Institution: University of Bristol		
Unit of Assessment: 5) Biological Sciences		
Title of case study: Diagnostic tests and viral knowledge for sustainable cassava production in Africa		
Period when the underpinning research was undertaken: 2000 - 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Gary Foster Andy Bailey	Professor of Molecular Plant Pathology Reader in Molecular Mycology	10/1996 to present 04/1999 to present
Period when the claimed impact occurred: 1st August 2013 - 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

The cassava brown streak virus (CBSV) molecular toolkit, developed by the University of Bristol (UoB), has enabled sustained virus-free cassava production in sub-Saharan Africa providing economic and food security. Long-term disease surveillance and monitoring programmes show declines in national incidence and increased cassava production. Molecular diagnostics have enabled clean seed production by farmers in Uganda generating USD1,360,000. Breeding programs have developed CBSV tolerant strains. A new professional network – CONNECTED, includes 1,486 members from 83 countries, has pump-primed technological innovations and delivered training to 87 individuals from 18 countries. Both CONNECTED and the molecular diagnostics toolkit have built capacity across industry researchers and agricultural practitioners to expand CBSV-free cassava production and created invaluable professional networks.

2. Underpinning research

Cassava (*Manihot esculenta*) produces carbohydrate-rich storage roots, which are a staple food crop for approximately 800 million people worldwide. Cassava brown streak disease (CBSD), caused by Cassava brown streak viruses, generates losses typically 60-70% of root harvest in susceptible varieties, as well as substantial reduction of market value due to necrotic lesions. CBSD has become an extremely serious constraint to cassava production in East Africa, as well as a threat to cassava production throughout Africa.

In 2001, ground-breaking UoB research, led by Foster and Bailey and funded by Department for International Development (DFID) [i], identified and characterised segments of the cassava brown streak virus genome by a complex combination of viral purification, cDNA synthesis and sequencing methods [1], showing CBSV to be an Ipomovirus in the Potyviridae. These data enabled development of the first diagnostic tests [2] to ensure accurate and rapid identification of diseased material.

From 2010 to 2015, UoB research led by Foster and Bailey developed a new method to generate infectious clones (ICs) to plant viruses using yeast-based recombinational cloning. Initially reported for the *Pepino mosaic virus* [3], these new approaches were then applied to Cassava brown streak viruses, with infectious clones generated for the first time against these important pathogens [4]. UoB research used these clones to allow pathogenicity factors to be identified as well as the *in vivo* tracking of live virus, with viral targets for plant breeding programmes identified [5, 6].

3. References to the research

[1] Monger W, Seal S, Issacs A, **Foster GD.** (2001a). Molecular characterisation of the *Cassava brown streak virus* coat protein. *Plant Pathology,* 50, 527-534. DOI:10.1046/j.1365-3059.2001.00589.x

[2] Monger WA, Seal S, Cotton S, **Foster GD.** (2001b). Identification of different isolates of *Cassava brown streak virus* and development of a diagnostic test. *Plant Pathology,* 50, 768-775. DOI:10.1046/j.1365-3059.2001.00647.x

[3] Duff-Farrier CR, Candresse T, **Bailey AM,** Boonham N. **Foster GD.** (2016). Evidence for different, host-dependent functioning of *Rx* against both wild-type and recombinant *Pepino mosaic virus*. *Molecular Plant Pathology,* 17: 120-126. DOI:10.1111/mpp.12256

[4] Duff-Farrier CRA, Mbanzibwa DR, Nanyiti S, Bunawan H, Pablo-Rodriguez JL, Tomlinson KR, James AM, Alicai T, Seal SE, **Bailey AM, Foster GD.** (2019). Strategies for the Construction of Cassava Brown Streak Disease Viral Infectious Clones. *Mol Biotechnol* 61, 93–101. DOI:10.1007/s12033-018-0139-7

[5] Tomlinson KR, Pablo-Rodriguez JL, Bunawan H, Nanyiti S, Green P, Miller J, Alicai T, Seal SE, **Bailey AM, Foster GD.** (2019). *Cassava brown streak virus* Ham1 protein hydrolyses mutagenic nucleotides and is a necrosis determinant. *Molecular Plant Pathology* 20, 1080–1092. DOI:10.1111/mpp.12813

[6] Tomlinson KR, Seal SE, **Bailey AM, Foster GD.** (2019). Utilization of infectious clones to visualize *Cassava brown streak virus* replication in planta and gain insights into symptom development. *Virus Genes,* 55, 825–833. DOI:10.1007/s11262-019-01697-5

Grant Funding:

[i] **Foster GD.** Molecular diagnostics and further characterisation of *Cassava brown streak virus*. DfID, 2000 - 2001, GBP49,810

[ii] **Foster GD.** CONNECTED – COmmunity Network for africaN vECTor borne plant viruses. BBSRC/GCRF, 2017 – 2020, GBP1,719,466

4. Details of the impact

Cassava brown streak disease (CBSD) is one of the seven most dangerous plant diseases in the world due to the impact it can have on food and economic security. 500 million people in Africa depend on cassava, which is the second most important food staple in terms of per capita calories consumed.

