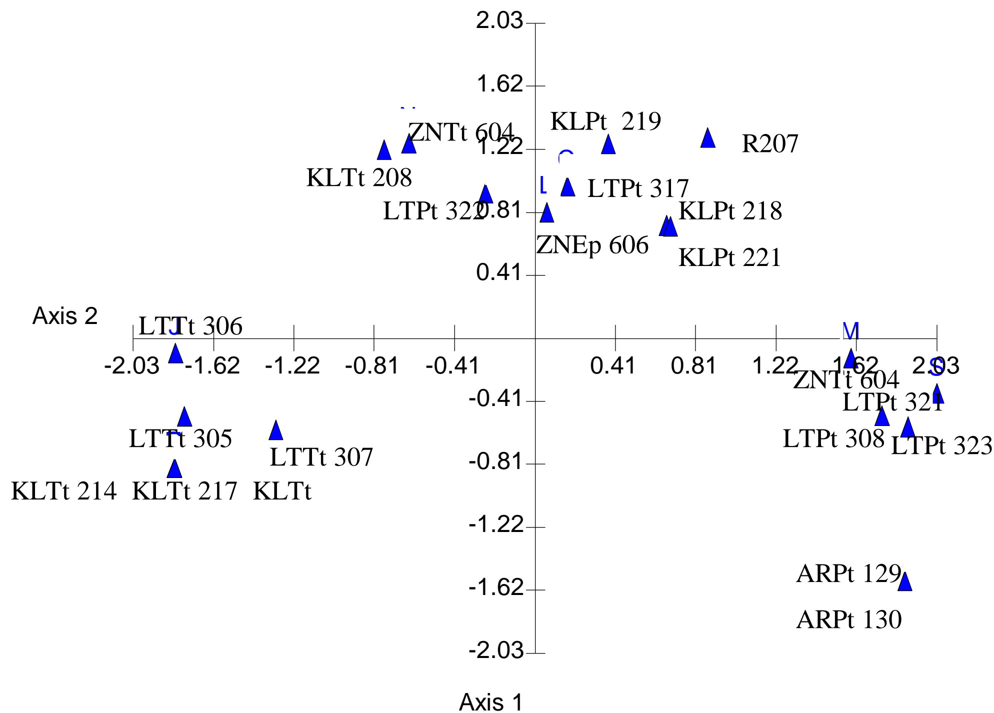

Fig. 4. Principal coordinate analysis of isolates of *R. solanacearum* from potato and tomato characterised by RAPD polymorphisms. Isolate R207 is a banana isolate included for comparison.

Conclusions: Two biovars of *R. solanacearum* were identified in Tanzania, biovar 2 confined to highland potato and biovar three predominating in tomato. Biovar 2 had not previously been reported in Tanzania. Characterisation of isolates of *R. solanacearum* by RAPDs revealed genetic variation associated with host and geographical variation. Metabolic profiling of isolates of *X.c.* pv. *campestris* revealed variation associated with differences in aggressiveness towards *Brassica* hosts.

5. Representative cultures of isolates of all the target pathogens retained at NRI.

Cultures representative of geographical and host ranges have been designated as reference material.

6. Strategies for management of bacterial diseases of solanaceous vegetables identified.

Participatory Rural Appraisal (PRA)

The importance of various cash crops is given in Table 3. Tomato, potato and cabbage were overall the most important vegetables but with regional differences that has significance for the distribution of bacterial diseases. Their prioritisation by farmers is given in Fig. 5. All the farmers interviewed acknowledged that the objective of vegetable cultivation is to generate income. For some of the farmers it is the primary economic activity. However, for others vegetable production provides additional income needed in the household.

Farmers' perceptions of the most important constraints on productivity are summarised in Fig. 6. It is perhaps surprising that diseases were ranked so highly. It is possible that this is because the interviewees knew that the interviewer was a plant pathologist but another survey by economists had given similar results (Stevenson *et al.* 1994).

