Report of the work of the United Kingdom Potato Quarantine Unit 1 November 2010 – 30 November 2012 for the Review Committee Meeting 11 December 2012 at SASA, Roddinglaw

Part of text redacted for confidentiality [redacted]

Back ground to the UKPQU

The UKPQU was established in 1981 to test potato material imported from outside the European Community on behalf of the UK Plant Health Authority. It also tests material moved within the community that is not eligible to be issued with a EU Plant Passport.

Since its establishment, the UKPQU has built up an international reputation for its expertise in potato quarantine testing and in November 2007 was accredited as an offshore potato quarantine station for New Zealand. In 2010 it became accredited by the United Kingdom Accreditation Service (UKAS) for PSTVd testing (digoxigenin cRNA probe) and virus testing (ELISA and bioassay) to ISO17025.

In this report reference may be made to data from earlier years if results have been updated since the previous report.

1. UK POTATO QUARANTINE UNIT (UKPQU) SERVICES

1.1. Quarantine on potato material from outside the EC (Table 1)

Testing at the UKPQU exceeds EU testing requirements specified in Directive 2008/61/EC and it now meets or exceeds most of the recommendations made in EPPO standard PM 3/21 ("Post-entry quarantine for potato"), although the test for phytoplasmas has still to be introduced. The pathogens tested and test plants used are shown in Appendix 1.

To maximise efficiency through bulk processing of samples there are 2 quarantine-testing periods (receipt by 10 December and 10 June). Although material is accepted after these deadlines, it is accepted on the understanding that processing / testing may be delayed until sufficient material is received to enable batch processing.

The number of lines received were 15 in 2010, 29 in 2011 and so far 22 lines have been received for testing in 2012 (Table 1). In 2010 the time from receipt to release ranged from 33 to 63 weeks and in 2011 33-48 weeks. No lines received in 2012 have been released yet.

Between 2010-2012 lines have failed quarantine because of virus infection PLRV, PVM, PVS [redacted] and PVY, rotting tubers and virus like symptoms in the glasshouse grown plants (Tables 1 and 2). [redacted]

Most of the lines infected with virus have been or are being subject to virus elimination. The time from receipt to release of 2 lines from which PVS was eliminated was 99 and 105 weeks but the line from which PVS and PVY was eliminated took 4 years!

Table 1. Summary of requests, receipts, releases and quarantine failures 2008-2012. All vegetative material.

Year of licence issue and number of licences issued	No of lines			Lines passed quarantine		Lines still in quarantine	Lines failing quarantine because fault	Lines not progressed
	requested	received	not received		after virus elimination			
2008[1] ***9*** licences	54	4***5***	***9***	2***7***		***0***	1 (PVM) 1 (PSTVd) 2 ***(failed glasshouse inspection)***	14 (withdrawn by importer)
2009 8 licences (includes 1 for NZ)	34 includes 1 for NZ	34 (includes 3 for NZ)	0	***33*** ***includes 3 for NZ)***	***1*** ***(PVS+PVY)***	0	0	
2010 5 licences	16 (includes 3 for Finland)	***15***	***1***	***12***	***2*** ***(PVS)***		***1 PVS***	
2011 10 licences (includes 3 lines for NZ)	42 includes: 10 lines with 2 clones 8lines for NZ	29 includes 7 for NZ	12 includes 1 for NZ	11		8	1 in virus elimination (Carlaviruses +PVY) 1 failed glasshouse inspection 3 PVY discontinue 3 PVY for virus elimination 1 line (tubers rotted reimported)	1
2012 to date 6 licences	22 includes 2 for NZ	22	0					

[1]For 2008, 2009 and 2010 results were presented in the 2002-2008 report but have been updated. Changes are shown in ***bold italics***

Countries from which lines were received

2008 Canada, Republic of Korea, Ukraine, USA
2009 Belgium, Canada, Ukraine, USA
2010 Belarus, Canada, Peru, USA
2011 India, Italy, New Zealand, USA
2012 Canada, Malawi, Peru, Uruguay, USA

Table 2. Pathogen interceptions and faults 2010-2012

Licence issued	No of lines	Pathogens and faults	Origin
2010	2[1]	Microplants-PVS	Belarus
2011	4[1]	Microplants-PVY	India
	1[1]	Tubers - PLRV, PVM, PVS, [redacted]	Italy
2012 to date	2[1]	Tubers PVS	Malawi

[1] For virus elimination [redacted]

1.2 Quarantine and testing of potato material from Member States

1.2.1 Non-Passported material One line was moved with a licence because it could not be issued with a Plant Passport (Line from Italy in Table 2)

1.2.2 Passported material is tested at the request of the consignee. No material has been received for testing over the period of the report.

1.3 Lines imported for "Common Use"

Since the last report no lines have been imported under the "common use" reduced charge of £180.

1.4 Quarantine charges

The charges for quarantine testing have remained at £180 / line for Common use and £380/line for Exclusive use (Plant Health Fees (Scotland) Amendment Regulations 2004 over the period of the report.

1.5 Rapid Multiplication

Importers use the rapid multiplication service to obtain larger quantities of material than would normally be released from quarantine for the production of minitubers for trial purposes and Approved Stocks. Microplants are supplied in Phytatrays. In 2011, 4774 microplants were produced of 5 lines and in 2012, 2824 microplants were produced of 3 lines.

1.6 Testing of pre-pollination parents at the James Hutton Institute (JHI)

The EC Plant Health Directive (Annex IV AII 18.2 and 18.3) requires that potatoes for planting, other than classified seed potatoes, are derived in a direct line from quarantine tested material if they are to be passported. To ensure that breeding material at the JHI meets this requirement, a programme of testing was started in 1994 by targeting pre-pollination parents (the start of the breeding programme) and testing for the seed-borne pathogens PSTVd, APLV, AVB-O, PBRV and PVT and since 1996 for also *Potato yellowing virus*. JHI send leaf samples to SASA for testing.

New pre-pollination parents are derived from the SASA pathogen-tested microplant collection or tuber material obtained by JHI from commercial sources. As part of SASA's agreement with JHI to safeguard plant health, all tuber material (with a plant passport) is sent to SASA and tested for freedom from *Clavibacter michiganensis* ssp s*epedonicus, Ralstonia solanacearum, Dickeya* spp and PSTVd before despatch to JHI and subsequently tested as indicated above for pre-pollination parents.

No pre- pollination parents were tested in 2011 and 57 were tested in 2012. No pathogens were detected.

2. TESTING OF GENE BANK MATERIAL

Testing of gene bank material has continued in support of Scottish Government's "Underpinning Capacity Programme and Strategic Research Programme 2: Food, Land and People, Theme 5 - Efficient and Resilient Supply Chains for Food" awarded to JHI. Although the aim is to regenerate 50 accessions per year from the CPC, in 2012 the UKPQU requested a reduction in this number because of a staff shortage.

Seed is sown at JHI under containment. Plants are sampled and tested for PSTVd (by nucleic acid probe, bulking into 10s) prior to sampling and testing for viruses: APLV, AVB-O, PBRV, PVT, PYV (by ELISA, each plant is tested individually). Testing is done in a concentrated period, PSTVd from May and viruses late-June to mid-August. One or two growing plant inspections are made annually.

Table 3 shows the number of accessions and plants tested. No pathogens were detected. In 2011 and 2012 respectively 5 and 11 plants which showed a different phenotype to the rest of the accession were tested by inoculation to test plants. In 2011 and 2012 respectively 4 and 9 plants giving ELISA absorbance readings > 2 x the negative control (suspect positives) were retested for PYV using primers designed at the UKPQU. No virus was detected. Only seed derived from fully tested plants is eligible to be issued with a plant passport.

Table 3. Number of accessions and plants tested in the CPC and which now are eligible to be issued with a plant passport 2010-2012

	2010	**2011**	**2012**
No. of accessions submitted for testing	77	70	29
No. of accessions quarantine fully tested	77	70	29
No. of fully tested plants	1181	1239	575

3. OTHER TESTING SERVICES

3.1 UKPQU: an accredited off-shore potato quarantine station for New Zealand (NZ)

The UKPQU was accredited as an offshore potato quarantine station for NZ in November 2007. The first lines were sent to NZ in December 2008. The testing programme is described in the previous report (2002-2008). There continues to be a high demand for this service. The lines reported with suspect phytoplasma infection in the 2008-2010 report were regrown starting from microplants and subject to very intensive testing programme agreed previously with NZ Biosecurity. (Appendix 2). Phytoplasma infection could not be confirmed and the lines were released. It is most likely that the suspect results were because of cross contamination. New procedures have been put in place and this has minimised the re-occurrence of false positives. A number of lines have failed their glasshouse inspection and have not been released. These lines and new cultures obtained from the importer have failed to pass their glasshouse inspection on a number of occasions and investigations are continuing.