Following a major outbreak of CBSD in East Africa in 2004, the cassava molecular toolkit developed at UoB [1, 2] was crucial to eliminating infected material, establishing clean fields and producing clean seeds for distribution. The diagnostic protocols [1, 2] were adopted by the International Institute of Tropical Agriculture (IITA), National Agricultural Research Organisation (NARO) (Uganda), and Food and Environment Research Agency (Fera), working across Uganda, Kenya, Tanzania, Rwanda, Burundi and DRC. This large-scale effort prevented the spread of CBSD from East to West Africa, safeguarding food and economic security.

Sustained virus-free cassava production in Africa

Surveillance and molecular monitoring

The cassava brown streak molecular toolkit [1-6] developed by Foster and Bailey remains central to the efforts in Africa to enable virus-free cassava production. Monger *et al.* (2001b) [2] was the first ever diagnostic to be used and deployed, paving the way for the development of at least seven 2nd generation diagnostic tests that are routinely used [B]. Molecular diagnostic tests are used for '*detection, tracking and mapping distribution of CBSVs, therefore contributing real*

time updated occurrence information and CBSD management measures' [B]. In Uganda, evidence gathered through this process shows that national incidence of CBSD declined by 7% from 2010 to 2017 and cassava production increased from 2,800,000 metric tons (2008) to 3,400,000 metric tons (2019) [B].

Recent research developments [4, 5, 6], which improve understanding of the pathology of CBSD are *'critical to develop robust control measures'* [Ai]. The Central and West African Virus Epidemiology (WAVE) team at Abidjan, Cote d'Ivoire are using this understanding to "build individual and institutional capacity around diagnostics, especially phytosanitary officers" who can monitor the cross-border trade in cassava planting material. In 2019-20, WAVE initiated national workshops for cassava value chain stakeholders in Benin, Burkina Faso, Cameroon, Cote d'Ivoire, Democratic Republic of Congo, Gabon, Ghana, Nigeria, Sierra Leone and Togo to prepare country response plans to CBSD [C].

FERA Science Limited have noted that UoB's research their work since 2014 has made seed testing with diagnostics, "*a mainstay of the Foundations investment to seed systems to Africa, spanning cassava, maize, sweet potato and yam amongst other crops. What also changed by the good example of the GLCI was the inclusion of National Seed Authorities, charged with certification of seed, as main partners*" [D]. They add that *"Deploying, or positioning, diagnostic capability into seed certification providers is therefore another attribute of change that can be associated with cassava, controlling CBSD and the early work of Bristol University."*

Clean seed and planting material

Improved diagnostics and virus therapy protocols enable the distribution of 'health' certified planting material to users, from at least 10 countries each year [Ai]. Official seed certification systems in Uganda and Tanzania have incorporated CBSV testing into quality assurance frameworks [B]. NARO report that *'between 2015-2016, Ugandan farmer seed entrepreneurs earned estimated USD1.1 million in sales of certified cassava planting material to local growers and USD260,000 from exports to Rwanda'* [B].

Breeding CBSD tolerant cassava varieties

The molecular diagnostics tests [1, 2], also play a crucial role in the plant breeding process to determine virus occurrence and accumulation in promising genotypes. This approach has led to the identification of six CBSD tolerant varieties now widely used in sub-Saharan Africa [B].

Recent development of virus infectious clones (ICs) [4], and viral targets for plant breeding programmes [5 ,6] has improved understanding of the pathology of CBSD and provided breeders and biotechnologists with effective tools to assess virus resistance. The Head of the Germplasm Health Unit at IITA notes that *'The new knowledge on CBSV-cassava interaction are helping IITA's cassava breeding program aimed to develop CBSD resistant cultivars."* [Ai]. The Principal Scientist at NARO also anticipates that virus ICs will improve the *'efficiency of germplasm screening'* [B].

Despite the promise of ICs to generate resistant crop varieties, secure use close to the target crops requires Category 3 (Cat3) genetic containment facilities. A report led by Foster and Bailey, 'BioSafety requirements for the use of *Cassava Brown Streak* Virus Infectious Clones in Africa', commissioned by the Bill & Melinda Gates Foundation [Ji], established the exact GM Cat3 lab facilities and regulations for the use of viral infectious clones in Africa. In 2019, Foster and Bailey hosted a Gates Foundation sponsored meeting with delegates from East and West Africa and international policy makers, to establish policy for use of viral ICs. Agreement was

reached on protocols and facilities required and these policies have been presented and supported by the relevant regulatory authorities in African countries, enabling the designed Cat3 facilities to be constructed. In a unique collaboration between architects and engineers, a design for an off-site construction of a complex and portable Cat3 lab has been developed through to full design integration, sourcing of elements, costings, full logistics and legalization plans, and is ready for receiving funding and initiating deployment.

