Hort Innovation

Final Report

Sampling for Candidatus Liberibacter solanacearum (CLso)

Project leader:

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Delivery partner:

South Australian Research and Development Institute

Project code:

PT19001

Project:

Sampling for Candidatus Liberibacter solanacearum (CLso) PT19001

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Summary

385 words

This project has developed and validated sample collection and preservation protocols, and associated sampling kits for high throughput testing of *Candidatus Liberibacter solanacearum* (CLso) in potato tubers.

CLso, the cause of Zebra chip can cause large economic losses in potato and other Solanaceae crops. Early 2017, the incursion in WA of the Tomato Potato Psyllid (TPP) and CLso vector, *Bactericera cockerelli* highlighted the lack of high throughput diagnostic protocol to support CLso surveillance in Australia.

This project complements project PT17000, Developing and implementing high throughput diagnostic tests for *Candidatus Liberibacter solanacearum* (CLso), which developed a high throughput testing protocol for CLso and sampling recommendations for potato tuber slices (taken at the stolon end) and stem sections (below ground). Industry feedback indicated that tuber samples were the preferred option because of the ease of sampling and application for seed certification. Tuber tissues are prone to rapid decay and the tuber slices used in PT17000 were difficult to standardise between samples and operators.

In this project, SARDI has demonstrated that:

- Tuber cores are a good alternative to tuber slices for detection of CLso, standardise sample size and streamline collection.
- Addition of a desiccant bag (silica gel or calcium carbonate base) preserved core samples for up to 2 weeks, while preservation of tuber slice samples was inconsistent. Some slices, especially from large tubers started to show signs of decay within one week of preparation.
- Tuber cores taken from the stolon end are suitable for detection of vascular pathogens, such as CLso and *Verticillium dahliae*. Core and slice samples incorporating the stolon scar taken from tubers infected with *V. dahliae* had the same rates of detection, however, DNA concentrations were higher in the core samples. This indicates core samples should also improve the detection of CLso.

To facilitate uptake by growers, SARDI has developed a coring tool based on grower feedback and produced a video to demonstrate the sampling protocol. The coring device will be provided as part of a surveillance sampling kit; three hundred units have been produced ready for dispatch upon request.

The outcome of this project and PT1700 is a robust, high-throughput sampling and testing capability for CLso that encompasses the diagnostic needs for national emergency response plan, large-scale surveillance, potato seed certification and conduct surveys to support crop and region area freedom assessments.

Keywords

CLso; Potato; Sample preservation; Sampling protocol; Surveillance; Biosecurity.

Introduction

277 words

Zebra chip is caused by the bacterium *Candidatus Liberibacter solanacearum* (CLso) and is transmitted by the Tomato potato psyllid (TPP), *Bactericera cockerelli*. The disease can cause large economic losses in potato and other plants from the family Solanaceae, including tomato and capsicum. While Australia is currently declared free from CLso, the incursion of TPP in Western Australia in early 2017 has significantly increased the risk that CLso may be present and highlighted the need for a contingency plan to ensure the industry is ready to face a potential CLso outbreak with the best diagnostic tools.

The SARDI-led, Hort Innovation-funded project PT17000, Developing and implementing high throughput diagnostic tests for *Candidatus Liberibacter solanacearum* (CLso), validated a high throughput diagnostic protocol for detection of CLso in potato. A key finding was that tuber or stem samples are more useful than leaf samples for detecting CLso, contrary to the existing national and international diagnostic protocols (SPHD NDP 18, 2012; IPPC DP 21, 2017) that are based on leaf samples. New sampling recommendations based on tuber slices (heel end) and stem sections (below ground) were developed.

There are challenges associated with sampling tubers and stems. Tubers are prone to rapid decay during transport to testing laboratories, which can reduce detection of CLso. Stem sections store well, but sampling is difficult. Industry feedback indicated that sampling tubers was the preferred option, because of ease of sampling and application for seed certification.

The aim of this project was to develop and evaluate a robust tuber sampling system and preservation kit. Given Australia's CLso free status, the investigations undertaken by this project were conducted using potato tubers infected with *Verticillium dahliae*, another vascular pathogen.