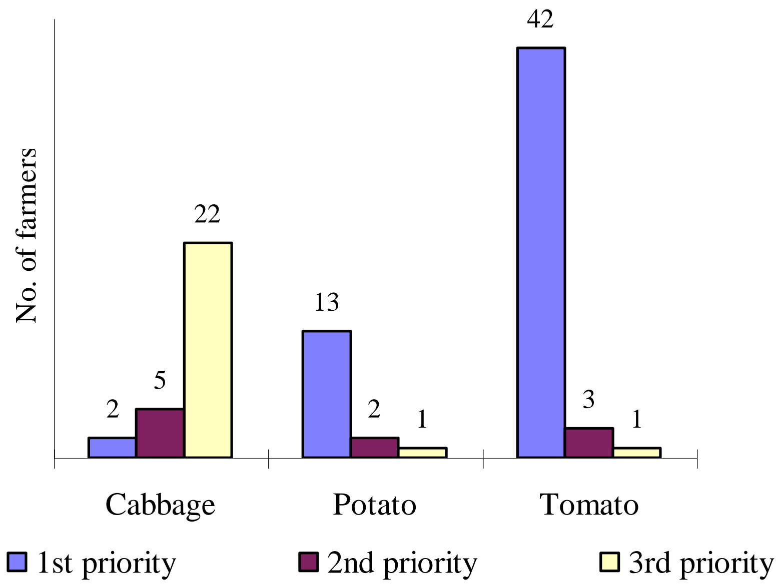

Fig. 5 Relative importance of cabbage, potato and tomato as cash crops as ranked by farmers.

Table 3. Cash crops grown by interviewed farmers.

	Regions surveyed		
Crop	**Arusha (45)**[1]	**Kilimanjaro (10)**	**Mbeya (5)**
Vegetables			
Amaranthus	+[2]	-	++
Beans	+++	-	++
Bitter tomato	+	-	-
Cabbage	++	+++	++
Carrot	-	+++	-
Chinese cabbage	-	-	++
Cucumber	++	-	-
Eggplant	++		-
Ethiopian mustard	+	-	++
Finger millet	++	-	-
Kale	+	-	-
Night shade	++	-	-
Onion	+++	-	-
Peas	+	+++	-
Potato	+	+++	+++
Spinach	+	-	-
Sweet pepper	++	-	-
Tomato	+++	-	+++
Water melon	+	-	-

[1] No. of farmers interviewed per region.
- the crop was not selected by the farmer interviewed or not grown in his village
[2] The crop was referred to by:
+ less than 10% of the interviewed farmers
++ between 10-50% of the interviewed farmers
+++ more than 50% of the interviewed farmers

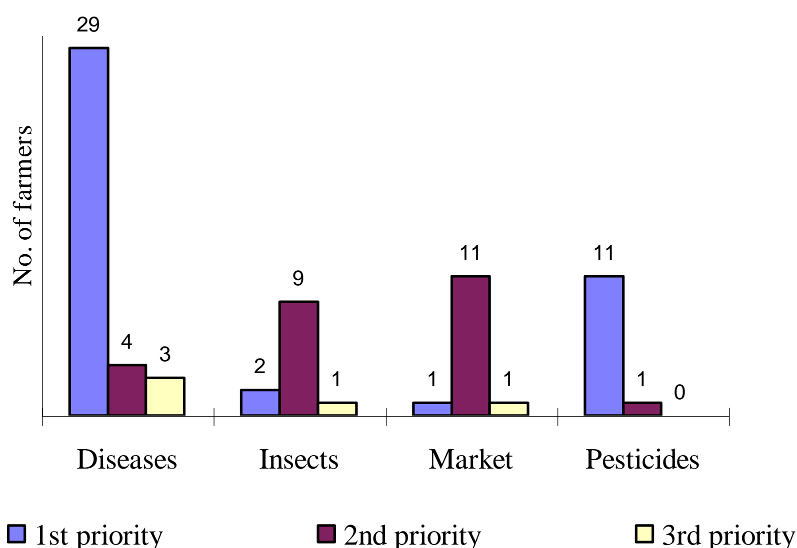

Fig. 6. Relative importance of major constraints according to farmers' ranking

The survey revealed that farmers had virtually no recognition of bacterial diseases as distinct from fungal diseases; they were familiar with some fungal diseases like late blight (*Phytophthora infestans*) but had little conception of wilting as a symptom or syndrome of plant disease. Farmers' opinions on the cause of wilting were recorded (Table 4). The link to soil or to soil borne agents like nematodes is interesting as *R. solanacearum* is a soil-borne pathogen.