The time from receipt to release in 2008 ranged from 52 to 72 weeks, in 2009 62-100 weeks, in 2010 39-63 weeks and in 2011 41-48 weeks.

Table 4. Number of lines for quarantine testing under the NZ quarantine testing programme 2008-2012

Year of application	**No. of lines**				
	Requested	Received	Passing NZ quarantine testing	Still in NZ quarantine testing	Failing NZ quarantine testing because of fault
2008	12 (plant passports)	12	12	0	
2009	13 (plant passports)	13	13	0	1 Failed glasshouse inspection
	3 (licences)	3	3	0	
2010	9 (plant passports)	9	8	0	1 Failed: Pectobacterium? (New culture obtained)
2011	26 (plant passport)	26	18 (includes 4 waiting for destination instructions)	7	1 Failed glasshouse inspection
	8 (licences)	7	2	4	1 Failed glasshouse inspection
	3 previously tested for EU	3	3 includes 1 line that had initially failed glasshouse inspection		
2012 to date	10 (plant passports)	10		10	
	2 (licences)	2		2	

Before changes to the NZ protocol are made by the UKPQU these are always agreed with NZ Biosecurity before implementation. For example different primers / test methods are now being used for some tests as a result of a review of test methods (see Section 5.2).

A test for *Tomato chlorosis virus* was introduced in July 2012 (also see sections 5.2 and 7.3).

3.2 *Ad hoc* testing

Various tests were carried out on material received from Brazil [redacted] Results were all negative except for material from Brazil in which *Tomato chlorosis virus* was detected (also see sections 5.2 and 7.3).

Table 5 *Ad hoc* testing 2010[1]-2012

Year	Country	Investigation	Test methods	Type of sample for test	Result
2011	[redacted]	Test for *Beet curly top virus*	PCR using Crosslin primers	58 DNA samples	Negative
	[redacted]	2 tomato plants showing bunchy leaves: Test for PSTVd	Digoxogenin RNA probe	2 Leaf samples	Negative
2012 to date	[redacted]	Test for PSTVd	Digoxogenin RNA probe	Microplants of 3 potato varieties	Negative
		Test for *Potato mop top virus* (PMTV)	ELISA	Microplants of 1 potato variety	Negative
		Test for PSTVd, PMTV, *Tobacco rattle virus* (TRV)	As above +Real time RT-PCR for TRV	Microplants of 1 potato variety	Negative
	Brazil	Leaf rolling in growing crop .Test for Begomoviruses, *Potato leafroll virus, Tomoto chlorosis virus*	Conventional PCR, ELISA and real time RT-PCR respectively	11 potato leaves	All positive for ToCLV
		1 Blackleg stem. Test for pectolytic bacteria	CVP medium + sequencing for e.g *Pectobacterium carotovorum* subsp. *brasiliensis*	Potato stem	Pectolytic bacteria present. Investigations continuing
	[redacted]	2 True potato seed derived plants showing necrotic symptoms. Test for seed borne pests	Digoxogenin RNA probe for PSTVd, conventional RT-PCR for Potato yellowing virus, bioassay	2 leaves	All negative

3.3 Future development of quarantine services

3.3.1 Australia

Following a request for the UKPQU to become an accredited off shore potato quarantine station and an audit of the UKPQU by Australian plant health officials in June 2010, Plant Biosecurity of the Department of Agriculture, Fisheries and Forestry released, 20 April 2012 a draft review for the importation of potato propagative material into Australia *http://www.daff.gov.au/ba/reviews/current-plant/review_potato_propagative_material/potato_propagative_material_draft_review* .

This review recommends an increase in testing including the introduction of generic PCR tests for Potyvirus, Carlavirus, Begomovirus, Crinivirus and Potexvirus and certain pathogen specific ELISA or PCR tests. The UKPQ SASA is recommended as an approved source for high health potato material but unfortunately the draft will require that imported material will require on arrival a mandatory minimum 3 month growth period in a closed government quarantine facility to verify the application of phytosanitary measures. During this time the material will be inspected and random testing may be done for a range of pathogens. C Jeffries has responded to the stakeholder consultation pointing out various errors in the draft review and requesting that the mandatory verification period is removed for quarantine testing laboratories that have a proven track record. It

is expected that the response to the stake holder consultation will be published early in the New Year.

3.3.2 South Africa

No progress has been made with the South African Plant Health Authority for the UKPQU to become an accredited off shore potato quarantine facility. However discussions were held with Sanette Thiart, Managing Director of the South African Seed Certification Service at the World Potato Congress in May and she will endeavour to pursue this with the Plant Health Authority.

4. POTATO PLANT HEALTH ISSUES

4.1 The European Community

4.1.1 Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

A draft proposal revising this regulation has been produced. In order to rationalise and simplify the overall legislative framework, the proposal integrates into the regulation rules currently applicable to official controls in specific areas currently governed by separate rules e.g. controls on residues of veterinary medicines in live animals and animal products, animal and plant health controls. Significant to plant health is that ISO 17025 accreditation will be required for official laboratories (i.e all the methods used by the laboratory for analysis when operating as an official laboratory) although it does appear that the scope of accreditation could be limited to tests which are the most significant and representative for the laboratory.

This proposal is part of comprehensive package, which also includes a review of the plant health regime / directive (see section 4.1.2).

4.1.2 EU Review of the EU plant health regime

The first stage of the EU review, an evaluation of the current regime, was completed in 2010 and recognised the key priorities identified by Scotland and the UK to seek faster decision making, better risk targeting and better co-ordination to enable more effective action to be taken against the increasing risks to plant health. Scottish Government (SG) actively participated in the EU Conference and Workshop during the evaluation stage of the regime.

The review is also linked to those of the EU Animal Health, Plant Reproductive Material and the Food and Feed regimes.

During the review process SG has provided regular updates to stakeholders to ensure they are fully engaged in the review. This constructive involvement has been valuable in identifying key priorities.

The Commission are now in the process of developing an Impact Assessment and draft legislative proposals. These are expected in February/March 2013.

4.1.3 Emergency measures 2007/410/EC measures to prevent the introduction into and the spread within the Community of *Potato spindle tuber viroid*

These measures were introduced in June 2007 following findings of PSTVd in traded ornamental plants of *Solanum jasminoides* and *Brugmansia* spp. in the EU. Primarily this required that plants of these species including seeds are inspected and tested on entry to the community and that member states undertake surveys of host plants on their territories. Viroid testing at SASA including that

conducted under 2007/410/EC is shown in Table 6. In addition all potential ornamental host plant species of pospiviroids were tested at a commercial ornamental / potato micropropgation facility in 2011 to ensure that these plants did not pose a risk to potato production.

Table 6 Viroid testing at SASA 2010-2012

Plants tested	**2010**		**2011**		**2012 to date**	
	No tests	**No plants**	**No tests**	**No plants**	**No tests**	**No plants**
Ornamentals (Petunia)	277	2761	183	1830	106	1042
Tomato	57	570	48	471	31	310
Ornamentals (microplants)			134	134		
SASA (Potato trial plots)	26	260	12	122	59	589
SASA-Nuclear Stock Initiation Unit	73	202	74	180	125	262
SASA-UKPQU	156	197	135	135	135	135
SASA- experimental material from 3rd country (nucleic acid)					35	35
JHI (CPC)	208	1565	232	2093	81	687
JHI (Pre-pollination parents)	8	8			18	48
N Ireland	2	20				
Ireland	80	80	45	45	40	40

Although PSTVd was not detected (2010-2012), as reported previously (2008-2010 Report) another pospiviroid *Tomato chlorotic dwarf was* detected in 4 lines of Petunia from Germany and the Netherlands in 2010. This is not listed as a quarantine pest, however, Scottish Government took statutory action against *Tomato chlorotic dwarf viroid*, ordering destruction of the material, since the pest is not normally present in Scotland.

5 years years of surveys for PSTVd by member states have now been presented to the EU's Plant Health Standing Committee (PHSC) (2007- half year, and for 2008, 2009, 2010 and 2011 – full years results) by the Food and Vetinary Office (FVO).

