Sugar Research Australia™

PROCEDURES FOR THE ESTABLISHMENT AND OPERATION OF APPROVED-SEED PLOTS

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This manual was prepared by SRA for use by SRA, Productivity Services and other organisations in the Australian sugar industry providing approved seed.

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1. INTRODUCTION

An approved-seed scheme provides cane growers with disease-free seed of varieties that are true-to-type. Disease-free seed (stalks, billets, setts or tissue culture plantlets used for planting) is a key control measure for systemic diseases of sugarcane, including ratoon stunting disease (RSD), leaf scald, Fiji leaf gall, smut, chlorotic streak and mosaic.

Provision of a nucleus of disease-free or approved seed in each mill area in the Australian sugarcane industry is co-ordinated by SRA, in cooperation with the distribution agents of SRA varieties. In most areas, the distribution agents are local Productivity Services. SRA provides the distribution agents with a disease-free supply of new varieties. These varieties have been DNA fingerprinted to ensure correct identification and that they are true-to-type. The distribution agent multiplies the new varieties following procedures set out in this manual and sells the approved seed to growers. The growers use the cane as a nucleus to further multiply the varieties on their farm in preparation for planting commercial fields.

All new SRA varieties are covered by Plant Breeder's Rights, and an agreement between SRA and the distribution agent allows the latter to provide these varieties to growers who have signed a PBR Licence Agreement. This procedures manual forms a part of the agreement between SRA and the distribution agents.

The procedures in this manual set the minimum standards for the operation of approved-seed plots (ASPs) and the procedures are based on world's best practice for sugarcane disease management. Quality-control measures are built into the procedures to ensure that, as far as possible, the disease-free status of seed plots are maintained. This involves regular visual inspections and RSD sampling. Variety integrity is also ensured by sampling leaves of varieties for DNA fingerprinting to ensure that variety identification is correct.

There are three guiding principles for managing disease and the subsequent classification of approved-seed plot material, thus ensuring nursery material is of the highest quality.

Disease management principles

- **Elimination**: of systemic diseases
- **Minimisation of transmissible diseases**: plot location, fungicides, rogueing, crop termination
- **Propagation material classification**: reflecting the crop health standards achieved

Hot-water treatment (HWT) plays an important role in the production of approved seed through the elimination of systemic diseases. Research shows that HWT can eliminate RSD, leaf scald, chlorotic streak, and smut in seed-cane. Heat treatment can affect germination; in many regions cane is first HWT and planted into a mother plot. Cane from the mother plot is used to establish the approved-seed plot (ASP) from which cane is supplied to growers. The use of the MP system reduces the risk of large areas of germination failure that could result from hot-water treatment.

In some districts, where insect-borne or air-borne diseases are present, the MPs are established in a remote area to minimise the risk of disease spread from commercial crops.

The following procedures set the minimum standards for operation of ASPs under agreements between SRA and distribution agents.

2. RECORDS

Records of the source of planting material used in the seed plots, treatments applied, samplings for RSD and DNA fingerprinting and visual inspections are essential for quality control. All distribution agents should record all this information. A form to assist with record keeping is illustrated in Appendix 1.

If one of the following diseases is recorded during an inspection, the distribution agent should immediately notify a SRA pathologist.

Diseases in this list will be referred to as major diseases in this document:

- Fiji leaf gall;
- RSD;
- Smut
- Leaf scald;
- Mosaic;
- Striate mosaic;
- Chlorotic streak;
- Dwarf;
- Sclerophthora stunt;
- Bacterial mottle;
- Exotic diseases (e.g. downy mildew, Ramu stunt, leaf scorch, etc).

3. MOTHER PLOTS (MPs)

3.1 Definition

A plot that provides planting material free from major diseases for planting of an ASP.

3.2 Site selection and crop growth

- Select a plot that is well drained, flood-free and has no record of major sugarcane diseases or problem weeds within the last two crop cycles.
- Select sites facilitating reasonable cane growth (with available irrigation if required) and secure from animal pests.
- Fallow the site for at least 6 months ensuring there are no volunteer sugarcane plants in the selected field.
- Signpost the plot indicating entry is restricted to authorised personnel only, to help maintain site biosecurity.

3.3 Variety purity and identity confirmation

- Carefully inspect the MP to ensure that no mixing of varieties has occurred.
- Check new varieties by comparing the cane in the mother plot with a known, identified stalk sample. Collect leaf samples from all new varieties for DNA fingerprinting by 1 December each year (protocol for sample collection is given in Appendix 4). Contact a SRA Variety Officer for further information on DNA fingerprinting, as needed.

- Carefully inspect the plot for off-types or sports. Keep a record of the number and type of off-types. Destroy off-types by spraying young plants with glyphosate or rogueing affected stools.