Methodology

879 words

CLso is a vascular pathogen which concentration in infected potato plants is relatively low and highly variable (Li et al., 2009). *Verticillium dahliae* was used as a model organism throughout the project as it is also a vascular pathogen like CLso (i.e., it is in low concentration and limited to vascular tissues of infected plants and tubers), and it is endemic in Australia. This greatly facilitated access to and handling of infected material.

All results are reported in Appendix 1.

Facilities

Laboratory research was conducted at the SARDI Molecular Diagnostic Centre (MDC), using the Root Disease Testing Service previously developed and delivered by SARDI (Ophel-Keller et al., 2008). The service relies on proprietary sample processing, DNA extraction and quantification protocols.

Sample type

PT17000 demonstrated that tuber slices taken at the stolon end of potato tubers, which are rich in vascular tissues, were suitable for CLso diagnostics. However, due to varying tuber sizes, they are difficult to standardise. Tuber cores would likely standardise sample size and streamline sample collection while also facilitating preservation (see below). To validate tuber cores vs tuber slices for CLso diagnostic a batch of tubers with known high incidence of *V. dahliae* was used to prepare composite samples made of slices or cores taken from the same tubers. Cores were collected using a medical corer (Biopunch®, 8mm diameter by 8mm depth). Six cores were taken from the stolon end of each tuber. To ensure that the majority of the vascular tissue was sampled, one core was taken directly over the stolon scar of each tuber, and another five around the perimeter of the first core. One slice of approx. 5mm was also taken from the stolon end of each tuber. The cores and slices from 15 tubers (i.e. 90 cores or 15 slices) were pooled separately; there were ten replicates of each sample type. Samples were dried, processed for DNA extraction and tested using the SARDI *V. dahliae* quantitative PCR (qPCR) test.

Preservation method

A high throughput protocol for CLso detection consisting of 3 to 6 composites of 50 samples was proposed following the completion of PT17000. The protocol was developed using tuber slices that had been freeze dried upon collection in the US and sent to SARDI. In a local outbreak scenario, samples would be collected in the field/packing shed and would need to be dispatched to the diagnostic laboratory for testing. A sound preservation method was needed to stop tuber tissues to decay during transport.

Preservation methods were first evaluated for their ability to stabilise tuber core/slices for up to 2 weeks. Methods included liquid food preservatives (potassium sorbate, sodium benzoate, pH 4 +/- sodium metabisulfite), moisture absorbing pads and desiccant bags (silica gel or calcium carbonate base).

Preservation methods were also evaluated for their impact on DNA preservation. Given the results of the preservation trial, only tuber cores and two stabilisers were evaluated in this experiment. Composites of 50 cores taken from a batch of tubers with known high incidence of *V. dahliae* were stored in stabiliser for 14 days prior to DNA extraction. Stabilisers included (i) immersion in a solution of potassium sorbate and sodium benzoate at pH 4 and (ii) calcium carbonate based desiccant bags. There were 4 replicates for each treatment. Samples were dried, processed for DNA extraction and tested for *V. dahliae* using the SARDI qPCR assay.

Evaluation

The best sampling and preservation conditions from above experiments needed to be evaluated in the field. This was done two ways:

- *Strength test the sample handling and preservation method.* A batch of tubers with known high incidence of *V. dahliae* was used to prepare paired composite samples. Cores were taken directly on the stolon attachment point of each tuber (one core per tuber) using a medical corer (12 mm diameter by 8mm depth), then cut in half. Each composite in a pair consisted of one half of each of the cores taken from 50 tubers. One sample from each pair was dried immediately at 40°C in a dehydrating oven. The paired sample was placed into a zip lock bag containing a 125 g AbsorGel desiccant Pouch (Absortech) then kept for a week in an incubator set on a 24h cycle with 15h at 35°C followed by 9h at 15°C. This was to mimic time and conditions during sample shipment to the testing laboratory. There were six paired samples in the first experiment and five in the second one. All samples were then tested for *V. dahliae*. Results were converted to amount of *V. dahliae* DNA

per gram tissue using a calibration standard. *V. dahliae* detection rate and DNA concentrations were compared between the two sample types.