Table 4. Farmers' identified causes of potato or tomato wilting

Region	Causes of wilting
Arusha	18 (nematodes)
	3 (soil)
	24 (do not know)
Kilimanjaro	10 (cut worms)
Mbeya	5(do not know)

Pair-wise ranking of vegetables highlighted one of the reasons that influences farmers to keep on growing related species of vegetable crops one after the other on the same piece of land (Table 5). The cultivation of related crops presents a problem to the management of bacterial diseases through related crops generally supporting high pathogen populations. The lack of crop rotation (or lack of effective rotation with a suitable crop) will be a major factor in the persistence of some soil-borne diseases in the study, particularly *R. solanacearum* (Table 4).

Table 6. Pair-wise ranking of vegetable crops grown as cash crops.

1	2	3	4	5	6	7
Potato	Tomato[z]					
Tomato	Potato					
Potato	Beans (Equal priority)					
Tomato	Onion	Cabbage				
Tomato	Cabbage	Potato				
Potato	Carrot	Cabbage	Peas	Beans		
Tomato	S/pepper	Eggplant	Cucumber	Cabbage		
Tomato	Eggplant	S/pepper	Cucumber	Onion	Beans	Cabbage

1 = First priority crop 7 = Last priority crop

[z] Shaded areas show related crops (member of the same family) ranked one after the other

The last aspect of the PRA to be highlighted is the sources of farmers' planting material (vegetable seed and potato seed tubers). Farmers reported that buying seeds each cropping season is not profitable, and that consequently they supplement their commercial seeds by extracting their own seeds. Almost all interviewed farmers (97%) responded that they buy seed from the market (commercially-packaged or not) and/or produce their own seeds. This is a common practice not only for tomato, but for some traditional vegetables such as *Amaranthus* sp., Ethiopian mustards and others.

Farmers producing their own tomato seeds select fruits for seed extraction from the same fields where the commercial fruit crop is produced; there is no practice of allocating special plots for seed production. Many farmers extracting their own seed, prefer to extract seeds from fruits produced in the first generation from commercial seeds. The practice varies from farmer to farmer, some buy commercial seed lot once and extract seeds from the harvested fruits only once. However, some farmers continue to extract for two or three consecutive cropping seasons.

The only method used for potato propagation is seed tubers. Potato seed tubers, are traditionally selected from harvested ware potatoes, there being no practice of assigning special fields for producing seed potatoes. Farmers select small tubers of about 3-5 cm during or after harvest of ware potato. In case of shortage of planting materials, potato growers buy ware potatoes from the local markets and use them to establish their fields. This is true for potato growers in Arusha, West Kilimanjaro and Lushoto. Farmers also buy seed potato from other farmers, especially if the seed potato has been produced at high elevation. In Mbeya district, apart from the selection of small tubers produced in the fields, farmers also buy seed potato from an agent selling seed potato to the farmers. This project revealed that these "seed potatoes" originate from small potatoes rejected in the markets. The agent buys these, then sorts them according to size and appropriate varieties, and packs them into sacks for selling back to growers.

Farmers have managed the poor quality of seed potato by treating the selected potato seed tubers before they store them for the next cropping season. The treatment used (sun-drying of the tubers) is thought by the authors to be a very localised practice. Farmers claim that if the tubers are not dried a good percentage is lost due to rotting.

Diagnostic technology adapted for bacterial disease management

From general observations made during the field surveys, the two wilt pathogens *R. solanacearum* and *C.* subsp. *michiganensis* constitute a real threat to the widely grown vegetable crop, tomato, which is of major economic importance in Tanzania. Moderate to severe crop loss can result from their attack. Reliable diagnostic technology if available would very much assist in their control by timely and appropriate means. As pointed out by Black and Elphinstone (1998), improved methods of detection offer much scope for better bacterial wilt management in particular, especially in developing countries where climatic conditions favour the pathogen. Techniques for detection of *R. solanacearum* have been categorised into six groups according to Black and Elphinstone (1998). This project tested four of the six techniques, including isolation and culturing, serology, molecular techniques and metabolic profiling with the Biolog system. The other two techniques (immunofluorescence and fatty-acid profiling) were not used due to requiring advanced facilities.