3.4 Disease management

3.4.1 Systemic disease elimination: it is important that only the best, disease-free propagation material is planted in MPs. For this reason, MP material should (where possible) be sourced from already established MP cane. If this is not possible, then a plant source treated in the following way should be accessed: -

- *LHWT:* planting material should be HWT for 3 hours, 50^0C on two (annual treatment) occasions with at least one of these including a pre-treatment of 40 hours cold water soak (CSLHWT).
- *Inspection*: potential MP planting material should be pre-inspected to ensure its disease-free status
    - *Smut incidence:* disease levels above 0.5% (see Section 3.4.2 below for method of calculation) will preclude the use of the cane as MP planting material.

3.4.2 Minimisation: disease risks to MPs arise from systemic diseases in the planting material used to establish the plot; disease transmission into MP cane from surrounding diseased crops is also an important consideration. These risks can be minimised by the following strategies: -

- **HWT**: as described above
- **Fungicide application**: all MP cane should be treated at planting with fungicide(s) to control pineapple sett rot and smut (see Section 3.6.4 and Appendix 2 for registered fungicide information)
- **Location**: there are several considerations with respect to plot location

    - **Board seed plot layout:** well-designed seed plots offer many advantages. It is recommended to locate the MP as far away from any potential disease sources present in other cane crops growing on Board plots. For example, locate MPs as far away as possible from varieties of uncertain disease resistance rating.

    - **Proximity of other crops:** location of commercial crops (non-Board cane / commercial Board cane) is critical in relation to the disease-free status of MP material. Boards must ensure this issue is addressed, otherwise several triggers / actions will apply (see below for disease-status zones). Locate the plot >500 m from any blocks with a recent history of leaf scald.

- ***Inspection:*** in order to ensure the disease pressure on MP cane is fully understood, disease inspections (including RSD and smut) of all Board plot cane / other cane crops close-by is required (MP, ASP, commercial cane of any kind). Inspection data will highlight the risk of disease transmission into MP cane.

    - ***Diseases with external symptoms***

        - Inspect MPs and other adjacent crops by walking every row at least three times during a season. Inspect as young plant cane, advanced tillering to out-of-hand, and at 6-12 months of age (where possible).

- If smut is present, record % smut infection and incidence of other major diseases using the following method: *assume two stools/m of row, count the number of infected stools and divide by 2 times the length of row inspected – then multiply by 100*. If a smut-infected stool is found in MP cane, destroy the whole stool by digging it out or spraying with glyphosate. Notify a SRA pathologist if more than 0.5% (1 per 100 m of row) smut-infected stools are found in an individual MP.
- Examine any unusual or suspect disease stools and send a specimen to a SRA pathologist for further identification, if a serious disease is suspected. Clearly identify specimens as high priority.
- Notify a SRA pathologist immediately if any serious diseases are found.

- **Smut**
  - **Disease incidence thresholds:** have been set; inspections in MP and surrounding cane provide an indication of the likely risks posed by smut.
  - **Trigger:** the following disease incidence thresholds will trigger further actions:
    Trigger 1: >1% diseased stools closer than **100m** to the MP cane
    Trigger 2: >10% diseased stools closer than **100m** to the MP cane
  - ***Trigger implications***
    - *Trigger 1*: (>1% diseased stools). If this threshold is exceeded, the following actions are required: -
      - *Rogueing*: all diseased stools should be rogued (on an ongoing basis) from cane growing within the 100m exclusion zone, where possible.
      - *Inspection*: if further inspections suggest that rogueing cannot keep disease incidence below 10% diseased stools, (it may not be possible to rogue some adjacent crops) then either that MP crop should be terminated (ploughed out) or trigger 2 will be enacted.
    - *Trigger 2:* disease levels above 10% maintained for any length of time (for instance, >2 weeks) at any stage in any crop (closer than 100m) will automatically eliminate the possibility of using the proposed cane as MP material.

- **RSD**

Assay

- Sample all varieties in the mother plot for RSD.
- Sample at least 50-100 stalks per variety and bulk xylem extracts, combining 16 extracts per tube per variety. Varieties in plots < 200 m long require 10-50 stalk extracts per plot.
- Collect extracts in tubes and send to the SRA RSD laboratory at Indooroopilly for qPCR assay. Detailed sampling procedures are listed in Appendix 3.

Other RSD assays

- If another type of assay is to be employed, contact a SRA pathologist for sampling advice.

The finding of any RSD (*Leifsonia xyli* s.sp *xyli*) immediately precludes the use of the diseased material as MP cane. If *Lxx* is found in adjacent crops, contact a SRA pathologist to discuss necessary corrective actions.

3.4.3 Classification: if the smut disease incidence (and other major diseases) exceeds the set threshold, then the cane can no longer be considered to be MP cane. It may continue to be grown for commercial harvest but will not be classified as approved planting material.

3.4.4 Compensation: any compensation for the loss of nursery material status is entirely a matter between the agent and the cane grower.