Building capacity in agricultural practice in Africa – CONNECTED

In 2018, the COmmunity Network for africaN vECTor borne plant viruses **–** CONNECTED, funded by UKRI-BBSRC [ii], established a sustainable network of international scientists and researchers to address the challenges of vector-borne plant viruses in Sub-Saharan Africa. CONNECTED now represents 1,486 members from 83 countries who are forging and consolidating links with funders, farmers, stakeholders and policy makers, to provide the collaborative, multidisciplinary approach essential for tackling vector-borne disease.

Technological interventions addressing agricultural pests and disease

CONNECTED also provides pump-prime funding for short-term research projects generating technology innovations which reduce the burden of agricultural pests and disease. The 20 projects to date have involved 33 institutions, 55 researchers, 11 crops and 14 countries. The most developed technologies include:

i) Improved disease detection in the field

A mobile app for detection of maize lethal necrosis (MLN). Database development via insertion of images showing different disease symptoms including MLN/AMLN is ongoing. Using digital imaging, the system is field-trained to recognize/identify MLN tobetter detect the disease.

ii) Novel diagnostic kit

A new diagnostic protocol was developed that specifically detects the episomal form of badnaviruses infecting yam. This directly benefited from our advances on CBSD diagnostics.

iii) First licensed entomopathogenic fungi (EPF) lab

Early career researchers developed new specialist skills in the biology of EPF and their evaluation as biopesticides. Insect pathology laboratories were established at IITA and Wesley University. To our knowledge, the facility established at Wesley University is the first licenced EPF laboratory in Nigeria. A method was developed for evaluating the susceptibility of cassava whitefly to fungal biopesticides, and commercial biopesticides based on entomopathogenic fungi were evaluated against cassava whitefly at laboratory and field scales.

Delivery of skills training

CONNECTED provides resources and information exchange to enable more effective plant vector-borne disease research, as well as training to improve agricultural methods, products and services that increase crop yield and reduce losses. CONNECTED has delivered training in partnership with UK and institutes in East Africa [H] and West Africa [Gi] enabling them to fulfil their pan-African training mandates [H], preventing duplication of efforts.

To date, CONNECTED has trained 87 individuals from 18 countries across India and South Asia as well the UK [I], and from across the agricultural value chain including universities, national research institutes, private companies, NPPOs, plant health and seed certification organisations and international research organisations [G]. In a survey in January 2020 [I], 70% of participants report improvements to their research as a result of CONNECTED training, and 88%

improvements to their teaching. Every respondent had shared their knowledge with others, at least 359 further scientists and professionals, and 85% had continued to build the professional network of scientists created through CONNECTED.

Partner organisations, East-West Seed Group, National Agricultural Seeds Council (NASC) and International Institute of Tropical Agriculture (IITA), all highlight the dissemination of training through their organisations, as well as the importance of the creation and strengthening of professional networks across Sub-Saharan Africa [E, F, G]. East-West Seed Group note that their employee was able to disseminate training to her team enabling them to '*apply these new skills to their plant sampling work'* and contribute to a current research project *'more effectively'* [Eii]. They also describe the networking across certification organisations, national and international research institutes and universities, as '*invaluable'* [Ei]. NASC likewise report that skills and new knowledge have been used and shared in the 6 months since the training [F] and *'extremely valuable'* professional connections have been established with researchers working in a *'variety of contexts'* [F]. *'The impact this training is already having is greatly appreciated in East Africa'* [Gii].

Increased awareness of policy makers and public

CONNECTED also provide tools to help engagement with policy makers, funders and the public. In 2019, a short animation to explain the impact, spread and importance of cassava whitefly transmitted diseases in accessible language was widely disseminated and viewed. This animation was selected to be shown at two UK film festivals, Encounters and Depict. In an African based poll, the CONNECTED Network won the award of *"GROUP that made the greatest contribution on Twitter promoting African Agricultural Transformation in 2018".*

5. Sources to corroborate the impact

[A] IITA (2020). Supporting Letters: i) Head of Germplasm Unit (Nigeria)
ii) Principle Scientist (Tanzania)

[B] NARO (2020). Supporting letter – Principle Scientist

[C] Central and West African Virus Epidemiology for Food Security (WAVE). (2020) – Supporting letter – Executive Director

[D] Fera (2020). Corroborating statement – International Partnerships

[E] East-West Seed (2020). Supporting letters: i) General Manager (Tanzania)
ii) Vice-President (International)

[F] Nigerian National Agricultural Seeds Council (2020). Supporting letter – Director General

[G] IITA (2020). Supporting letters:
i) Director of Genetic Enhancement and Biotechnology (West Africa Hub)
ii) Director, Research for Development (East Africa)

[H] Biosciences eastern and central Africa International Livestock Research Institute (BecA-ILRI Hub) (2020). Supporting letter – Senior Scientist

[I] CONNECTED (2020). Summary of training data/evaluation

[J] i) Brewer H & Hird D (2017). Biosafety requirements for the use of Cassava Brown Streak Virus Infectious Clones in East Africa
ii) Standard operating procedures for the safe use of infectious clones