Conclusions from the 2011 survey were

- Significant increase in number of laboratory samples (*Solanum tuberosum* over 70%)
- Increased sampling in domestic production and internal movement
- Total number of positive samples had decreased (162 in 2011, 223 in 2010)
- PSTVd incidence had decreased (0.8% in 2011; 1.5% in 2010 and 2009)
- PSTVd incidence in *Solanum jasminoides* had decreased (10% in 2011, 19% in 2010, 13% in 2009)
- PSTVd was found in 10 MS, either in their domestic production, or in consignments in free circulation (111 outbreaks/findings) 126 in 2010, 131 in 2009, 131 in 2008 and 253 in 2007
- 100 outbreaks/findings on *Solanum jasminoides*
- One outbreak on *Solanum tuberosum*
- No positive case on *Brugmansia sp*.
- No positive findings on import

The European Food Safety Authority (EFSA) has now produced (03/08/2011, updated 26/10/2011) its Scientific Opinion on the assessment of the risk of solanaceous pospiviroids for the EU territory and the identification and evaluation of risk management options (http://www.efsa.europa.eu/en/efsajournal/doc/2330.pdf). The abstract and summary are in

Appendix 3. The conclusion seemed to be that pospiviroid infected ornamentals did not pose a risk to for example potato production although the level of uncertainty was high.

There has been discussion at the EU Plant Health Standing Committee whether the survey requirement on ornamental plants were still justified, based on experience and the EFSA opinion. In May 2012 the UK (Neil Giltrap) gave a presentation to the PHSC on the FVO survey results and made some suggestions relating to discontinuing the surveys. The Commission has concluded that there is now enough information to enable the regulatory status of PSTVd to be decided and that surveys are no longer required.

The (Plant Health Directive) Annex Working Group is working on a proposal to amend the current requirements for PSTVd and to add permanent measures for the other posiviroids *Columnea latent viroid, Mexican papita viroid, Pepper chat fruit viroid, Tomato planta macho viroid, Citrus exocortis viroid, Tomato apical stunt viroid* and *Tomato chlorotic dwarf viroid. Chrysanthemum stunt viroid* will be considered separately as part of the review of all IIAII organisms. It has been agreed that all should be regulated and when complete the proposal will be put the PHSC.

The Scottish Government's view continues to be that there is a potential pathway from these ornamental plants to potato and without action these viroids will continue to increase in the environment and this will increase the likelihood that sooner or later potatoes will be infected. Therefore targeted surveys will continue and if any pospiviroids are found crop destruction will be required. Destruction of material sends a clear message to producers to clean up their production procedures. This position will be kept under regular review.

4.1.4 Emergency measures for *Pepino mosaic virus* (PepMV) (2004/200/EC)

Introduced in 2004, these measures require tomato seeds to be free from the organism and an annual survey. Previously reported as an experimental host, potato has now been reported as a natural host of *Pepino mosaic virus* (see 2008-2010 report section 7.2). The proposal is now to incorporate this measure into the revised Plant Health Directive.

4.2 European Plant Protection Organisation (EPPO)

EPPO is a regional plant protection organisation comprising 50 member countries, which is responsible for international co-operation in plant protection in the European and Mediterranean region (see http://www.eppo.org). The main technical work of EPPO is done through panels of experts.

The EPPO *ad hoc* panel on European Phytosanitary Measures for Potato was set up in 1998, primarily to develop an EPPO Potato Commodity Standard and also develop and approve other standards for potato (Table 7). Drs Giltrap (Fera) and Jeffries (SASA) are members of this panel and have contributed significantly to the production of these standards. Details of the standards can be found at http://archives.eppo.org/index.htm .

Since the last report:

- There have been 2 further meetings of the panel (8-10 March 2011, Paris and 14-16 February 2012, Moscow). These meetings were combined with workshops on *Meloidogyne chitwoodii* and *M fallax* in Paris and *Synchitrium endobioticum* (potato wart) in Moscow.
- One new standard has been published PM 9/13 National Regulatory Control System: *Potato spindle tuber viroid*
- Two standards have been revised: PM 9/2 National Regulatory Control System: *Clavibacter michiganensis* ssp. *sepedonicus* and PM 9/3 *Ralstonia solanacearum*
- A draft National Regulatory Control System for *Meloidogyne chitwoodii* in Paris and *M fallax* has been prepared
- Revision of PM 8/1 Commodity specific phytosanitary measure: Potato has started

- A new PCN post harvest sampling standard for potato tubers for export will be prepared.
- SASA will host the next EPPO meeting and a workshop on PCN (impact of the new EU PCN control directive) in February 2013.

4.3 International Plant Protection Convention (IPPC)

There have been revisions to the International Standards on Phytosanitary Measures (ISPM) since the last committee meeting and one new standard has been produced (see www.ippc.int).

- In March 2011, the Commission on Phytosanitary Measures (CPM) adopted revisions to International Standard on Phytosanitary Measures (ISPM) no. 7 (*Phytosanitary certification system*) and ISPM no. 12 (*Phytosanitary certificates*). The revisions took into account the concept of issuing certificates after dispatch; clarified types of certificate (copies, replacements etc) and requirements for re-export, especially where there are no requirements in the importing country, but specific requirements in the country of re-export (often an issue with seed lots); a requirement for country of origin to be clear (also applies to seed lots); and examples of harmonised wording for additional declarations.
- In March 2012, the CPM adopted ISPM no. 36 on *Integrated Measures for Plants for Planting*. This applies generally to the production of plants for planting. General integrated measures are explained, including requirements to keep a plan of the place of production, examination of plants, keeping records, control of pests and sanitation. Additional elements may be required, where justified, such as a place of production manual, specific pest management measures, training of personnel, packing and transportation requirements and internal and external audits.
- In 2012, the CPM also adopted a revision to supplement no 1 to ISPM no 5 (*Glossary of phytosanitary terms*) on the definition of the concept "not widely distributed". This is relevant to consideration of whether a pest present in a country should be categorised as a quarantine pest (not widely distributed and under official control).
- During summer 2012, a draft appendix to ISPM 12 on electronic certification (using electronic systems for issuance of phytosanitary certificates) was circulated for member comments. More than 500 comments were received and the draft will be discussed in May 2013 and, hopefully, adopted in March/April 2014.

C Jeffries is the lead author for the IPPC diagnostic protocol for *Potato spindle tuber viroid*. The protocol has now been subject to expert review and was recently (27/11/2012) presented to the IPPC Technical Panel on Diagnostic Protocols (TPDP). The final draft should be available towards the end of January 2013. It then has to be approved by the Standards Committee before May for approval for member consultation between July and November 2013.

4.4 Harmonisation / standardisation of potato quarantine testing

C Jeffries has initiated a working group on international harmonisation of potato quarantine testing with CIP (Peru), Plant Protection Service (The Netherlands), APHIS-USDA (USA) and CFIA (Canada). In early 2013 the UKPQU and CIP, Peru will send proficiency test samples to each other for up to 3 viruses to establish the proof of concept for the group conducting proficiency tests in the future.

C Jeffries presented a poster "Towards a single post-entry quarantine testing regime for potato microplants in international trade"at the World Potato Congress 2012 (Edinburgh). This presented the testing regime as used for New Zealand as the basis for discussions on a global harmonised testing regime (Appendix 4).

5. DEVELOPMENT WORK ON POTATO VIROIDS, VIRUSES, PHYTOPLASMAS AND LIBERIBACTER

5.1 EUPHRESCO Project: (Epidemiology and diagnosis of potato phytoplasmas (P) and *Candidatus* Liberibacter solanacearum (Ls) and their contribution to risk management in potato and other crops (PHYLIB) March 2012 (see also section 7.1).

This 2 year project is coordinated by C Jeffries (SASA) and Jennifer Hodgetts (Fera). Partners are from Belgium (Phytoplasmas + Liberibacter, P+Ls), Canada (Ls), France (P+Ls), Finland (Ls), Hungary (P), Netherlands (?), Spain (Ls), Turkey (P), UK (P+Ls). Karen Pearson has been appointed (July 2012) to conduct the SASA part of the project with assistance from Fiona Highet SASA Virology and Zoology Section who is organising carrot surveillance for Ls symptoms in carrots and trapping of carrot psyllids in Scotland.