- *Develop and optimise a sampling kit.* A first sampling kit comprising of a medical corer (8 x 8 mm Biopunch®) and six barcoded zip lock bags each containing a 125 g desiccant pouch was first assembled and sent to growers together with a sampling protocol for evaluation. Six samples, each of six composite of 50 cores were received back at SARDI. Compliance with sampling protocol and preservation was assessed and feedback sought from users. The feedback was used to design and produce a customised coring device that was sent to the same growers for further evaluation and feedback. Another two samples were received.

Outputs

319 words

The major outputs of PT19001 consist of:

- A sample handling and preservation method for CLso detection in potato tubers (Appendix 1). Tuber cores were shown to be a superior alternative to tuber slices for CLso diagnostic, as they have the added advantage of standardising the sample size. Tuber tissues and associated pathogen DNA can be efficiently preserved for downstream DNA extraction and CLso detection using calcium carbonate-based desiccant.
- A customised sampling tool for the collection of tuber samples for CLso surveillance (Appendix 2). The device consists of a stainless steel coring tip attached to a 50 ml tube. The coring tip is 8mm diameter by 8mm depth and is reusable. The tool standardises sample size and streamlines collection; the tube conveniently serves as a gauge for 50 cores. A prototype of the device was first produced and evaluated by growers. After minor changes, 300 coring tips have been produced.
- A sampling kit dedicated to CLso surveillance. The kit is designed for collecting six composites of 50 cores per paddock/seed lot, the most stringent sampling regime recommended by PT17000. It comprises six barcoded zip lock bags, each containing a 125 g desiccant pouch, a coring device and illustrated sampling instructions (Appendix 3). The bags are barcoded to facilitate tracking of the samples in SARDI's Laboratory Information Management System.
- A field sampling protocol for CLso (Appendix 3). The protocol uses the sampling kit developed by this project and is based on the high throughput diagnostic test and sampling recommendations developed by PT17000. See 'Recommendations' section.
- A 'Sampling for CLso, the cause of zebra chips' video (Appendix 4). The 5-minute video has been produced by SARDI in collaboration with AgCommunicators to clearly demonstrate the sampling protocol to the end users. It highlights the critical aspects of the protocol such as coring on the stolon attachment point and appropriately using the sample bags containing the desiccant pouch to ensure sample preservation during transit.

Outcomes

141 words

The outcomes of PT19001 are:

- Creation of a fully operational surveillance capability for CLso. The capability is based on the sampling protocol developed by this project and on the high throughput diagnostic and sampling recommendations developed by project PT17000. The surveillance capability ensures the industry is 'incursion-ready' and can quickly roll out an emergency response plan if required.
- Delivery of a national service to growers, supporting seed certification and biosecurity agencies on a fee for service basis. The service can be established as soon as the industry has made decisions on sample size and accuracy of detection they require, as discussed in the PT17000 final report.
- Development of a robust sample preservation method for tuber cores based on using calcium carbonate-based desiccant.
- Development of a sampling kit and coring device using close industry collaboration to maximise grower uptake of the protocol.

Monitoring and evaluation

585 words

The main outcome of PT19001 is a fully operational surveillance capability for CLso. While the basis of a high throughput diagnostic service for CLso surveillance was developed by PT17000, the delivery of an operational capability was contingent to the development of a sampling protocol by this project. This encompassed:

- Identifying the best sample type. Tuber slices taken at the stolon end and below ground stem sections were both found suitable for CLso detection by PT17000. In this project, tubers were favored over stems because of their broader application for seed certification and ease of sampling. However, tuber slices are not well adapted to high throughput sampling and are difficult to preserve due to the amount of potato flesh. This project found that tuber cores are a suitable, if not superior, alternative to slices for CLso detection.

  Using the endemic pathogen *V. dahliae* that has similar properties to CLso we were able to confidently assess various options without the constraint of handling high biosecurity risk samples.

  Results clearly showed that is critical to take the cores exactly on the stolon scar to optimise the chance of detection. This is likely due to higher concentration in vascular tissues beneath the tuber stolon scar.

- Validating a preservation protocol. Tuber tissues are challenging to preserve, as they are prone to rapid decay. Typically, samples will be collected in the field or packing shed and will have to be sent to the diagnostic laboratory, with shipping time varying from a couple of days to more than a week. In addition, conditions during transport, particularly temperature, cannot not be controlled. The preservation method needed to be able to withstand long transit times at high temperature.