3.5 Establishment and entry of new varieties

1. No smut susceptible varieties (rating 8-9) should be planted in a mother plot.
2. Select planting material from a plot that has been derived from cane that has received a long HWT (LHWT) or cold-soak long HWT (CS-LHWT) (LHWT = 50°C for 3 hours, CS-LHWT = 40-hour soak at ambient temperature followed by 50^0C for 3 hours) and smut fungicide treatment in two consecutive years.
3. Inspect the plant source for RSD (see notes on RSD sampling), smut (see above) and other systemic diseases, particularly Fiji leaf gall, leaf scald, mosaic, striate mosaic, chlorotic streak, sclerophthora stunt, bacterial mottle and dwarf diseases, plus genetic mutations. The cane should have been treated and inspected for these diseases in the previous 2 years. Consult a SRA pathologist if any of these diseases are found in the proposed plant source.
4. Ensure that the correct variety is cut for planting and there is no mixing of varieties. DNA fingerprinting is recommended, and this should be timed so that results will be obtained well before the intended planting date. SRA requires that samples are taken from the first plant crop of each new variety to ensure that there has been no mixing or mislabelling during the planting process. Contact a SRA Variety Officer for further information on DNA fingerprinting.
5. If the seed plot operator does not manage plant sources for the MP, they should ensure they are supplied with a signed statement verifying that the treatments and inspections listed in points 2-4 have been met.
6. The source of planting material used to establish tissue-culture plantlets for planting in mother plots should meet all the conditions listed in points 2-4. The source material should have originated from the region in which the mother plot is located, or from a location with a similar incidence of serious diseases, and the plantlets also hardened in a location with similar diseases. Plantlets should be inspected for serious diseases by an experienced inspector during the hardening stage.
7. CS-LHWT all cane, as described in section 3.6.1, followed by treatment with a registered smut fungicide, as per the label (see section 3.6.4) (excludes tissue cultured plantlets).
8. Clean and disinfect the planter as described in section 3.6.5.
9. Thoroughly disinfect all cane knives or use a separate set of cane knives maintained for MP use.

3.6 Procedures for heat treatment and disinfection

Detailed instructions for HWT and operation of HWT plants are given in Appendix 2. The following instructions are for HWT of cane for use in MPs.

3.6.1 Procedure for CS-LHWT

1. Soak cane in cold water for at least 40-48 hours, with circulation of the water and a slow input of fresh water.
    - Treat whole stalks, or two-eye setts in baskets.
    - Stack whole stalks loosely in layers approximately three stalks deep, with 50 mm spacers between the layers by using pieces of timber or similar materials.
2. HWT the cane for 3 hours at 50°C, within 6 hours of being removed from the cold-soak tank. Keep the cane in separated layers during the heat treatment. Ensure there is adequate circulation and check tank temperature regularly; the temperature should remain at 50 ± 0.2^0C.

3.6.2 Procedure for LHWT

HWT the cane for 3 hours at 50°C in bundles of whole stalks, ensuring there is adequate circulation; check the tank water temperature regularly. The temperature should remain at 50 ± 0.2^0C.

3.6.3 Procedure for SHWT (Short-hot-water treatment)

Various short HWTs can be used to control smut or chlorotic streak.

Smut

For general smut control, HWT bundles of cane at 52°C for 30 min. The long HWT used for RSD and leaf scald are also effective for eliminating smut.

Chlorotic streak

For control of chlorotic streak, HWT cane at 50°C for 30 min.

3.6.4 Procedures for fungicide treatment to reduce smut infection

After HWT, smut can re-infect treated cane, either through spores in the air or soil. Fungicides can be used to reduce re-infection and this protection lasts for a number of months. It is recommended that all HWT cane be treated with a registered smut fungicide.

Two smut fungicides are registered; these are:

- propiconazole (Throttle®, Tyrant®);
- flutriafol (Sinker®)

The fungicides should be applied as per the label. Propiconazole must be applied as a dip for at least 5 min; Sinker® can be applied as dip or as a spray through a cane planter.

3.6.5 Procedure for disinfecting implements

All implements that are likely to cut the leaves or stalks of cane in the seed plots should be disinfected thoroughly before entering the plot.

1. Thoroughly clean all implements removing all dirt and cane residues. A high-pressure cleaner is recommended.
    a) Harvesters - include topper, throat, basecutter, feed rollers, chopper box, boot, elevator, and primary and secondary extractors.

b) Planters - include planter trailer, feed chute, blades and rubbers, and exit chute. If a recirculating fungicide spray or dip is used, ensure that tank and spray-lines are completely emptied and flushed with disinfectant.

c) Plant cutters and whole-stick harvesters - include toppers, base-cutter and gathering chains.

d) Stripping machines - include guards on inter-row tractor and fan, and fan blades.

e) Cultivation equipment - include tines, coulters, discs and tool bars. Special attention should be paid to disinfecting stool-splitting fertiliser boxes. Cultivate plant cane first, followed by first ratoon.

f) Other equipment - include cane knives, slicing knives, brix dibblers, chain saws used to trim stalks, trucks used to transport cane.