Key SASA objectives

Phytoplasmas

- establish reliable -methods for maintenance of phytoplasmas in potato, periwinkle (+ other hosts?) (*in vivo* and *in vitro*)
- evaluate current generic phytoplasma tests for use in potato quarantine and nuclear stock testing and to compare with the Sabrina Palmano method (see report for 2008-2010, Section 5.5)
- develop reliable sampling strategy for detection of pathogen in post-entry potato quarantine and nuclear stock testing
- introduce proficiency testing scheme

Ls

- whether Ls is present in Scotland on carrot crops (visual inspection followed by real-time PCR on suspect samples)
- vector populations (yellow traps)
- establish reliable methods for maintenance of infected material *in vivo* and *in vitro*
- develop reliable sampling strategy for detection of Ls in post-entry potato quarantine and nuclear stock testing

Countries that have Ls (Finland, Spain) in carrot are trying to establish whether this Ls haplotype can infect potato and the source of the Ls infection e.g. infected seed other hosts (see section 7.1 for further information on haplotypes).

SASA progress to date

Phytoplasmas

- Produced fact sheet on potato witches broom (PWB) (the only indigenous potato phytoplasma)
- Growers and potato inspectors asked to report suspect plants to SASA (a few plants were reported but tested negative)
- Evaluated a number of phytoplasma extraction and detection methods (continuing)
- Have received from partners in Hungary 4 potato stolbur infected varieties for propagation and testing
- Obtained PWB infected periwinkle (*in vitro* and *in vivo*)

Ls

- Taken part in a ring test of a new Ls detection method (organised by Spain)
- Prepared information pack on Ls and psyllids in carrots
- Growers and agronomists asked to report suspect plants to SASA
- Installed water traps in carrot crops and surveyed a number of carrot crops for Ls type symptoms and carrot psyllids (None found).

- Fiona Highet attended a training course on psyllid identification organised by a the EU COST Phytoplasma group in Montpellier, France
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5.2 Validation of molecular methods for the detection of potato viruses to meet the requirements of ISO 17025

In 2011 an assessment of a number of currently used molecular tests used in the UKPQU was done to ensure that they were still fit for purpose. This was followed by validation/verification to meet the requirements of ISO 17025. The validation/verification procedure was based on EPPO standard *PM 7/98 (1): Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity*, using the criteria sensitivity, specificity, selectivity, repeatability and reproducibility.

- For *Beet curly top virus* the unpublished Crosslin primers have been replaced with published Strausbaugh primers because of their slightly better sensitivity
- For *Tomato infectious chlorosis virus* the unpublished Wintermantel primers used in conventional RT-PCR (gave non specific banding with healthy potato) have been replaced with a real time assay as the primary test. However, since the related virus *Tomato chlorosis virus* (ToCV) is detected weakly, confirmation of a positive by conventional PCR and sequencing is required using the RT-PCR primers TIC5783F/criniSolR (Wintermantel & Hladky 2010)
- A real time and conventional RT-PCR test has been validated for *Tomato chlorosis virus* and introduced for the NZ testing programme
- The primers for potyviruses PV2/Pot1 were judged fit for purpose but the current 3 step method was changed to one step and the annealing temperature reduced to 45^{o}C to give improved results
- The real time RT-PCR test for *Potato yellow vein virus* was judged fit for purpose

Validation and verification of methods/tests to meet the requirements of 17025 has continued with the addition of an internal control to the above tests in a multiplex reaction. Currently nad5 is used for the RNA virus tests in a simplex (separate) reaction to eliminate the possibility of false negatives in PCR due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors. However it will not guard against failure of the target sample being added to the reaction mix. For this a multiplex reaction is needed. Currently no internal control is used for DNA reactions and for this reason DNA tests are being multiplexed with primers for the detection of cytochrome oxidase (COX).

In addition the following has been done:

- Carlavirus primers + nad5 The Badge *et al* (1996) primers have been replaced by the primers of Nie et al (2008) because the Badge primers give a similar sized amplicon to the nad5 primers and the Nie primers give a larger amplicon for sequencing.
- Begomovirus primers + COX The Wyatt *et al* (1996) primers have been judged fit for purpose although the annealing temperature has been reduced slightly.
- Potexvirus primers + nad5 The van der Vlugt and Miranda Berendsen (2002) primers potex 2RC/5 has been replaced by the van der Vlugt and Miranda Berendsen primers 1RC/5 because of the production of less non-specific PCR products and improved sensitivity. The annealing temperature has also been reduced.
- *Potato yellowing virus* (PYV) primers +nad5 Not primers were available for PYV and very little sequence data was available on the NCBI database with which to design primers. One primer pair did amplify with positive material but produced a larger product than expected because one primer did not bind as expected. The PCR product was sequenced and confirmed as PYV. Recently CIP, Peru have provided us with unpublished sequences that

have enabled us to design a much better primer set. These have been validated and have also been sent to CIP for testing against a large range of PYV isolates.

6. UPDATE ON WORK CONDUCTED BY BACTERIOLOGY

6.1 Blackleg and Associated Diseases

PhD Studentship - *Dickeya*

The studentship forms part of a multi-centred research initiative, involving FERA and JHI.
Investigating the biology of '*Dickeya solani*': An emerging bacterial pathogen of potato. Sept 2010 for 3 years (update)
This project is funded jointly by the Potato Council and the Scottish Government's Rural & Environment Research and Analysis Directorate (RERAD).

The focus of this studentship was to use DNA sequencing to define diversity within '*D. solani*' isolates and use these methods to study disease epidemiology. Near-field experiments have been carried out to determine differences in aggressiveness and transmission efficiency between isolates and studies conducted to determine how '*D. solani*' survives in the environment with a view to developing effective control strategies. Investigation was also carried out on the susceptibility of '*D. solani*' to common disinfectants.

This study has now also encompassed the work initiated in the studentship "Molecular Characterisation of *Dickeya* spp.: A Potential Threat to Scotland's Potato Industry. Sept 2008 for 3 years" development of molecular characterisation methods for isolates of *Dickeya* to determine the population structure and explore diversity within *Dickeya* isolates from Europe and elsewhere and to identify markers of bio-forensic or epidemiological value. MLST and SNP analysis method based on pyrosequencing was being evaluated.

EUPHRESCO Phytosanitary ERA-NET " *Dickeya solani*" (an emerging bacterial pathogen on potato) 2010
Focus on co-ordination and collaboration between national research programmes, cluster existing work to ensure added European value.

Main objectives of this project

- Investigating the distribution of *Dickeya solani* throughout Europe, findings were presented at annual meetings from surveys of tuber production and watercourses from EU member states involved in the project.
- Development and standardisation of protocols proficiency testing - extraction methods, evaluation of species-specific or generic assays for laboratory use - other generic detection methods, genotyping (MLSA) and sampling and surveillance approaches.
- Aggresivness and transmission, survival in various enviromental conditions, strain differentiation.

There is a proposal that the project is continued in EUPHRESCO II.

6.2 Bacterial Ring Rot and Brown Rot

EUPH03 Interlaboratory Test on Detection of *Clavibacter michiganensis* ssp. *sepedonicus* and *Ralstonia solanacearum* in Potato Tubers April 2012 – ongoing (ILT3)
This is a non-competitive project of the EUPHRESCO Phytosanitary ERA-NET which aims to verify the performance of official (and potentially official) test methods required by the EU Directives 2006/56/EC and 2006/63/EC though interlaboratory comparison.

ILT3 (third Interlaboratory test) was organised in conjunction with the EPPO Panel on Diagnostics in Bacteriology and was targeted at official laboratories in the EPPO region. The focus of this scheme was for participants to use their own testing protocols on 10 "blind" samples, ensuring PCR, real-time PCR and bioassay were included, as well as the principle screening tests.

SASA achieved comparative results with all test methods. Results were submitted in the autumn, awaiting EU result analysis/feedback.

7 NEW DISEASES OF POTATO

7.1 *Candidatus* Liberibacter solanacearum (Ls)

This bacterium is the causal agent of zebra chip of potato in North America, Mexico and New Zealand and is transmitted by *Bactericera cokerelli* (the tomato/potato psyllid). The psyllid is absent from Europe. Two Ls haplotypes[1] have been described (A and B) infecting potato and other solanaceous crops in the Americas (A and B) and New Zealand (A).

Different haplotypes of the bacterium have been reported infecting carrot in Finland, Norway Sweden (C) and carrot in Spain and the Canary Islands (D). These apparently stable haplotypes suggest stable long standing populations of Ls. In Scandanvia Ls is transmitted by the carrot psyllid *Trioza apicalis* and in Spain and Canary Islands by the psyllid *Bactericera trigonica.* Whether these haplotypes will infect potato remains unknown but is being investigated as part of the EUPHRESCO PHYLIB project (section 5.1). Recently Ls infecting carrots was also reported from France (Appendix 5).