  The use of desiccant to preserve tuber tissues stemmed from the Hort Innovated-funded project PT15008 (Michael Rettke, SARDI) that had shown that air drying potato peel prior to shipping to a diagnostic laboratory produced more consistent samples for DNA testing. We used a calcium carbonate-based desiccant to efficiently dry composites of 50 tuber cores and preserve DNA for downstream detection. This a cost effective solution, which has shown great reliability and has the added advantage of making for safe and easy transport, particularly when compared to other options such as liquid preservatives that were investigated during this project. The same desiccant is now under consideration by PT15008 for use in the PREDICTA® Pt peel sampling kit; it has already shown promising results with peel samples, which are much larger samples than those considered in this project.

- Developing a sampling kit and associated sampling tool. To ensure relevance, efficiency and effectiveness in the development of a potential service, SARDI consulted with industry during the project, including Potatoes South Australia (Robbie Davies) and AUSPICA (Nigel Crump).

  Potato growers evaluated the sampling protocol and sampling kits in the field, including Mr Ian Simpson from the Mitolo Group, and Mr Aaron Haby and Mr Andrew Widdison, two independent growers. The feedback on the first version of the sampling kit was that the coring device was not practical. The customised coring device designed by this project made the sampling easier and faster. It was much better received, in particular the use of the tube as a gauge for 50 cores instead of counting them. Three hundred coring tips were produced.

In conclusion, the high throughput CLso diagnostic and sampling protocols developed by both PT17000 and PT19001, and evaluated and implemented by this project have demonstrated their feasibility and reliability. Recommendations to facilitate rolling out the CLso surveillance service are presented below.

Recommendations

322 words

The surveillance capability developed by PT17000 and PT19001 is now ready to implement. Three hundred coring devices have been produced and CLso sampling kits are available from SARDI upon request.

We recommend that:

- The default level of detection for CLso be set at 1% infection with 95% confidence. Based on project PT17000 results, this would require six composite samples of 50 cores (Table 1). Assuming 3000 tubers per ton, 20 t/ha and one sample per ha, this would correspond to one per 1000 tubers. This sampling intensity may impact uptake of a discretionary service.
- Industry determine the level infection and confidence of detection required, as this will determine the appropriate sampling intensity (Table 1 and additional data from Project PT17000 final report).
- Surveillance be undertaken in high risk areas, possibly informed by the presence of TPP, and that the DNA-based method developed by PT17000 be one component of the disease monitoring. This should be implemented before CLso is detected in Australia.
- The industry start using the service for seed certification and to support market access.
- These recommendations be reviewed in the event that CLso is detected in Australia.

Table 1. Probability of CLso detection in composites of 50 samples depending on the infection rate in the paddock or seed lot analysed developed by Project PT17000.

Infection rate in paddock/seed lot	Probability of detection with composites of 50 samples[^]		
	2 composites (100)[*]	3 composites (150)	6 composites (300)
1%	63%[#]	77%	95%
2%	86%	95%	100%
5%	99%	100%	100%

[^] The overall probability was obtained by combining the probability of an infected tuber being in at least one of the composites together with the detection rate and assuming that the material obtained from the USA is representative of CLso infected plants; [*] The number in parentheses indicates the total number of samples analysed per batch; [#] The probability of detection is based on results obtained with three PCR replicates per DNA extract.

Refereed scientific publications

NA

References

DP 21 '*Candidatus* Liberibacter *solanacearum'*, 2017. Diagnostic Protocols, International Standards for Phytosanitary Measures 27. Produced by the Secretariat of the International Plant Protection Convention (IPPC).

Li, W., Abad, J.A., French-Monar, R.D., Rascoe, J., Wen, A., 2009. Multiplex real-time PCR for detection, identification and quantification of '*Candidatus Liberibacter solanacearum'* in potato plants with zebra chips. Journal of Microbiological Methods **78**, 59-65.

National Diagnostic Protocol 18 V1.2, 2012. Diagnostic protocol for the identification and detection of *Candidatus Liberibacter solanacearum*, the causal agent of zebra chip of potatoes. Issued by SPHD.

Ophel-Keller, K., McKay, A., Hartley, D., Herdina, H., Curran, J. 2008. Development of a routine DNA-based testing service for soilborne diseases in Australia. Australasian Plant Pathology, 37, 243-253.