The successful adaptation and subsequent application of the above diagnostic techniques to conditions prevailing in Tanzania will depend on the infrastructure and basic resources that are available for the work. Also to be considered is that the principal users of such diagnostic services are smallholders who are normally not charged for the services offered. Screening germplasm should be major function of diagnostic services set up, as well as routine backup for field diagnoses. It is important to realise that many seedborne bacteria are infective at extremely low incidences and as such present problem of seed sampling and detection (Maude, 1996). The application of more than one technique is important to avoid or eliminate the possibility of false negative results. However, such combinations of techniques must be economically and effective. The important outcome of seed detection is the confirmation that both farmers' saved seed and commercial ones (especially if imported) may play an important role in spreading and perpetuating the identified plant pathogenic bacteria and the diseases they cause in the farming systems. Some of the bacterial pathogens isolated were confirmed to be virulent by pathogenicity tests.

Generally, it is more convenient for a resource-poor plant pathology laboratory to use semi-selective media for detection of plant pathogenic bacteria, as this can be carried out with basic laboratory equipment. During the course of this work various semi-selective media were used to isolate the plant pathogenic bacteria involved in the study. However, to ensure good recovery of target bacteria on semi-selective media it was necessary to use media which contained many antibiotics (from three to five antibiotics). Some of the antibiotics used are very toxic and it is necessary to weigh them in a fume cupboard. However, such a facility was lacking and there was only one balance for weighing everything. For this reason the use of the ideal medium in Horti-Tengeru was not always possible, and studies involving such media were either carried out at NRI, or using antibiotics weighed out elsewhere.

For detecting a target bacterium on semi-selective media use of serial dilution plating is essential. Consequently a lot of space was needed to incubate many plates at the same time. This was often not possible as only one incubator would be used limiting the amount of available incubation space. It was also not possible to use disposable plastic petri dishes as there were no facilities to cater for disposing hundreds of such items. These two factors combined made the process of seed screening very slow. It was only possible to continue with seed screening when sufficient space and plates were made available, which often resulted in each seed screening requiring at least three to four weeks.

Application of the PCR-based technique for detection of the bacterial wilt pathogen, *R. solanacearum*, did not pose any major problem from a technical point of view. However, proper disposal of waste material, such as ethidium bromide needs to be looked at, especially when considering a place like Horti-Tengeru, where within the premises of the institute there are residential areas. One other aspect to be considered is that once the facility is available, the researchers within

the institute and from other institutes nearby will be tempted to use it. However, not all PCR procedures are as simple as the one used for detection of *R. solanacearum*. Here the protocol did not involve any use of harmful chemicals for DNA isolation such as phenol and chloroform, which need the use of a fume cupboard. In certain cases the PCR protocol is only effective with purified DNA (as it was shown with the RST2/RST3 primers for the detection of *X. c.* pv. *vesicatoria*) and there are no facilities for handling harmful chemicals, such as chloroform and phenol, necessary for genome extraction.

Erratic power supply is one common problem that regularly affected all laboratory work. Sometimes there was no regular power for as long as two weeks. In such instances, prepared samples and some reagents that needed low temperature, such as PCR reagents were affected. PCR reagents such as reconstituted primers (some primers were stored at room temperature in freeze-dried form) and the enzyme *Taq* polymerase were the most sensitive to such power cuts due to freeze-thaw damage. Sometimes new stocks of PCR reagents had to be prepared as a result of power cuts. Long power cuts also affected incubated plates and the running of PCR experiments. However, power interruption did not affect the completion of the ELISA technique as much provided all needed reagents were already prepared. Other available facilities could be exploited, for instance, for incubating ELISA plates, such as placing these in the glasshouse (in which the temperature was 34-38°C during the day).

A frequent shortage of water supply also impaired regular washing up and the running of the autoclave for sterilisation. Similarly, availability of a functional water distiller was important, with malfunctioning affecting the efficiency of all diagnostic techniques. Nevertheless, it is important to realise that despite the above factors the work planned was carried out with reasonable results. Therefore the practical applicability of the techniques is not a major problem. However, the costs involved for running each technique may well be main determining factor. This is discussed below.