2. Spray or dip equipment using Cane Knife Steriliser® or Steri-Max® at a 1 in 100 dilution, allowing a 5 min treatment time. Renew disinfectant solution daily, or whenever it becomes dirty.

Alternatively, implements can be disinfected with 70% methylated spirits, a faster-acting sterilant; implements can be used after a 1 minute treatment time. However, methylated spirits is flammable and care should be taken using it near ignition sources.

3.7 Planting within the plot

1. Fallow all MP land for at least 6 months, eliminating all volunteers early in the fallow period.
2. Use cane varieties already in the MP as a source of plants. Treat the cane as follows:
   a) LHWT at 50°C for 3 hours: sufficient for varieties with a leaf scald rating <4.
   b) CS-LHWT if the leaf scald rating of a variety is ≥4.

Check with a SRA Variety Officer or QCANESelect™ for the latest variety ratings.

1. Apply a registered smut fungicide after HWT (see section 3.6.4).
2. Wash and thoroughly disinfect planting and cutting machinery.

3.8 Cutting planting material and harvest

1. Ensure that only staff of the distribution agents, or workers under their direct supervision, cut planting material. Ensure they use disinfected cane knives, plant cutters and / or harvesters.
2. Harvest unused cane in the MP with thoroughly-disinfected cane knives or mechanical harvesters.

3.9 Crop cycle

Do not allow cane to remain in the MP past first ratoon, if the MP is to continue at the same location.

4.0 APPROVED-SEED PLOTS (ASPs)

Definition

Propagation material of varieties of confirmed identity, free of important systemic diseases which is distributed to farmers for the establishment of their own nursery material, or for direct commercial planting.

4.1 Site selection and crop growth

Introduction: The requirements for ASP sites are the same as those for MPs; that is, sites that are well-drained, suitable for cane growth, free from major sugarcane diseases, fallowed for at least 6 months and sign-posted. **Please refer to the Mother Plot guidelines for further information**.

4.2 Variety purity and identity confirmation

- Before planting the ASP, carefully inspect the MP (source of planting material) to ensure that no mixing of varieties has occurred.
- Check new varieties by comparing the cane in the MP with a known, identified stalk sample. DNA fingerprint all new varieties by 1 December each year. Contact a SRA Variety Officer for further information on DNA fingerprinting.
- Carefully inspect the plot for off-types or sports. Keep a record of the number and type of off-types. Destroy off-types by spraying young plants with glyphosate or rogueing the plants.

4.3 Disease management

Introduction: the same instructions as for MPs, should also be followed for ASPs. That is, paying close attention to: i. systemic disease elimination through HWT / CSLHWT and the application of appropriate fungicides, ii. disease minimisation through ensuring diseased crops are not in close proximity to ASPs (see below), and iii. undertake quality assurance testing via RSD assays (**see sections 3.4.1 and 3.4.2**).

Requirements in relation to the presence of smut are outlined below.

- **Smut**
  - **Disease incidence thresholds:** have been set; inspections in ASP and surrounding cane provide an indication of the likely risks posed by smut.
  - **Trigger:** the following disease incidence thresholds will trigger further actions:
    Trigger 1: >1% diseased stools closer than **50m** to the ASP cane
    Trigger 2: >10% diseased stools closer than **100m** to the ASP cane
  - ***Trigger implications***
    - *Trigger 1*: (>1% diseased stools, <50m). If this threshold is exceeded, the following actions are required: -
      - *Rogueing*: all diseased stools should be rogued (on an ongoing basis) from cane growing within the 50m exclusion zone, where possible.
      - *Inspection*: if further inspections suggest that rogueing cannot keep disease incidence below 10% diseased stools, (it may not be possible to rogue some

adjacent crops), then either that crop should be terminated (ploughed out) or trigger 2 will be enacted.

- *Trigger 2:* disease levels above 10% maintained for any length of time (for instance, 2 weeks) at any stage in any crop (closer than 100m) will automatically eliminate the possibility of using the proposed cane as ASP material.

- **RSD**

RSD assays

Follow the same procedures for RSD assays, as per MP.

The finding of any RSD (*Leifsonia xyli* s.sp *xyli*) immediately precludes the use of the diseased material as ASP cane. If *Lxx* is found in adjacent crops, contact a SRA pathologist to consider any necessary corrective actions.

4.3.3 Classification: if the smut disease incidence (and other major diseases) exceeds the set threshold, then the cane can no longer be considered to be ASP cane. It may continue to be grown for commercial harvest but the plot cannot continue to be used as an ASP.