Intellectual property, commercialisation and confidentiality

See attached IP register

Acknowledgements

Assistance from the following people is gratefully acknowledged:

- Robbie Davies, Potatoes South Australia for facilitating communication with growers.
- Mr Ian Simpson (The Mitolo Group), and Mr Aaron Haby and Mr Andrew Widdison (independent growers) for evaluating the sampling tools and providing useful feedback.
- Michael Rettke (Research scientist, SARDI) for his contribution to developing the preservation method and accepting to present the sampling demonstration video.
- The SARDI MDC staff for processing material, extracting DNA and completing qPCR testing.

Appendices

Appendix 1 – Results

Appendix 2 – Sampling device

Appendix 3 – Sampling instructions

Appendix 4 – Sampling video

Appendix 1 – Results

1- Sample type

Tuber cores were investigated as an alternative to tuber slices for detection efficiency.

When paired composite tuber slice and tuber core samples taken from *V. dahliae* infected tubers were analysed, detection rate was 100% with both sample types. However, the concentrations in the cores was much higher than in the slices (Figure 1).

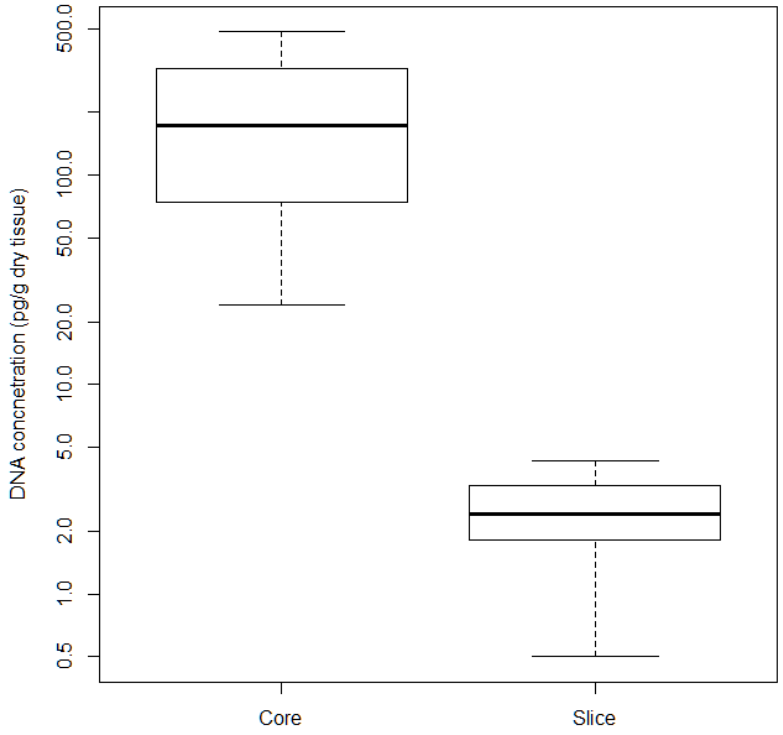


Figure 1. Comparison of *V. dahliae* DNA concentration (logarithmic scale) in composite tuber core and slice samples. Both cores and slices were collected at the stolon end of the tuber.

Additional data (not shown) indicated that the presence of tissue located directly beneath the stolon scar was a determining factor for higher detection levels. This would be critical for improving CLso detection, when concentration is low.

2- Preservation method

Tuber tissue is prone to rapid decay, which can affect DNA preservation and detection.

Preservation methods included liquid food preservatives, moisture absorbing pads and desiccant bags. Overall, core samples preserved better than slice samples irrespective of the preservation method. They showed less oxidation, decay and microbial growth than slices. For core samples, the best preservation was achieved using desiccant bags; for slice samples, the food preservative solution was more efficient, providing the slices were fully immersed in the solution.

Most importantly, when composites of 50 tuber cores that had been stored for 2 weeks at ambient temperature either in liquid preservative or on desiccant were tested, *V. dahliae* was only detected in samples stored on desiccant (Table 2).

Table 2. *Verticillium dahliae* DNA concentration in composites of 50 tuber cores, stored for 2 weeks at ambient temperature with desiccant bags (Silica gel based) or in liquid preservative.