Without considering the costs involved for running each technique (see below), routine application of semi-selective media with the back-up of the *BACTID* kit must be given preference over other techniques as they provide information not available by other techniques. The availability of bacterial cultures is important for generating detailed information about the different bacterial pathogens. However, even using semi-selective media, lack of experience may still lead to failure, for example by selection of unsuitable plant material that is likely be overgrown by saprobic bacteria (Stead, 1992).

Similarly, Black and Elphinstone (1998) pointed out that there should be no attempt to encourage advanced technology to detect *R. solanacearum* unless it can be used to good effect as an appropriate solution to the existing problem. Otherwise isolation and plating methods together with pathogenicity tests will not be used where it is sensible and cost-effective. Due to the neglect of general bacteriology in developing countries, some laboratory technicians who could detect *R. solanacearum* in plant tissues by PCR or ELISA were found to be less competent to recognise and work with the pathogen in culture (Black *at el*., 1996).

Use of the semi-selective medium, SMSA, during the course of this work proved to be more sensitive and reliable for determining latent infection of bacterial wilt pathogen, *R. solanacearum,* than even PCR (where both inhibitors an power cuts can interfere with the results). PCR as well as ELISA offer the prospect of rapid detection and identification of bacterial wilt pathogen from field-infected material without recourse to bacterial culture. This is particularly important for tomato wilt where it is not easy to determine from field symptoms which wilt pathogen, *R. solanacearum*, *C. michiganensis* or P. *corrugata*, is involved. Therefore techniques such as ELISA may be used to confirm or eliminate *R. solanacearum* association with the wilt problem.

The results of this work showed that some potato tubers are latently infected, and although the rate of infection is low, latent infection is likely to be a significant contributing factor in the spread of bacterial wilt pathogen in the field. As ELISA did not detect latent infection, the use of PCR as well as ELISA (for applications above) will be necessary for rapid, sensitive and specific direct detection of the pathogen in plant material.

Seal (1998) made a comparative analysis in respect to the costs involved for application of SMSA medium, indirect ELISA and PCR for detection *R. solanacearum* on the basis of UK prices for consumables. ELISA was shown to be the cheapest, costing about two thirds of cheapest PCR test. The costs of SMSA medium calculated on the basis that two serial dilution platings are used for each isolate with three reusable glass plates each would cost about four times the cost of ELISA. Final application of one detection technique or the other for screening purpose should not only be based on costs but also other repercussions that the technique may have.

Though ELISA is less sensitive than PCR in detecting latent infection in plant material and may give false positives, it has been shown to be cost-effective. On the other hand, although PCR is more sensitive than ELISA it is very vulnerable to erratic power supply and requires environmentally sound means for handling harmful chemicals and disposing harmful waste reagents. Therefore ELISA should be a technique of choice for routine application, and PCR to be used occasionally to complement the ambiguous results from ELISA, or where the result is critical, for example with seed certification.

As an identification method, the Biolog method was found to be easy to use and adequate for identification of Gram negative plant pathogenic bacteria. Little capital equipment is needed in a basic system. However, its application in resource-poor plant pathology laboratories could be limited to research work for characterisation and comparison of isolates rather than for routine idetification use. This is essentially due to the inherent drawbacks of the system, especially high costs of purchasing the MicroPlates, (Black *et al*., 1998; Black and Sweetmore, 1994).

Conclusions: Participatory appraisal of farmers' knowledge and perceptions revealed that certain cultivation practices promoted the spread and development of the target pathogens; adoption of practices that have a beneficial effect may be possible through the extension services. Internal plant quarantine/plant health measures could restrict the further spread of *C.m.* subsp. *michiganensis*. Most importantly, poor seed and potato seed tuber quality, including contamination with bacterial pathogens, should be addressed; this will require a combination of screening and quality control by Tanzanian suppliers to produce disease-free material and controls on imported seed.

This study has established that seed-borne and tuber-borne bacterial pathogens are among the important disease causing agents that affect vegetable crops in Tanzania. Both commercial and farmers' germplasm are pathways for spreading these bacterial pathogens within the country. Farmers' cultivation practices help to perpetuate bacterial diseases from one cropping season to the other and from one field to another. The attacks of wilt-causing bacteria, *R. solanacearum* and *C. michiganensis*, leading to crop loss are likely to have more severe consequences than bacterial leaf spot disease, *X. c.* pv. *vesicatoria*, as farmers can still sell the produce affected by the latter, though at much reduced prices.