4.4 Establishment and entry of new varieties

Introduction: procedures for variety introductions are similar for ASP as for MP. However, a few of the details are slightly different, so the methods are repeated here.

1. No smut-susceptible varieties (rating 8-9) should be planted in the ASP.
2. Only plant cane from a MP or a source that has received the same treatment as a MP and is not more than one year away from HWT should be planted in an ASP. The plant source must have been intensively inspected and free from RSD and other systemic diseases, particularly smut, Fiji leaf gall, leaf scald, mosaic, chlorotic streak, striate mosaic, sclerophthora stunt, bacterial mottle, dwarf disease, plus genetic mutations.
3. Ensure that the variety is correctly identified and there are no volunteer stools of other varieties.
4. Where planting material for an ASP is sourced from a farmer, or other supplier, ensure that a signed statement is obtained verifying that the treatments and inspections listed in 3.4.1 and 3.4.2 have been met.
5. The source of planting material used to establish tissue-culture plantlets to be propagated in ASPs should meet all the conditions listed in points 4.2 and 4.3 and have originated from the same region in which the mother plot is located. If not, then they must be sourced (and hardened) in a location with a similar (or lower) serious disease incidence in which the approved seed plot is located. Plantlets should be inspected during the hardening stage for serious diseases by an experienced inspector.
6. Smut intermediate-susceptible varieties must be treated with a suitable smut fungicide at planting (see section 3.6.4).
7. If smut is present in the MP, all varieties in which smut was detected must be given a SHWT (52°C for 30 min, see section 3.4.3) plus treated with a registered smut fungicide (see section 3.4.4) before planting into a ASP.

8. Where no MP is available, the cane should be LHWT (or CS-LHWT if the leaf scald rating is ≥4) (see sections 4.3.1) followed by a smut fungicide treatment (section 3.6.4) before planting
9. If there is a risk of chlorotic streak infection in the ASP, the cane should be SHWT (section 3.4.4), then treated with a smut fungicide (section 3.6.4).
10. Disinfect planting and cutting equipment before commencement of operations.

4.5 Planting within the plot

1. Fallow the site for at least 6 months and ensure that there are no volunteer sugarcane plants before planting the plot.
2. Cane to be planted should be treated as outlined in section 4.3.1

Weeds

Undertake weed inspections in plots and carry-out appropriate management to ensure that the plot is weed-free.

4.6 Distribution of approved seed

4.6.1 Classes of approved seed

Approved seed should be sold or distributed under three categories.

a) *Approved seed*: Cane from the plant crop in the approved-seed plot.

b) *First-ratoon approved seed*: May be sold in larger quantities as required, but with a notice stating that this cane has been harvested in the previous season.

c) *Second-ratoon approved seed*: If sold, it should be for commercial planting only, and the purchaser must accept there is no guarantee of the disease status of the cane.

4.6.2 Distribution

Seed is distributed in different ways in different regions; the following are some of these methods. It is important that each method minimises contamination risks posed by diseases and that varieties are not mixed.

a) *Farmer cuts cane by hand under staff supervision*. Only open the plot during specified periods on specified days. Staff either supervise disinfection of knives, disinfect knives themselves for the farmers, or provide disinfected knives that are returned when the farmer leaves. Staff should direct the farmer where to cut and estimate or weigh the amount of cane supplied. Knives should be disinfected between varieties.

b) *Distribution agent arranges cutting*. Contract cane cutters or whole-stalk cane-cutting machines cut the cane, which is then available for collection by the farmer or the cane is delivered to collection points, or to the farm. Careful supervision and training of contract workers should be carried out to ensure they use the correct disinfection procedures (see section 3.4.3). Disinfect knives and plant cutters between varieties.

c) *Contractors or farmers cut cane by chopper or whole-stalk machines*. The distribution agent must supervise disinfection of the machines, ensuring disinfection is carried out thoroughly.

They should also supervise the machine operation to ensure there is no contact with any adjacent commercial cane or that the wrong variety is cut (see section 3.4.3).

4.7 Crop cycle

A crop cycle of only plant, first ratoon and fallow is recommended for ASP.

4.7.1 Harvest of excess cane

1. A reliable contractor or farmer should be contracted to harvest excess cane.
2. Staff should supervise the disinfection of the harvester before cutting at the plot.
3. Supervise the harvest and do not let the harvester contact any commercial cane adjacent to the ASP.
4. Harvest plant cane first, followed by first ratoon.

APPENDIX 1 – Certification of MPs and ASPs

RECORD OF PLANTING AND INSPECTION OF MPs AND ASPs

DATE ..... DISTRIBUTION AGENT .....

I certify that inspections and treatments listed in Tables 1-4 and attached documents and maps were performed according to the procedures given in the SRA Manual "Procedures for the establishment and operation of approved-seed plots: Sixth Edition 2021".

