SRA
RSD DIAGNOSTIC LABORATORY

Sugar Research Australia

XYLEM EXTRACT COLLECTION PROTOCOL FOR RSD DIAGNOSTIC LAB - 2021

OVERVIEW

For each block/field, cut 16 stalks and pool xylem extract into one 5ml sample tube.

Recommended stalks per crop/block: 16

- Target as much of the block as possible i.e. all 4 corners/sides/internal rows rather than one corner.
- Depending on block type and size, more or less than 16 stalks may be appropriate (in general, more stalks will give higher chance of sampling at least one infected stalk).

EQUIPMENT LIST

Item	Notes/supplier
5 ml sample tubes	SSI Centrifuge Tube - 5.0 mL Sterile, Clear (200/bag) – from LabGear
Barcode stickers	Supplied from RSD Lab
Racks for 5ml tubes	-
Xylem pump	Air compressor with attached rubber milking liner
Cane knife/secateurs	-
Esky	-
Ice bricks	-
Decontamination equipment	Please see details below. Spray bottles, 70% metho + water OR household bleach + water + containers

COLLECTION METHOD

1. Label each 5 ml sample tube with a barcode sticker running vertically up the tube as pictured, before collecting samples. For each barcode, an extra small sticker is provided with the sample number which can be placed on the tube lid OR kept for your records. Record the barcodes on the sample submission form.
2. Place the 5 ml tubes in a rack, open only one tube at a time to prevent cross-contamination. Using a second rack for holding only the current tube will help keep any xylem spray away from the clean tubes.
3. Collect xylem extract directly into the sample tube using an air compressor pump with rubber hosing attachment. Only a few drops are required from each stalk; 1 ml - 4 ml is the recommended volume per sample. Use the graduations on the sample tube as a guide and avoid overfilling.
4. Keep samples cool in the field (e.g. on ice bricks), then freeze samples as soon as possible to prevent sample degradation
5. To help prevent lids from popping open, avoid overfilling the tubes and freeze them in an upright position.

POSTAGE TO RSD LAB & SAMPLE SUBMISSION FORM

1. Place frozen samples in a zip lock bag labelled with the batch ID (the name you assign the batch on the sample submission form) and pack in a well-insulated esky or cooler bag with plenty of ice packs (2-3+) to keep them cold in case of several days in transit and padding to prevent tubes breaking.
2. Label the parcels with "Freeze On Arrival" and "RSD Lab" stickers (we can supply these if you need), as well as my contact details (Lucy Gibbs - **0436 851 754**) to help prevent any issues with couriers and deliveries. Address the parcels to the RSD Lab as follows:

RSD Lab | Sugar Research Australia

50 Meiers Rd, Indooroopilly QLD 4068

3. Send parcels using a reliable courier to ensure they arrive as quickly as possible - overnight/next-day delivery is ideal. The best service depends on the region, however TOLL or TNT are preferred by most areas compared to Australia Post Express (we have had several 7+ day delays when using AusPost). If using a postage service that you are unsure about and the samples are important, it is a good idea to keep a duplicate of each sample (see TIPS/NOTES below). Please send on a Monday or Tuesday if possible, to avoid delays over the weekend.
4. An email will be sent to let you know when samples have been received in the lab and their condition (e.g. any missing/ leaked samples/ barcode mistakes). If samples arrive at room temperature or warm, samples will usually have degraded to an extent that we cannot provide an accurate result and will need to be re-sampled. Depending on insulation and weather, sample quality will generally be fine up to approx. 3 - 4 days in transit.
5. Please label your eskies and ice bricks with your organisation name and we will return them by post - the freight charge will be added to your annual invoice.
6. Please fill out a sample submission form for each batch of samples – the form template is available for download on the SRA website (updated 2021) or we can send it via email if required. Please email the completed form in **excel** format to RSDLab@sugarresearch.com.au prior to or when posting your samples – we do not require a paper copy of the form. Please also let us know the tracking/consignment number and which courier service you are using in the email, so that we know when to expect the parcel and chase up any delays.