The origin of the Ls infestations in carrot is unknown but there are a number of theories including that the pathogen is seed-transmitted and was introduced with imports of infected carrot seeds from another country, or that the infestation is the result of vector transmission from another as yet unnkown host.

The EPPO PRA for *Candidatus* Liberibacter solanacearum and the vector *B cockerelli* has now been approved and will be put on the EPPO website within the next week or two.

A web site on zebra chip can be found at http://zebrachipscri.tamu.edu/. Each year there is a conference (reporting session) on zebra chip and the presentations are available.

7.2 *Potato yellowing virus* Family *Bromoviridae*: Genus: *Ilarvirus* (Tentative)

Although not a new virus infecting potato it has now been assigned to the **Family** *Bromoviridae***: Genus** *Ilarvirus* based on phylogenetic analysis of cloned products from universal PCR primers designed for the *Bromoviridae* **(**Silvestre R, Untiveros, **,** Cuellar WJ, 2011. First Report of *Potato yellowing virus* (Genus *Ilarvirus*) in *Solanum phureja* from Ecuador. Plant Disease 95:355). Previously PYV had been assigned to the **Family** *Bromoviridae***: Genus** *Alfamovirus.*

7.3 *Tomato chlorosis virus*: Genus: *Crinivirus*

Tomato chlorosis virus has been reported infecting potato volunteer plants growing in a crop of pepper in Spain (Fortes IM, Navas-Castillo J, 2012. Potato, an experimental and natural host of the crinivirus Tomato chlorosis virus. European Journal of Plant Pathology: 134:81–86). It has now also

[1] Haplotypes represent discrete and readily identified genetic changes that can be used to assist in epidemiological studies and offer a means of separating and defining slight differences in the bacteria which are not readily defined, for example tracking movement over time or slight differences in their impact on fitness of the host organisms. The Lso haplotypes are described from single nucleotide polymorphisms (SNPs) and synchronous acrossthree gene regions, namely the available partial sequences of 16S, 16S/23S intergenic spacer region (ISR) and 50S rRNA genes.

been reported infecting commercially grown potatoes in Brazil (Freitas DMS; Nardin I; Shymoiama N; Souza-Dias JAC; Rezende JAM, 2012. First report of *Tomato chlorosis virus* in potato in Brazil. Plant Disease 96:594)

7.4 Tomato ringspot virus Genus: *Nepovirus*

Tomato ringspot virus has been reported infecting potatoes in Japan (Maoka T, 2010. Detection of 12 potato viruses from potato samples collected between 2005 and 2009. Ann. Rept. Plant Prot. North Japan).

8. RELOCATION AND QUALITY MANAGEMENT

8.1 Relocation

The proposals to relocate the PSTVd laboratory to SASA and installation of a waste water treatment facility mentioned in the 2002-2008 report are currently not being progressed because of the lack of funding for such projects.

8.2 Quality management

8.2.1 ISO 9001

SASAs work continues to be accredited to the quality management sytem ISO 9001:2000 with the scope: "To provide expert scientific and technical advice and information on agricultural and horticultural crops and aspects of the environment".

8.2.2 ISO 17025

As mentioned in the previous report (2008-2010) the UKPQU was assessed by UKAS in September 2010 for a range of tests. After clearance of non conformities accreditation was granted in early 2011 for 18 virus tests using ELISA, potato viruses using bioassay and detection of PSTVd using a digoxigenin cRNA probe. A further assessment was carried out in March 2011 and accreditation granted for a further year. The full schedule can be found at *http://www.ukas.org/testing/schedules/Actual/1406Testing%20Multiple.pdf*. The UKAS testing logo was added to the UKPQU test certificates (i.e. Plant passports/Plant Health Statements) in July 2011.

For the UKAS assessment carried out February 2012, we requested an increase in scope for additional tests:
Conventional PCR for detection of curtoviruses (*Beet curly top virus*)
Conventional RT-PCR for detection Potyviruses
Real time RT-PCR for detection of *Potato yellow vein virus*
Real time and conventional RT-PCR for detection of T*omato chlorosis virus* and *Tomato infectious chlorosis virus*

Non conformities have been cleared and we are waiting for the revised schedule to be issued by UKAS.

8.2.3 Future tests for accreditation

For 2013 it is proposed to increase the scope by
1) adding an internal control (nad5 or COX) to the current tests in a multiplex reaction.
2) adding the following tests

- Carlavirus primers + nad5
- Geminivirus primers + COX
- Potexvirus primers + nad5
- Conventional PYV primers +nad5

- *Tobacco rattle virus* + nad5

8.2.4 UKPQU Customer satisfaction

ISO 9001 and ISO17025 requires customer satisfaction surveys. For the UKPQU this was done in March 2012. This covered all our services (quarantine testing, rapid multiplication, virus elimination, a*d hoc* pathogen tests and technical advice). Results are shown in a separate document "UKPQU results.pdf ". Customers were highly satisfied with the service provided by the UKPQU.

A number of comments were received on improving our service:

- Fewer plantlets in more tubes
- Would pay more for 100% courier performance /no delays in delivery
- You should despatch international to NZ on a Monday morning
- Quality acceptable on receipt, if not this is because of delivery delays

Additional comments were also received:

- The state authority improperly charged me breaching a quarantine order imposed because of a PSTVd outbreak. The prompt advice provided by SASA (Dr Jeffries) enabled me to place a submission with the prosecuting authority who withdrew the prosecution. I was very impressed that SASA took the time to respond to my request for help. THANK YOU.
- As technical advice is our main concern, keep running just like that!

9. STAFF MEMBERS

9.1 Staff update

- Rowan Gray (A3) was appointed as a permanent member of staff in 2011. Rowan has now been promoted to B1 in DMB Branch SASA, starting 1 December 2012 but will continue to provide support to the UKPQU until early in the new Year.
- Dr Wendy Monger (B2) was appointed 2011 as a PSTVd specialist. Wendy is a very experienced plant virologist/molecular biologist from Fera. She is responsible for PSTVd testing and progressing molecular testing for viruses particularly validation of these tests to ISO17025.
- Carolyn Nisbet is on maternity leave January 2012- January 2013.
- Dr Jeffries who is a UKAS and DANAK (the Danish accreditation body) technical assessor for accreditation of phytosanitary diagnostics to ISO 17025, now also does similar work for FINAS (Finland) and SA (Slovenia).

10. MEETINGS/CONFERENCES/MISSIONS

10.1 C. Jeffries

2010

Acted as technical assessor for UKAS at Fera (UK)

Acted as technical assessor for the EUPHRESCO final project report "Detection and Epidemiology of Pospiviroids (DEP)"

Attended Crop Protection in Northern Britain 2010, Dundee, UK

Attended the 14th EAPR Virology Conference Hamar, Norway

Attended 15th Meeting of the EPPO Panel on European Phytosanitary Measures for Potato. Vilnius, Lithuania

2011

Acted as technical assessor for DANAK at the Danish Plant Directorate, Lyngby

Attended 16th Meeting of the EPPO Panel on European Phytosanitary Measures for Potato. Paris France

Attended 18th Triennial Conference of the EAPR, Oulu, Finland
Presented "Emerging Potato Pests? Somethings old somethings new" at BP2011 Harrogate.

2012
Acted as ISO 17025 technical assessor for FINAS and SA
Attended Crop Protection in Northern Britain 2012, Dundee, UK
Attended 17th Meeting of the EPPO Panel on European Phytosanitary Measures for Potato, Moscow, Russia
Attended TPDP meeting to present the PSTVd diagnostic protocol, Paris, France
Presented Poster "Towards a single post-entry quarantine testing regime for potato microplants in international trade" to the World Potato Congress, Edinburgh
Presented "Phytosanitary regulation and potato quarantine" to the XXIII Brazilian Congress of Virology, Fos do Iguacu, Brazil
Presented "SASA Potato Quarantine" to the Syngenta Quarantine Committe, Canoa Quebrada, Brazil
Presented "Potato Quarantine and potato certification in Scotland" to the 35° Alvaro Santos Costa Meeting of potato growers (Irai de Minas, Brazil)

10.2 Attendance, Carolyn Nisbet

2010
Attended COST project on Phytoplasmas Sitges, Spain
Presented paper: Validation of ELISA and bioassay used in potato quarantine to meet the requirements of ISO 17025 at the 14th EAPR Virology conference Hamar, Norway

2011
Attended COST project on Phytoplasmas Sitges, Neustadt/Weinstrasse, Germany

11 PUBLICATIONS 2010-2012 (SASA authors in bold type)

PEER REVIWED ARTICLES

Monger W, Tomlinson J, Booonham N, Marn MV, Plesko IM, Molinero-Demilly V, Tassus X, Meekes E, Toonen M, Papayiannis L, Perez-Egusquiza Z, Mehle N, Jansen C, Nielsen SL (2010) Development and inter-laboratory evaluation of real-time PCR assays for the detection of pospiviroids. Journal of Virological Methods169:207-210.