Unless the quality of vegetable seeds from commercial companies conforms to farmers' requirements, farmers will continue to produce and depend more on their own germplasm in order to satisfy their needs. Therefore, it is worthwhile to support farmers' efforts by ensuring that reasonable quality germplasm is being used to sustain vegetable production. It is important to improve the existing scientific infrastructure, incorporating both traditional and advanced diagnostic technology for plant pathogenic bacteria. This will allow reliable screening of planting material as well as permitting routine diagnoses of farmers' field problems.

As far as bacterial wilt of potato is concerned, future work can pursue in detail the influence of farmers' practice in relation to the occurrence of latent infection of potato seed tubers with *R. solanacearum*. It is noteworthy that farmers' treatment of tubers intended for seed may lead to a reduction of inoculum of *R. solanacearum*. Thus the actual contribution of tubers in spreading the disease needs detailed examination. Such efforts will be important as the prospect of the government or other authority launching a certified seed tuber scheme is remote.

C. michiganensis may be confined to the southern highlands at present. If so, internal quarantine means could be introduced to prevent its spread. On the other hand, further surveys of the northern regions are necessary to confirm the distribution of *C. michiganensis*. Collaborative work with the commercial seed companies could lead eventually to healthier tomato seeds

7. Diagnostic techniques evaluated and compared for their suitability in resource-poor situations

All diagnostic techniques used proved to be effective for isolation and direct detection of plant pathogenic bacteria from planting materials and field-infected samples, respectively, under some circumstances at least. However, under certain circumstances it was not possible to apply a standard methodology due to lack of certain facilities or reagents. In such cases it was necessary to adapt the described procedure and accommodate the work according to the existing conditions. Despite such deficiencies, future practical employment of some of the techniques used is promising. Techniques such as use of semi-selective media supported by the *BACTID* kit, and ELISA , could be routinely applied for the diagnosis of bacterial diseases.

For preliminary identification of the isolates the *BACTID* identification system was of great help as several key tests for each suspect isolate were conducted simultaneously. This was only possible due to the fact that the *BACTID* tubes occupied less space compared to plates; where often the initial isolations had to be done in batches due to limited space in the incubator. One important observation on the *BACTID* tubes is that there is less deterioration under normal storage facilities compared to media prepared in petri dishes or in bigger bottles. *BACTID* tubes prepared from the beginning of the work (October, 1996) lasted for the whole two years of the field study without being contaminated, as is usually the case when media are kept at room temperature for more than six months. In fact, *BACTID* tubes introduced in Tanzania in August 1995 for the first time remained viable for three years and were used at the end of the field studies in September, 1998.

A principal achievement of the *BACTID* system was the rapid elimination of saprophytes. The limitation to the system is that it was intended primarily for Gram negative bacteria. However, considering the advantage that the system possesses and the constraints that affect most resource-poor laboratories, modification of the system to accommodate the identification of Gram positive bacteria would be appreciated by many such laboratories. The ability of *C.m.* subsp. *michiganensis* to grow in 6% NaCl broth can be used as a starting point in the future development of the system, together with other identified tests.

Use of ELISA and PCR was especially useful with the isolates of the bacterial wilt pathogen, *R. solanacearum*, where most of the primary *BACTID* tests results were reproduced by an *Agrobacterium* sp. contaminant. Use of *R. solanacearum* polyclonal antisera in ELISA and OLI 1 + Y2 primers and PCR resolved the ambiguity that existed between these species. Both SMSA medium and PCR techniques proved to be accurate, sensitive and reliable for determining the presence of latent infection of the bacterial wilt pathogen in potato seed tubers. Unlike the ELISA technique used, SMSA medium and PCR successfully detected the pathogen in symptomless tubers as well as those with clear symptoms but with apparently low concentrations of the pathogen. It is important to emphasis that application of PCR technique for detection of bacterial wilt pathogen, *R.*

solanacearum, is advantageous in terms of reliability (proving to be very specific), and sensitivity. Another positive feature of the PCR technique is rapidity.