……..
Signature of staff member authorised to sign by the distribution agent

Location of plot (government map details or GPS coordinates) .....

Please record the following information in addition to the table below:

1. A map of the plot showing blocks, block numbers and varieties.

2. Reports from the RSD laboratory on assay results.

3. Report from DNA fingerprinting laboratory.

Table 1 Source of planting material and treatments

Treatments: the HWTs applied to the cane at planting in the current planting season (Nil, SHWT, LHWT, CS-LHWT; only required for plant crops).

Category: i. MP = mother plot, ii. ASP = approved-seed plot.

Source: provide a reference to block number and location of the source or organisation who supplied the cane, e.g. Blk3 MP = Block3 mother plot, or SRA = supplied by SRA.

Variety	Crop Class	Category MP/ASP	Source	Treatment	Block number	Area (Ha) or length of row (m)	Date planted

Table 2 Disease inspections

- Visual inspections: enter inspection dates
- Smut: calculate the percent smut infection

= (number of infected plants all inspection/2 x the metres of row) x 100

- RSD: show number of samples.

Block	Variety	% Smut infection	Visual Inspection			RSD
			Early	Mid	Late	

Table 3 Summary of Approved Seed distributed

Tonnes sold in the previous calendar year to growers as approved seed.

Variety	Crop Class	Block Number	Tonnes sold	Whole-stalk or billet

Table 4 DNA fingerprinting (new varieties only)

Variety	**Category MP/ASP**	**Block**	**DNA Fingerprinting**		
			Sample collected	**Sample sent**	**Result received**

APPENDIX 2 – HWT of sugarcane to control diseases and pests

INTRODUCTION

HWT of cuttings (stalks or stalk pieces) to minimise pest and disease incidence and spread, was pioneered in Australia. HWT can reduce the risk of planting material being infected with ratoon stunting, leaf scald, chlorotic streak, smut and insect pests. It does not usually control viral diseases such as mosaic and Fiji leaf gall. HWT is only a part of an integrated disease management (IDM) program and must be used in combination with disease inspections, crop management and hygiene to reduce the risks of reinfection (see attached 'Procedures for the establishment and operation of approved-seed plots').

Protocols for HWT vary depending on the disease or pest target. The temperature/time combinations most commonly used are close to the thermal death point of sugarcane. It is, therefore, critical that the temperature is not exceeded or germination will be severely affected. However, for effective disease control, the temperature must not drop below the target temperature. Temperature control thus must be precise with narrow tolerances. Water circulation is also critical to ensure that all parts of the tank are maintained at the correct temperature. Temperature checks during each run of the facility is essential to ensure good disease control. A regular maintenance program is also essential to ensure the tank is operating correctly.

The following specifications for disease and pest control were developed by SRA.

HWT FACILITIES

Tank size

The ratio of sugarcane stalks (by weight) to water volume should be 1:6. To treat a 1 tonne load of sugarcane, the tank should have a volume of 6000 L.

Circulation

Water circulation in the tank is critical. A general rule is that the circulation pump should circulate the entire tank water volume six times per hour.

The inlet pipe should be located at the base of the tank. A suitable screen should be in place to prevent blockages in the circulation system arising from leaf and stalk material. Water is returned to the tank via pipes located at the top of the tank.

Heating

Commonly-used heat sources for HWT tanks are steam from the sugar factory and electrical heaters; gas / oil furnaces are sometimes also used. Facilities that rely on steam from the sugar mill may only operate when the mill is operating; factory breakdowns threaten successful treatment.

Electrical heating or steam injection pipes can be placed at the bottom of the tank or in a secondary tank from which the heated water is circulated back into the main treatment tank.

Handling of the sugarcane stalks

To load and remove the sugarcane stalks from the tank, a cradle, basket or sling is often used, with care taken to avoid impedance of the circulating water. Baskets should be made with mesh with sufficiently large holes to allow water movement, but small enough to prevent cane stalks or cuttings from passing through the holes.

Temperature control

Automatic temperature control is essential for effective operation of the facilities. Temperatures should be maintained at 50 ± 0.2^{o}C. If the temperature is elevated above this range for extended periods, poor germination may result; low treatment temperatures will lead to ineffective pathogen elimination.

Heat loss may be reduced via a tank lid. Surrounding tank insulation also reduces heat loss. Locating the tank in a shed restricts airflow around the tank, further reducing heat losses.

Temperature monitoring

Tank temperature should be monitored with a quality electronic data logger, with suitable associated thermocouples. Water temperatures in various parts of the tank should be monitored regularly to ensure water circulation is adequate and even. The accuracy of all temperature recording devices, including thermometers, should be checked regularly.

Water quality

Tank water should be of good quality, free of high salt levels and have near-neutral pH. In practice, the water can be used for a number of loads over a number of days but should be replaced at least every 3-4 days - or sooner if large amounts of soil or plant material build up. Infrequent water changes may lead to fermentation of the sugars leached from the cane and result in poor cane germination.