NON-PEER REVIWED ARTICLES

Chard JM, Jeffries CJ, Pickup J, (2010) New pest risks for potato**.** *Proceedings of the Crop Protection in Northern Britain Conference* 2010: 165-170.

Chard JM, Nisbet C, Fraser K, Goodfellow S, James C, Monger W, Schlenzig A, Pickup J, Jeffries CJ (2012) Safeguarding the health of potatoes in Scotland. *Proceedings of the Crop Protection in Northern Britain Conference* 2012: 207-214.

Nisbet C, Ross S, Gray R, Jeffries CJ (2010) Validation of ELISA and bioassay used in potato quarantine to meet the requirements of ISO 17025. Bioforsk Fokus 5:29.

Palmano S. **Jeffries C**, **Mulholland V**, **Saddle GS** (2010) LNA probe-based Real-Time PCR for the detection of phytoplasma in *Solanum tuberosum*. *In* Abstracts of the first COST Action FA0807 meeting 1-2 February 2010, Sitges, Spain. Pp 50.

C Jeffries
UKPQU
6 December 2012

APPENDIX 1

1) PATHOGENS TESTED SPECIFICALLY IN POTATO QUARANTINE

Required under EU legislation
[1]*Potato spindle tuber viroid* (PSTVd)
Clavibacter michiganensis ssp *sepedonicus* (ring rot)
Ralstonia solanacearum (brown rot)

[1]*Andean potato latent virus* (APLV)
Andean potato mottle virus (APMV)
[1]*Potato black ringspot virus* (PBRV)
Potato leafroll virus (PLRV)
Potato virus A (PVA)
Potato virus M (PVM)
Potato virus S (PVS)
[1]*Potato virus T* (PVT)
Potato virus V (PVV)
Potato virus X (PVX)
Potato virus Y (PVY)
[1]*Potato yellowing virus* (PYV)
Tomato spotted wilt virus (TSWV)

Additional viruses tested by the UKPQU
[1]Arracacha virus B-oca strain (AVB-O)

Potato latent virus (PotLV) (test introduced 1997?)

Potato virus P (PVP) =Potato rough dwarf virus (test introduced January 2000)

Potato mop-top virus (PMTV) (test introduced August 2000)

Tomato black ring virus (TBRV) (test introduced January 2007)

Potato yellow vein virus (PYVV) (test introduced March 2007)

Tobacco rattle virus (TRV) (test introduced August 2008)

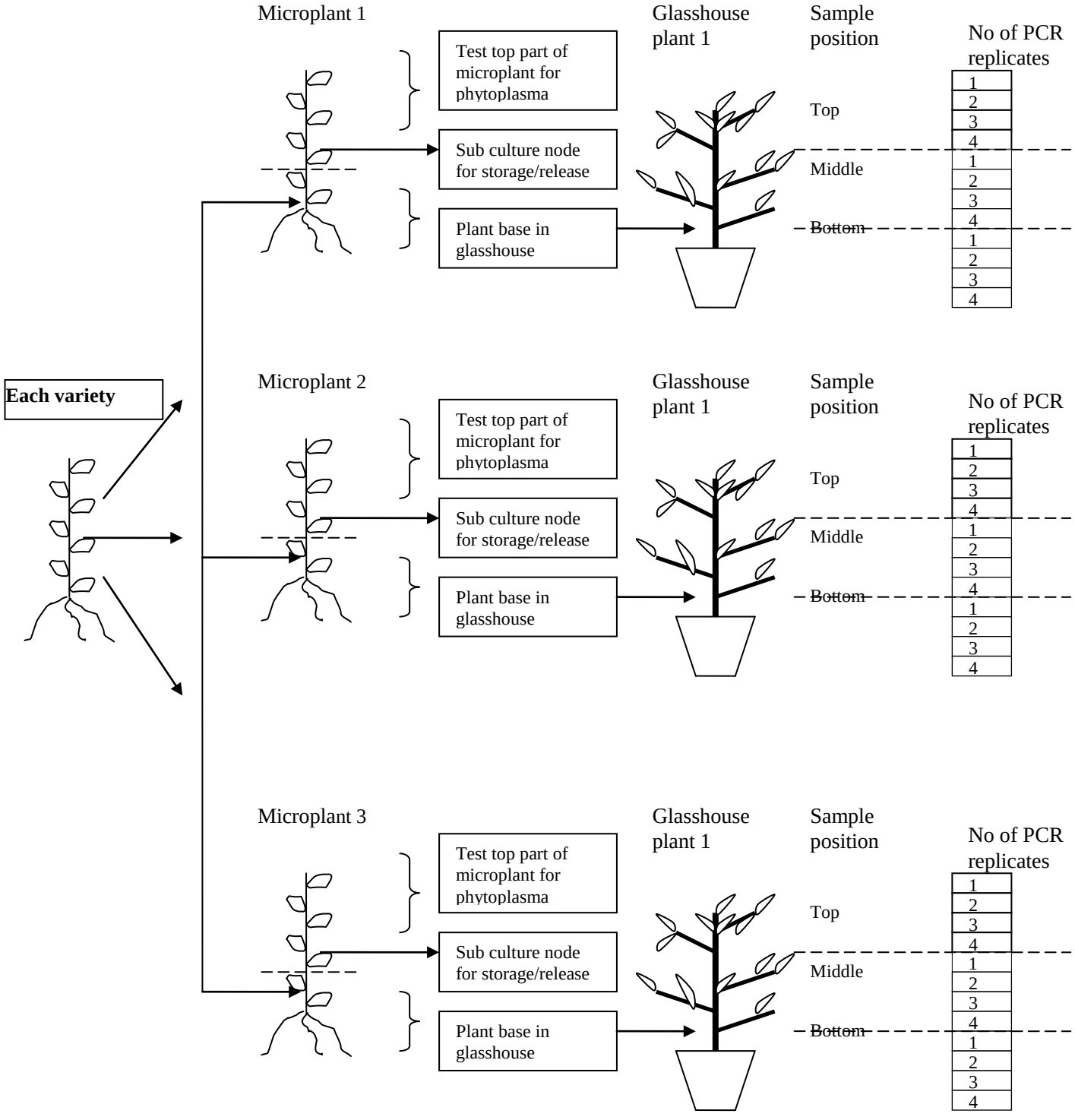
Additional bacteria tested by the UKPQU
Candidatus Liberibacter solanacearum (test introduced August 2010, changed to more sensitive primers September 2012)
Erwinia species
Dickeya species
Pectobacterium species

[1] True seed only tested for these pathogens

2) NON-SPECIFIC TESTING: INDICATOR PLANTS USED

Viruses *Chenopodium amaranticolor, C. murale, C. quinoa, N. bigelovii, N. clevelandii, N. debneyi,* and *N. tabacum* "White Burley"
Nicotiana benthamiana (introduced December 2000)
Nicotiana occidentalis- P1 (introduced January 2006)
Bacteria *Solanum melongena* cv. Black Beauty

TESTING PROGRAMME FOR THE 10 VARIETIES WHERE ORIGINAL DNA HAD PREVIOUSLY SHOWED BANDS WITH NESTED PCR



Number of PCR tests for each microplant :1 microplant x 3 glasshouse x 4 PCR replicates x 2 primer pairs = **24**

Number of PCR tests for each variety: 3 microplants x 24 = **72**

APPENDIX 3

EFSA SCIENTIFIC OPINION ON THE ASSESSMENT OF THE RISK OF SOLANACEOUS POSPIVIROIDS FOR THE EU TERRITORY AND THE IDENTIFICATION AND EVALUATION OF RISK MANAGEMENT OPTIONS

This scientific opinion, published on 26th October 2011, replaces the earlier version published on 3rd August 2011.