Use of semi-selective media did ensure better isolation of the pathogens from field-infected materials than by using basic media like nutrient agar. Most of the semi-selective media used produce colonies with definitive appearance that made the recognition of the target pathogen much easier. This was illustrated in the separation of the different wilt pathogens of tomato. However, the effectiveness of most semi-selective media for direct detection of plant pathogenic bacteria in planting material seemed to be influenced very much by the level of other bacteria contained by the seed lot compared to that of the target pathogen. For detection of bacterial pathogens such as *R. solanacearum, C. m. michiganensis* and *X. c.* pv. *vesicatoria*, the detection assays with semi-selective media more reliably recovered the pathogens from the field-infected samples than from seed or tubers. If the level of the pathogen was relatively high, the presence of other bacteria did not deter detection of the pathogen. Where, however, some of the seed lots had a comparatively high level of background microflora, they tested negative when screened by semi-selective media.

EPPO quarantine procedure for detection of the bacterial spot pathogen, *X. c.* pv. *vesicatoria*, from tomato and sweet pepper seeds can be easily applied in Tanzania, provided all reagents for preparation of various semi-selective media are available. Use of a complementary technique to Tween B medium in detecting bacterial spot pathogen was also essential; the PCR technique exposed more infected samples. Use of RST2/RST3 primers gives more definite results on the status of detected bacterium as the primer set amplifies a pathogenicity gene (the *hrp*-gene). However use of the RST2/RST3 primer requires extraction of the DNA genome of the bacterium before amplification (Leite, *et al*., 1995). Such a requirement can limit the technique's application in places like Tanzania with no facilities for handling the harmful chemicals needed for genome extraction. On the other hand the XCC/Y2 primer set is not well researched but it is assumed that it amplifies the sequences of both pathogenic and non-pathogenic *Xanthomonas* spp. In this work the XCC/Y2 primer set mainly amplified the DNA sequence of pathogenic isolates of *X. c.* pv. *vesicatoria* and *X. c.* pv. *campestris* and did not amplify sequences from *Xanthomonas axonopodis* from citrus and *Xanthomonas* spp. from carrot. Unlike the RST2/RST3 primers the XCC/Y2 primers can be applied with a simple and environmentally friendly method of extracting the genome template (by simply boiling some cells). Another advantage is that the XCC/Y2 primers amplify 16S rDNA sequences which are often species-specific and present as multiple copies in microbial genomes (Seal *et al*., 1993).

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8. Scientific papers published and other dissemination outputs produced.

Manuals on Diagnostics

"BACTID. Bacteriological Identification System for Resource-Poor Plant Pathology Laboratories" (booklet and software) prepared under the previous Adaptive Research Initiative Project was published under this project by NRI in 1996.

"Manual for detection of *Ralstonia* (*Pseudomonas*) *solanacearum*, causal agent of bacterial wilt" by Seal and Robinson-Smith (formerly of IACR Rothamsted) is in final proof stage and will be published by the end of 1999.

Peer-reviewed and other papers

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Conferences attended (* indicate publications listed above)

1. *Invited paper given by **Seal** at Second International Symposium on bacterial and bacteria-like contaminants of plant tissue cultures, at University College, Cork, Ireland, 3-7 September 1996.
2. ***Seal** invited speaker at Brighton Crop Protection Conference on Pests and Diseases, Brighton 18-21 November 1996.
3. ***Seal** presented paper at BCPC conference on Diagnostics in Crop Production, Warwick, 1-3 April 1996
4. **Black** attended conference of EPPO Phytosanitary Inspectors in Nitra, Slovak Republic, 14-18 October 1996 and contributed to discussions on diagnostics, especially on *R. solanacearum*.
5. **Black** was invited to present a paper on "Bacterial diseases in Potato and Tomato Germplasm in Tanzania" and to sit on the Expert Panel on "Brown Rot", Annual Plant Health Division Conference (MAFF), Ashford, Kent, 3 February 1998.
6. ***Black** and **Seal** presented papers at the Second International Bacterial Wilt Symposium, Guadeloupe, 22-27 June 1997 and chaired discussion sessions by invitation.
7. **Black** chaired platform paper session on "Application of Diagnostics in Crop Protection" at BCPC Pest and Diseases Conference, 16-19 November 1998.