In some countries, smut fungicides have been added to the tank water to protect against smut infection in the planted cane. This is not necessary (other fungicide treatment methods are available) and the disposal of large volumes of fungicide solution is an environmental problem.

HWT PROTOCOLS FOR DISEASES AND PESTS

RATOON STUNTING (RSD)

HWT is used to control RSD; the disease is caused by the bacterium *Leifsonia xyli* subsp. *xyli,* which is widely distributed in all sugarcane-producing countries around the world. The disease can cause losses of 5-60% and is spread via infected planting material and using contaminated cutting implements, such as cane knives, harvesters and planters. HWT of infected stalks at 50^{o}C for 3 hours gives greater than 99% disease control. In some countries, the treatment is reduced to 50^{o}C for 2 hours, but Australian research suggests that this treatment combination is not as effective at the 50^{0} C, 3 hour recommendation.

Selection of cane for treatment

The cane to be treated should be free from RSD, with a RSD assay completed first before treatment. If the cane is 100% RSD-infected, there is a risk that a small percentage of escapes may occur and the residual disease may spread to other plants when stalks are cut. If there is no alternative to using known diseased cane, it is most important that the cane be treated in two consecutive years before being used as a disease-free plant source.

To improve treated-cane germination, the cane source should be selected carefully; stalks should come from a well-grown and reasonably mature crop (preferably close to 12 months of age), with buds and root primordia in good condition; cane with stem rots or insect damage should be avoided.

Presentation for HWT

Dead leaves (trash) on stalks should be removed, though this is not essential. Leaves can cause blockages in the circulation system, so care should be taken to clean circulation system screens more regularly, if non-stripped cane is used.

The most common way cane is handled for RSD treatment is to treat whole stalks in large bundles placed in cradles or slings. The stalks should not be so long that they prevent circulation at the tank ends.

Alternatively, one- or two-eye setts or billets can be treated in open mesh baskets.

Treatment

The standard treatment is 50^{o}C for 3 hours. Timing commences as soon as the cane is placed in the tank. Treatments longer than 3 hours may lead to poor germination. Maintaining the temperature at 50 $\pm$ 0.2^{o}C is essential for disease control and good germination.

Initially the tank should be heated to 51-52^{o}C and then the cane lowered into the tank. The temperature will drop as the cane submerges; tank temperature should be returned to 50^{o}C as quickly as possible. The water temperature should then be strictly maintained at exactly 50^{o}C. A reliable thermometer should be used to regularly check (every 10-20 minutes) tank temperatures and adjustments made to the thermostat, as necessary.

Post-treatment

After the cane is removed, it should be cooled as quickly as possible by spraying the cane with cool water. Immediately after removal, treated buds will be soft and minimal cane handling will reduce bud damage. The cane can be planted once it has cooled, though some prefer to leave the cane for 1-2 days to allow the buds to harden. Cane has been planted up to 2 weeks after treatment with acceptable germination, but this is not recommended.

The cane setts should be dipped or sprayed with a fungicide before planting (e.g. propiconazole or flutriafol) to provide sett rot protection. HWT cane is particularly susceptible to sett rots.

The soil should be in ideal condition for planting to maximise germination. Ideally, cane should be treated and planted at a time when soil temperature and moisture are optimum.

Management of HWT cane

All implements that cut the leaves or stalks of sugarcane must be sterilised before entering the HWT cane plot. Implements should be thoroughly washed and sprayed with 70% methylated spirits or 1% quaternary ammonium disinfectant; the latter sterilant requires a 5-minute contact time for effective control.

Summary – RSD

1. Select mature cane with no damage to buds or stalks.
2. Stack stalks in a crate or sling. Alternatively, one- or two-eye setts or billets can be treated in loosely-packed open mesh baskets or crates.
3. Heat the tank to 51-52^{o}C.
4. Load the cane and treat for 3 hours, 50^{o}C; commence timing when the crate is submerged in the water.
5. Cool cane quickly at the end of the treatment by spraying with cool water.

6. Handle the cane carefully until it has cooled; cane should be left for 1-2 days before planting.
7. Spray or dip cuttings with a registered fungicide.
8. Plant cane when conditions are ideal for germination - good soil tilth, ideal soil moisture and temperature.
9. Protect cane from reinfection by sterilising all cutting implements used in disease-free plots

LEAF SCALD

Leaf scald is caused by the bacterium *Xanthomonas albilineans*. Leaf scald can cause complete plant death in susceptible varieties, and is spread by planting infected stalks, strong wind-blown rain and cutting implements. Research has shown that HWT alone does not completely eliminate the disease. For effective control, the cane must first be soaked in water at ambient temperature for 40-48 hours with immediate HWT at 3 hours, 50^{o}C (CS-LHWT). To improve the effectiveness of the heat treatment, care must be taken to maximise water circulation by carefully stacking the cane in layers separated by spacers. Treated cane should be planted in an area that is unlikely to be reinfected by other disease sources (diseased cane or alternative hosts).