Treatment / Replicate	*Verticillium dahliae* (pg DNA / g sample)
Desiccant / 1	9
Desiccant / 2	83
Desiccant / 3	4
Desiccant / 4	38
Preservative / 1	0
Preservative / 2	0
Preservative / 3	0
Preservative / 4	0

Overall, the results indicated that composite tuber cores that have been stabilised for up to two weeks using desiccant bags are suitable for DNA detection of vascular pathogens.

3- Evaluation

a. Impact of preservation on pathogen detection

When paired composite samples either dried immediately or exposed for a week to fluctuating daily temperatures of 35°C for 15h and 15°C for 9h were tested for *V. dahliae*, there was no significant difference in detection rate between the two treatments (Table 3). Detection rate for control samples was estimated at 91% (CI 59 – 99%) and at 100% (CI 72 – 100%) for samples stored on desiccant.

Table 3. *V. dahliae* detection rates in paired samples dried immediately after collection (control) or stored on desiccant for 7 days with fluctuating daily temperatures of 35°C for 15h and 15°C for 9h. There were five replicated paired samples in experiment 1 and six in experiment 2.

Experiment	Treatment	Number of replicates	Number positives
1	Control	5	5
	Desiccant	5	5
2	Control	6	5
	Desiccant	6	6

There was a large variability in the *V. dahliae* DNA levels measured between samples. However, a combined analysis of results of the two experiments revealed a significant difference in detection levels between treatments, with higher levels of *V. dahliae* DNA detected in samples preserved by desiccant (Table 4). Although this is difficult to explain, this observation is actually beneficial for CLso detection efficiency.

Table 4. *V. dahliae* detection levels in paired samples dried immediately after collection (control) or stored on desiccant for 7 days with fluctuating daily temperatures of 35°C for 15h and 15°C for 9h. These are combined results from the two experiments in Table 2, each with five and six replicated paired samples, respectively.

Measure	Control	Desiccant	p value	SED
per tissue	383	722	0.057	157.7

b. Field evaluation

Samples were collected by growers using both versions of the sampling kit.

In both cases, growers did not have difficulty following the sampling instructions and samples arrived in good condition at SARDI (Figure 2).

Figure 2. Tuber cores stored on desiccant (a) just after collection and (b) after 7 days.

With the first version of the sampling kit, the feedback on the coring device was that it was unpractical and made for a lengthy sampling process.

Irrespective of the sampling tool, the main concern was the sample size (ie six composites of 50 cores). Figure 3 shows the amount of potato required for a sampling intensity of six composites of 50 tubers. This may affect future uptake of the service.

Figure 3. Three hundred tubers laid out for sampling six composites of 50 cores (Photo A. Haby)

Appendix 2 – Sampling device

The sampling device provided with the first version of the sampling kit was an 8 x 8mm Biopunch® medical corer (Figure 4).
The device is really sharp so cores easily into potato tubers and the cost per corer is $3-$5 depending on supplier, which made it the ideal disposable tool to be provided to the industry as part of a sampling kit.
However, as shown in Appendix 3, each core needed to be dislodged from the coring tip using a wooden plunger, which the growers did not find practical, particularly when coring 300 tubers.

Figure 4. 8 x 8mm Biopunch® medical corer used in the first version of the sampling kit

A new sampling device was designed and produced (Figure 5).
The 8 x8 mm stainless steel coring tip can be attached to a 50ml tube, which simplifies the sample collection (Figure 5b).
As shown in Appendix 3 the tube can be used a gauge to collect 50 cores, which is the recommended number of cores per composite.
The cost per coring device is approx. $26, including $25 for the coring tip only. The tip is reusable after cleaning.

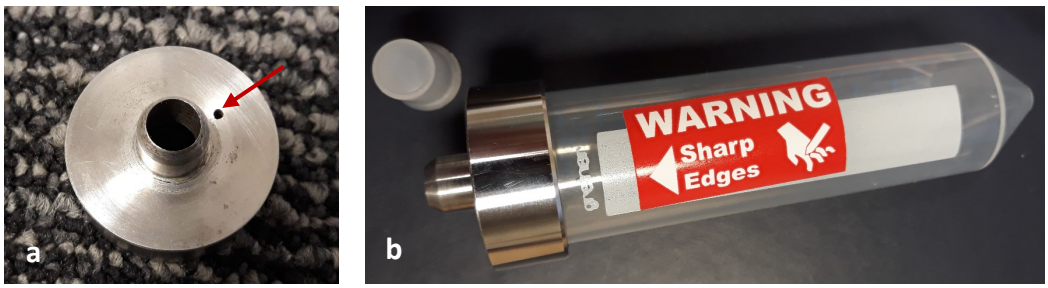


Figure 5. Customised sampling device for CLso surveillance sampling kit. (a) 8 x 8mm stainless steel coring tip; (b) coring tip attached to a 50ml tube to facilitate sample collection. The hole in the coring tip (red arrow) reduces pressure build-up in the tube while cores are collected.