DECONTAMINATION

OVERVIEW

The RSD Lab performs an extremely sensitive test to detect presence of DNA from *Lxx* (the RSD- causing bacterium). This means that if a very small amount of bacterial DNA from an infected sample gets into another sample tube, this may give you a false positive result. It is particularly important to thoroughly clean all sampling equipment in between samples to prevent "carry-over" contamination - i.e. residual DNA from an infected sample may be transferred to the following tubes from secateurs, cane knife, or pumping equipment. Take care also when taking samples and opening and closing tubes to avoid small droplets from getting into nearby tubes.

Please use the following decontamination recommendations as a guide. We always recommend using the most thorough and effective cleaning method possible, however considering time and resources in the field and the significance of carry-over contamination occurring – you may choose a less effective method if you understand the risks involved and monitor any consecutive positives.

EFFECTIVENESS OF DECONTAMINATION METHODS

TREATMENT	Chance carry-over after a positive (%)	Recommendation
No wash	35	No
70% Metho (spray)	20	No
70% Metho (spray) + water rinse (spray) (METHOD B – see below)	10	If low chance of positives/ monitoring carry-over
10% Bleach (soak 5-10 mins) + water rinse (METHOD A – see below)	0	Yes

METHOD A – BLEACH + WATER RINSE

OVERVIEW

Soak equipment in bleach for 5 - 10 mins (10% solution of household bleach = approx. 0.4-0.6% hypochlorite), then rinse thoroughly with water.

EQUIPMENT

- Household bleach (approx. 4 % - 6% hypochlorite) e.g. White King Standard Bleach
- Water
- Plastic container/bucket x 2
- Measuring cup/jug

METHOD DETAILS

1. Make up a fresh 10% bleach solution by diluting 1 part household bleach (4 % - 6%
2. hypochlorite) to 9 parts tap water. For example, add 100 ml bleach to 900 ml water to make up 1 L of diluted bleach solution
3. If necessary, first rinse sampling equipment to remove any plant material/debris
4. Place equipment (secateurs, cane knife, pump hose/nozzle) in a tray or bucket of 10% bleach solution to submerge
5. Keep in bleach for 5 - 10 minutes (do not leave metal items for too long in bleach as metal will corrode)
6. Transfer to a second tray/bucket of clean water to rinse off the bleach
7. Equipment is now ready to reuse

Note: A fresh bleach solution needs to be made up each day, as the chlorine will evaporate over time and will become less effective.

METHOD B – METHO/ETHANOL + WATER RINSE

OVERVIEW

Spray equipment with 70% metho/ethanol, then spray thoroughly with water to rinse.

EQUIPMENT

- Sprayer bottle with 70% alcohol: either methylated spirits (metho) or ethanol
- Sprayer bottle with clean water

METHOD DETAILS

1. Spray equipment (secateurs, cane knife, pump hose/nozzle) thoroughly with 70% metho or 70% ethanol
2. Spray equipment thoroughly with water, until running off and no residual metho or plant material remains.

TIPS/NOTES

- Samples can be taken from stalks of any age that are large enough to pump xylem extract from and have a minimum of 3-4 nodes. Stalks that are smaller than this may have higher concentrations of compounds that can inhibit the test.
- Back-up/duplicate samples: if there are higher risks of samples being lost (e.g. using a different or unreliable postal service), we recommend keeping back-up duplicates of each sample. Back-ups should be made by splitting each sample into two tubes and keeping one in your freezer (do not collect two separate samples in the field, as they will not be homogenous). Single use disposable plastic pipettes are good for this task.
- 1 ml - 4 ml is the recommended volume (this allows enough for automated transfer in the lab and re-testing) however if you cannot collect enough juice, lower volume samples can still be tested down to approx. 0.5 ml.
- If you require more barcode labels for the sample tubes, please let us know how many you will need and we will supply them.
- The 5 ml tubes we recommend can be ordered here if you need more: https://www.labgearaustralia.com.au/shop/product/ssi-centrifuge-tube-5-0-ml-graduated-sterile-clear-200-bag-5406?search=SSI+Centrifuge+Tube+-+5.0+mL%2C+Graduated.
- We aim to keep a consistent turnaround time of a maximum of 10 working days (from sample arrival to returning results). If there are any changes to this due to busy periods or large batches, we will be in contact with you to work out any extended turnaround times for samples where results are not urgent.
- If you have particularly high or low priority samples, please mention this in the comments section on the sample submission form and/or via email.
- Please feel free to email RSDLab@sugarresearch.com.au if you have any questions, or need clarification for anything related to the RSD lab.