Abstract

Following a request from the EU Commission, the EFSA PLH Panel conducted a risk assessment for the EU territory of pospiviroids affecting solanaceous crops, identified and evaluated risk reduction options and evaluated the EU provisional emergency measures targeting *Potato spindle tuber viroid* (PSTVd). The risk assessment included PSTVd, *Citrus exocortis viroid, Columnea latent viroid, Mexican papita viroid, Tomato apical stunt viroid, Tomato chlorotic dwarf viroid, Tomato planta macho viroid, Chrysanthemum stunt viroid* and *Pepper chat fruit viroid*. Four entry pathways were identified, three involving plant propagation material, with moderate probability of entry, and one involving plant products for human consumption, with low probability of entry. The probability of establishment was considered very high. Spread was considered likely within a crop and moderately likely between crop species, with exception of spread to potato, rated as unlikely. The probability of long distance spread within vegetatively propagated crops was estimated as likely/very likely. The direct consequences were expected to be major in potato and tomato, moderate in pepper, minimal/minor in other vegetables and minimal in ornamentals. Main risk assessment uncertainties derive from limited knowledge on pospiviroids other than PSTVd, although all pospiviroids are expected to have similar biological properties. Management options to reduce risk of entry, spread and consequences were identified and evaluated. No management options can prevent establishment. Examples of successful PSTVd eradication are linked to timely and strict implementation of measures. Uncertainty exists on the effectiveness of risk reduction strategies targeting only one pathway. The EU provisional emergency measures appeared to have significantly reduced PSTVd incidence in *Solanum jasminoides* and *Brugmansia* sp., even though eradication from the EU is so far incomplete. The low PSTVd incidence in food crops did not permit to conclude whether the reduction in PSTVd prevalence in ornamentals led to a reduction in outbreaks in food crops.

Summary

This scientific opinion, published on 26th October 2011, replaces the earlier version published on 3rd August 2011.

Following a request from the European Commission, the Panel on Plant Health was asked to deliver a scientific opinion on the risk of solanaceous pospiviroids for the EU territory. The Panel was requested to provide a pest risk assessment of the solanaceous pospiviroids, to identify risk management options and to evaluate their effectiveness in reducing the risk to plant health posed by this organism. It was also requested to provide an opinion on the effectiveness of the measures listed in Commission Decision 2007/410/EC[1] in reducing the risk to plant health posed by PSTVd.

The Panel conducted the risk assessment following the general principles of the "Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options" (EFSA Panel on Plant Health (PLH), 2010). The risk assessment was

conducted without considering the existing plant health legislation. The effectiveness of the current measures in place – specific or not to the pathogen – are evaluated under the Management Options sections.

This risk assessment covers the pospiviroids which are proven in field or in experimental conditions to affect plants of the family Solanaceae, cultivated for both food consumption (e.g. potato, tomato, pepper, aubergine, pepino etc.) and ornamental purpose. The pospiviroid species covered by this document are therefore *Potato spindle tuber viroid* (PSTVd), *Citrus exocortis viroid* (CEVd), *Columnea latent viroid* (CLVd), *Mexican papita viroid* (MPVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Tomato planta macho viroid* (TPMVd) *Chrysanthemum stunt viroid* (CSVd) and *Pepper chat fruit viroid* (PCFVd) which are hereafter collectively refered to as "solanaceous pospiviroids". Having never been observed to infect solanaceous hosts, Iresine viroid 1 (IrVd-1) is excluded from the scope of the present risk assessment. Although a detailed assessment of the impacts of pospiviroids species on non-solanaceous hosts (e.g. the impact on flower crops of Chrysanthemum stunt viroid and the impact on citrus of the Citrus exocortis viroid) is not included in this document, non-solanaceous hosts are examined for their role in entry and spread pathways.

After consideration of the evidence, the Panel reached the following conclusions:

With regard to the assessment of the risk of solanaceous pospiviroids for the EU territory:

- Four entry pathways have been identified, three of which implicate propagation material [True (botanical) seeds, Seed (potato) tubers and Plants for planting]. The fourth pathway involves plant materials not intended for planting and is considered of minor significance due to the perceived low probability of transfer to a suitable host. The uncertainties associated with this evaluation concern mostly the probability of association of the pathogens with the pathway at origin (due to the limited information available on geographical distribution and prevalence of the pospiviroids) and with the probability of transfer to a suitable host, due to the numerous parameters involved. For the three main pathways, probabilities of survival during transfer and storage, of survival through management procedures and of transfer to a suitable host are considered to be high or very high and there is little uncertainty associated with these ratings. The only limiting factor is the probability of association with the pathway at origin, which as for the pathway involving plant materials not intended for planting, carries a medium uncertainty level. Overall, the probability of entry of solanaceous pospiviroids in the EU territory through the effects of all identified pathways is considered as moderately likely.

- Given previous reports of pospiviroids in many EU Members States, the wide availability of suitable hosts, the suitability of the EU area for these agents and the inability of cultural practices and control measures to decrease the chance of establishment, the probability of establishment of solanaceous pospiviroids upon entry in the 27 EU Member States is considered to be very high (certain or close to certain). This evaluation is not associated with any significant level of uncertainty.

- Within a crop species on a short distance, the probability of spread is overall evaluated as likely to very likely, with low uncertainty. The probability of transfer between crop species on a short distance is generally evaluated as being moderately likely, with high uncertainty. However, due to the lower receptivity of the potato crop and to agricultural practices that limits potato crops contacts with other susceptible crops, the probability of spread to potato is rated as very unlikely to unlikely, but with an associated high uncertainty. The probability of long distance spread, to give widespread epidemics (as opposed to localized outbreaks) is

evaluated as likely to very likely for vegetatively propagated species and as moderately likely for non vegetatively propagated ones, with overall medium uncertainty.

- Direct pest effects are expected to be markedly different for the various host plant species. The impact of solanaceous pospiviroids is expected to be major on potato and tomato and moderate on pepper. The uncertainty associated with these evaluations is low in the case of potato and tomato but medium (PSTVd and PCFVd) to high (other pospiviroids) in the case of pepper. The impact on other vegetables is expected to be minimal to minor and that on ornamental species to be minimal. The associated uncertainties are medium and low, respectively. Indirect pest effects are expected to be minimal with low uncertainties, with the exception of the impact on industries producing and commercializing plant propagation materials (seed potato tubers, true botanical seeds, plants for planting) with medium uncertainties.

With regard to the identification and evaluation of management options, these aim at reducing the risk of entry and spread of pospiviroids within the EU and to limit their impact. A unique feature of pospiviroid infections in solanaceous ornamentals is the lack of visible symptoms hence complicating surveillance and phytosanitary control of production processes and consignments, whereas in potato and tomato pospiviroid infections result in variable symptoms. Natural infections of potato were only reported for PSTVd, however all pospiviroids are infectious to potato and tomato hence present a threat similar to PSTVd for both crops. In general given the very similar biology of pospiviroids, all analyzed management options are expected to have a very similar effectiveness against all solanaceous pospiviroids. In addition, if applied against a particular pospiviroid, most management options are expected to have an impact on any other one that might simultaneously be present. *Sensu stricto*, pospiviroid pathogens are established with the cultivation of an infected plant hence there is no management option that can prevent establishment other than exclusion, avoidance and destruction of the infected plants. Efficient implementation of these management options requires to take into account the difficulties presented by these pathogens and their host plants or products thereof (seeds) for inspection and testing. The following key conclusions were reached:

- Pre-entry measures include import requirements comprising plant materials originating from pest free areas of production and certified free of PSTVd and other solanaceous pospiviroids.
- Implementation of import requirements has a high effectiveness and feasibility only when inspections and viroid tests are conducted with prudence following standardized procedures.
- Testing for pospiviroids at points of entry is highly effective with the possible exception of seed testing due to the potentially low numbers of seeds infected and to the lack of standardized seed testing methods.
- Post-entry quarantine would also be highly effective for vegetative propagation material but only when all plants are tested, such as for nuclear propagation stocks.
- Subsequent surveillance and targeted inspection for pospiviroids would assure efficiency of the measure and freedom from pospiviroids.
- No management options were identified to reduce the likelihood of establishment.
- Management options to reduce probability of spread before cultivation starts and during cultivation most significantly would prescribe the use of planting material (certified) free from pospiviroids.