Appendix 3 – Sampling instructions

Version 1 using medical corer

South Australian Research and Development Institute

Disease Surveillance - Potato Tuber Samples

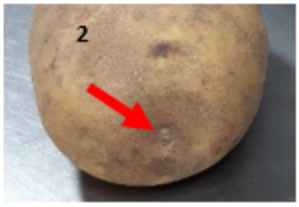
SAMPLING KIT (Fig 1)

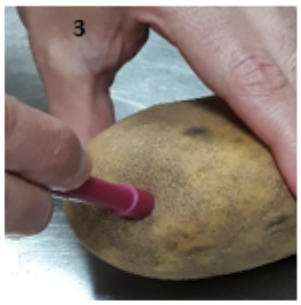
- 1 corer and wooden plunger
- 1 large barcoded zip-lock bag
- 6 barcoded bags with desiccant pouch

SAMPLE COLLECTION

1. Select 300 tubers to represent one paddock/seed lot; divide into six lots of 50
2. Remove the cap from the coring device (⚠ **sharp end**)
3. **COLLECT ONE CORE PER TUBER**
   a. Place corer over the stolon scar (Fig 2, red arrow) and push in, up to the plastic rim (Fig 3)
   b. Remove corer at slight angle to detach core from tuber
   c. Use wooden plunger to expel the core (Fig 4) into a zip-lock bag with desiccant; **expel on the printed side of desiccant pouch**
4. Repeat process until each of the six bags has 50 cores
5. Press seal each of the six zip-lock bags and place in the large zip-lock bag
6. Recap the corer, place in its original bag, seal and add to the large bag along with the six sub-samples
7. Seal the large zip-lock bag
8. **EXPRESS POST OR COURIER ASAP**

   Attn. Russell Burns
   SARDI Plant Research Centre
   2b Hartley Grove
   URRBRAE SA 5064





Version 2 using customised coring device

South Australian Research and Development Institute

Disease Surveillance - Potato Tuber Samples

SAMPLE COLLECTION

1. Randomly select 300 tubers to represent one paddock/seed lot; divide into six lots of 50
2. Collect one core per tuber
   a. The stainless steel coring tip is attached to a 50mL screw cap tube (Fig 1) (⚠ **sharp end**)

1

   b. Remove plastic cover
   c. Place corer over the stolon scar (Fig 2, red arrow) and push in, up to the rim
   d. Remove corer at slight angle to detach core from tuber
   e. Proceed to the next tuber
   f. Use the tube as a reservoir/gauge: 50 cores will approx. fill the tube to the 50mL mark


2

   g. Once tube is full, unscrew the coring tip and transfer the cores to one of the zip lock bags containing a desiccant pouch. *Note - All cores need to be on the printed side of the pouch*
3. Repeat process until each of the six lots of 50 tubers has been collected. Use one coring tip / tube per paddock seed lot
4. Press seal each of the six zip-lock bags with the cores and desiccant pouch, and place in the large zip-lock bag
5. Recap the corer, place in its original bag, seal and send back with the samples
6. Seal the large zip-lock bag
7. **EXPRESS POST OR COURIER ASAP**

   Attn. Russell Burns
   SARDI Plant Research Centre
   2b Hartley Grove
   URRBRAE SA 5064

Appendix 4 – Sampling video

The sampling video was produced by AgCommunicators and is presented by Michael Rettke, Research Scientist, SARDI.

The 5-minute video clearly demonstrates the sampling protocol to the end users. It highlights the critical aspects of the protocol such as coring on the stolon attachment point and appropriately using the sample bags containing the desiccant pouch to ensure sample preservation during transit.

The video can be viewed on the Hort Innovation website.