Other dissemination outputs resulting from esteem in bacterial diagnostics

Black prepared data sheets on *X. oryzae* pv. *oryzae* and pv. *oryzicola* for the CAB Internaitonal Crop Protection Compendium and on *X. axonopodis* pv. *citri* for FAO-sponsored Training in Africa.

Training

The *BACTID* system formed the basis for Masters level training in plant bacteriology given at Reading (MSc in Technology for Crop Protection), and at the University of Greenwich (MSc courses in Sustainable Agriculture/Natural Resources, MSc in Post-Harvest Technology). Molecular technology was taught in the Molecular Diagnostics for Plant Pathology unit in MSc Natural Resources, University of Greenwich. In-service training was provided to Kenyan and Malawi participants in other CPP-funded projects.

Contribution of outputs

Knowledge on recognition and the importance in cropping systems of bacterial diseases of vegetables gained for scientists, extension workers to farmers
(i) Before this project started various assumptions were made about the causes of wilting in vegetables on the basis of symptoms observed in the field and, in general, little was known about bacterial diseases even on the part of plant protection specialists. Consequently, wilting was often attributed erroneously to the wrong cause without further investigative work. It is now known that *R. solanacearum* and *C.m.* subsp. *michiganensis* are both significant causes of wilting in solanaceous crops while the commonly observed necrosis of pith associated with wilting in tomato is not due to *P. corrugata.*

(ii) Valuable data has been gained on the importance of bacterial diseases in the cropping systems. Most significantly the approach of "targeted seed pathology" has indicated the contribution that germplasm-borne inoculum of the bacterial pathogens studied makes to the spread and development of plant diseases in the field.

(iii) Components of management strategies have been identified (plant quarantine, seed treatment, crop rotation) that can be used to reduce losses from the target plant pathogens in the future.

Diagnostic facilities established for bacterial diseases
For the first time, comprehensive laboratories facilities were established and put into operation for the diagnosis of bacterial plant diseases.

Suitability for resource-poor situations
Extending the achievements of the predecessor projects, lessons have been learned from the adaptation of diagnostic technology that will make a lasting contribution to the detection of bacterial plant pathogens and the diagnosis of diseases of vegetables in resource-poor situations.

Further work planned

A new project under discussion plans to investigate further the effect of drying seed potato tubers on inoculum of *R. solanacearum* and the contribution this and other management practices could make to healthier potato planting material. There will also be additional dissemination material in the form of illustrated leaflets and posters in Swahili and English explaining the target bacterial diseases and methods of management.

The *BACTID* system is being improved and expanded under a project funded by the Higher Education Funding Council for England. New *Windows*-compatible software is being produced that will include

- an improved version of the core identification system (expert system)
- an encyclopaedia of media, tests and reagents used in the *BACTID* kit taking advantage of practical experience gained from the project
- a teaching module incorporating animated graphics of the tests taking place in the *BACTID* tubes

Acknowledgements

Experimental work at the Natural Resources Institute was carried under Plant Health Licences to import, move and keep prohibited plant pathogens granted by the Ministry of Agriculture, Forestry and Fisheries.

Annex 1. Should PCR be installed at Horti-Tengeru, Arusha?

ISSUE OR PROBLEM	FOR	AGAINST
Cost of equipment		Cost of equipment will reduce funds available for field and laboratory work
Cost of consumables	Continued cost of consumables could be provided (in part/totally) from other sources	PCR is expensive to run (consumables)
Unreliable -20°C storage at Horti-Tengeru	Damage to primers from lack of -20°C storage could be avoided by drying down primers	A freezer capable of -40C at least is required to store primers (and bacterial cultures)
Focus on molecular methods		PCR should not dominate PhD studies; PCR is not an appropriate technique to be set up in Zanzibar
Post PhD access to PCR	PhD student would have access after return to Zanzibar	Access to equipment more difficult at SUA
Location of PCR	Convenience of having facility at project base	PCR should be used in critical situations rather than be available routinely
	PhD student does not have to spend time and money travelling to and staying in DSM. Problems can be addressed without changes to schedule.	Samples can be stored and analysed together in block work
	Establishment of sustainable PCR at Horti-Tengeru would be major achievement of project.	One strategically placed centre might be better
	Of use to others in Arusha area who are familiar with technique (AVRDC, TPRI/NPQS)	
	Could be used in training courses	