- Integrated crop management including disease/ pest/ vector control provides a set of measures to reduce disease spreading significantly.
- Official surveillance in nurseries accompanied by targeted testing for pospiviroids assures freedom of these pathogens in plant stocks.
- Hygiene best practice as a suite of prophylactic measures and best practices in crop management, including the use of healthy planting material certified for viroid freedom and best hygiene practices and sanitation before, during and after cultivation to reduce occurrence and spread can provide an effective control of pospiviroids. Although some individual measures can be very effective (e.g. use of certified planting material) most other measures would individually have only a partial effectiveness but their concurrent implementation will reduce the risk of pospiviroid infections to a manageable level and reduce the impact of the disease.
- Options to reduce the probability of spread and to reduce the impact after an outbreak comprise destruction of infected plant materials, sanitation, cleaning, disinfection which when applied as a routine and with prudence effectively eliminate pospiviroids.
- Banning of continuous cropping and intercropping, weed and volunteer control and temporary ban of host plants are rigorous measures to prevent reinfection from alternative hosts and to keep crops free from pospiviroid infections.

As illustrated by examples from Canada to manage outbreaks of PSTVd in potato (Singh and Crowley, 1985), the rigiditiy with which specific measures are applied is crucial for successful risk management in potato. Successful eradication of PSTVd in *Solanum jasminoides* show similar pattern (De Hoop et al., 2008). Following outbreaks, the strict adherence to prescribed measures (destruction of infected source plants, including materials for distribution, sanitation, temporary ban of host plants etc.) and their timely implementation is most decisive for successful outbreak management.

Uncertainties exist over the effectiveness of inspection, sampling and testing measures at points of entry since pathogens in low concentrations in seeds and symptomless crops can escape detection. Since the relative significance of the two main pathways for pospiviroids infection of tomato and potato crops (seed borne infection and transmission from symptomlessly infected ornamentals) is not known, uncertainty exists on the ultimate effectiveness of a strategy targeting only one of these two pathways. In particular uncertainties exist over the effect of disease management in ornamentals to prevent occurrence of PSTVd in tomato and potato. There is low uncertainty on the overall effectiveness and feasibility of management options to reduce impact of pospiviroids: while the effectiveness of each individual hygiene best practice measure is likely to be low, if applied as a suite of measures, they are considered to be effective, with only low uncertainty.

With regard to the evaluation of the effectiveness of the provisional emergency measures, these were concerned with findings of PSTVd in the ornamental species *S. jasminoides* and *Brugmansia* spp. The available data indicate that although surveys of different intensities have been performed by EU Member States, the emergency measures have resulted in significant increases in inspection activities focused at the reduction of this pathogen in the targeted species. There does not appear, however, to have been a corresponding increase in the numbers of samples tested. Although these activities appear to have significantly reduced the levels of PSTVd inoculum in these species, this reduction is mostly represented by a reduction in findings of infected *S. jasminoides* plants and not necessarily by a similar level of reduction for plants of *Brugmansia* spp. In terms of the overall level of PSTVd circulating within the EU territory the measures significantly reduced the incidence of this pathogen, eventhough this effect has so far not been complete.

Due to the extremely low incidence of PSTVd in food crops of potato, tomato and pepper in the EU Member States, it is not possible to conclude whether the reduction in prevalence in ornamentals as a consequence of the emergency measures has led to a reduction in outbreaks in these species.

A side effect of the emergency measures was to raise the general level of awareness about other pospiviroids infecting ornamentals. Reported findings of viroids such as TCDVd, TASVd, CSVd and CEVd in ornamental species now far exceed the numbers of PSTVd records. This is probably a result of the increased vigilance combined with the wide use of generic detection methods with broad specificity towards a range of pospiviroids. It would appear that these viroids are now increasing in their prevalence and importance within the EU, eventhough all findings may not be reported as a consequence of the non-quarantine status of non-PSTVd pospiviroids. On the other hand, this increase in reporting may also result from an increased interest on pospiviroids research during the past few years.

The major area of uncertainty regarding the EU emergency measures is the interpretation and application of these measures within individual EU Member States, in particular concerning the intensity of efforts aimed at eradication following detection of PSTVd in ornamentals. It also concerns the voluntary extension by EU Member States of the emergency measures to other host plants or to other viroids. National surveys data compiled by FVO give the number of inspections conducted and number of samples tested but this does not always directly relate to crops or lots being inspected. An area of uncertainty arising from this is that although the number of inspections carried out increased three-fold, this measure alone would not have been adequate to ensure freedom from PSTVd due to the asymptomatic nature of infection in the species targeted by the measures. The number of tests carried out increased slightly, but not proportionally to the increase in number of inspections. Without knowing the number and type of samples taken and how this relates to inspections the effectiveness of surveillance measures cannot be properly evaluated. There are also uncertainties regarding missing data in the FVO reports and missing replies to the EFSA questionnaire for some Countries. Additional uncertainties exist on the side effect of emergency measures on other solanaceous pospiviroids because not all EU Member States report to FVO or Europhyt findings of pospiviroids other than PSTVd. High uncertainty also concerns the protection afforded by the emergency measures to the EU tomato and potato crops. This is because of the very limited number of PSTVd outbreaks in these crops (none in potato), which does not allow to draw meaningful comparisons between pre and post-emergency measures periods

APPENDIX 4

World Potato Congress 2012 (Edinburgh) Official Guide Poster 11

Towards a single post-entry quarantine testing regime for potato microplants in international trade

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Potato is one of the most regulated crops moving in international trade because of the ease with which pests, particularly latent pests such as viruses and bacteria, can spread from one region to another. The International Plant Protection Convention (IPPC) aims to protect plant health and progress global harmonization of phytosanitary measures through international standards (ISPMs). For potato, ISPM33 "Pest free potato (*Solanum* spp.) micropropagative material and minitubers for international trade" was published in 2010. The standard does not specify the level of testing that micropropagative material should receive, instead leaving this up to the importing country to decide, based on pest risk analysis. Countries may prohibit trade if the phytosanitary risks cannot be reduced to an acceptable level although post-entry quarantine may be available to enable normally prohibited material to enter the country. The testing regime applied in post-entry quarantine may differ between countries and traditionally it has been carried out on import but an alternative is to use specialist quarantine facilities in other countries. For example, the United Kingdom Potato Quarantine Unit (UKPQU), SASA is an accredited off-shore quarantine facility for New Zealand and is negotiating similar arrangements with other countries. Using this model, countries have access to one of the most thorough and comprehensive testing regimes worldwide. This approach could form the basis for discussions on a single harmonized post-entry quarantine testing regime for international movement of potato microplants. All work at the UKPQU is accredited to ISO 9001 and most tests are accredited to ISO 17025.

APPENDIX 5

EPPO Reporting Service no. 10 (Rse-1210.docx).

First report of '*Candidatus* Liberibacter solanacearum' on carrots in France, in association with *Trioza apicalis*

The NPPO of France recently informed the EPPO Secretariat of the first record of '*Candidatus* Liberibacter solanacearum' (EPPO A1 List – potato haplotypes*) in carrots (*Daucus carota*) on its territory. From March to June 2012, unusual symptoms characterized by poor crop growth and leaf yellowing started to be observed by growers in two fields (8 ha and 10 ha) of seed-producing carrots located in region Centre. In August 2012, the presence of '*Ca.* Liberibacter solanacearum' was officially confirmed by the national reference laboratory (real-time PCR). Field observations showed that the disease affected approximately 50% of the area in the 8 ha-plot, and 90% in the 10 ha-plot. In addition, the presence of the carrot psyllid, *Trioza apicalis* (Homoptera: Psyllidae) was observed in infected fields. The origin of this infestation is unknown but the following three scenarios have been envisaged: 1) the pathogen was introduced with imports of infected carrot seeds from another country, thus supposing that the disease is seed-transmitted (which remains to be verified); 2) infected carrot psyllids were introduced via commercial exchanges and then spread the disease; 3) infected carrot seed lots were imported and grown, thus constituting a source for local populations of carrot psyllids to acquire and further transmit the disease. Phytosanitary measures have been taken and include the following:

- all seeds harvested from the two infested fields are kept in confined conditions for further studies, in particular on the possible seed transmission of '*Ca.* L. solanacearum', and their commercialisation is prohibited;
- plant debris in infected fields have been buried;
- all machinery used to harvest infected seeds and bury plant material has been disinfected.

The pest status of '*Candidatus* Liberibacter solanacearum' in France is officially declared as: **Transient**.

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* The potato haplotypes of '*Ca.* L. solanacearum' associated with zebra chip disease, as well as their psyllid vector, *Bactericera cockerelli* (Hemiptera: Psyllidae), are absent from the EPPO region.