Selection of cane for treatment

The cane to be treated should be leaf scald-free. If no other alternatives are available, it is essential that diseased cane be treated in two consecutive years before use as a disease-free plant source.

Well-grown, reasonably mature (preferably close to 12 months of age) cane, with buds and root primordia in good condition, should be sourced to maximise germination. Avoiding damage from stem rots or insects is recommended.

Presentation for HWT

Dead leaves (trash) on stalks should be removed. The cane should be stacked in layers no more than three stalks deep, with 50 mm spacers between the layers (e.g. pieces of timber). Alternatively, one- or two-eye setts can be treated in loosely-packed open mesh crates or baskets.

Treatment

A single cold-soak long HWT (CSLHWT) largely eliminates leaf scald. The cane is soaked for 40-48 hours in water at ambient temperature; a HWT (50^{o}C, 3 hours) follows. A slow replacement of the water during the cold-soak period prevents water stagnation, which can affect germination. Extending the cold-soak period beyond 48 hours will not affect the treatment, but buds will start to shoot after this period and may be killed by the HWT. HWT should ideally commence immediately after the cane is removed from the cold-soak tank, but a short delay of a few hours will not affect the treatment. If the heat treatment time extends beyond 3 hours, poor germination may result. Maintaining water temperature at 50 ± 0.2^{o}C is essential for good disease management and germination.

Initially the tank should be heated to 51-52^{o}C before the cane is lowered into the tank. The temperature will drop when the cane is submerged and the water temperature should be returned to 50^{o}C as quickly as possible. The thermostat should be set at exactly 50^{o}C and this must be checked against the reading of a reliable thermometer placed in the tank; tank temperatures should be checked regularly (every 10-20 minutes) using the thermometer and adjustments made, as necessary.

Post-treatment

When the cane is removed, it is important to cool the cane as quickly as possible using either a water spray, or water dip, using cool water. Cane buds are soft after removal and this requires the cane to be handled carefully. It can be immediately planted, though some prefer to leave the cane for 1-2 days before planting - to allow the buds to harden. Cane has been planted up to 2 weeks after treatment with acceptable germination, but this is not recommended.

Cane setts should be dipped or sprayed with a sett rot fungicide after treatment (e.g. propiconazole or flutriafol); HWT cane is particularly susceptible to sett rots.

Ideally, cane should be HWT when the soil temperature and moisture are ideal for germination.

A site well removed from known sources of leaf scald should be selected to plant the treated cane. Leaf scald has commonly been found in alternative grass hosts along river banks; the cane should not be planted close to these areas.

Management of HWT cane

All implements that cut the sugarcane leaves or stalks must be sterilised before entering the HWT plot. Implements should be thoroughly washed and sprayed with 70% methylated spirits or 1% quaternary ammonium disinfectant; the latter requires a 5-minute contact period for effective decontamination.

Summary – Leaf scald

1. Select mature cane with no damage to buds or stalks.
2. Stack stalks in layers no more than three stalks deep, with 50 mm spacers between the layers (e.g. pieces of timber). Alternatively, one- or two-eye setts can be treated in loosely-packed open mesh crates or baskets.
3. Soak cane in water at ambient temperature for 40-48 hours with a slow input of fresh water.
4. Heat the tank to 51-52^{o}C.
5. Load the cane and treat for 3 hours, 50^{o}C; commence timing when the cane is submerged in the water.
6. Cool the cane quickly at the end of the treatment, by spraying with cool water.
7. Handle cane as little as possible until it has thoroughly cooled; cane should be left for 1-2 days before planting.
8. Spray or dip cuttings with a registered fungicide.
9. Plant in an area well separated from known sources of leaf scald.
10. Plant the cane when conditions are ideal for germination, which includes good soil tilth, ideal soil moisture and temperature.
11. Protect cane from reinfection by sterilising all cutting implements used in the disease-free plot.

SMUT

Smut, caused by the fungus *Sporisorium scitamineum*, is a serious disease of sugarcane. The disease can cause complete crop loss in susceptible varieties and is spread by wind-borne spores produced in fruiting bodies known as whips. The spores may travel long distances via wind dispersal and can

infect buds on standing stalks, buds on stalks planted into infested soil and via young developing shoots or tillers in plant and ratoon cane.

Effective smut elimination from stalks is achieved by HWT at 52°C for 45 min.

The long-HWT used for RSD and leaf scald are also effective in eliminating smut.

After HWT, smut can re-infect treated cane - if the cane is planted into soil infested with smut spores or if smut spores land on cane buds before they are planted or if infection occurs via young tillers in the plant crop. Fungicides may provide several months protection from smut after planting, and it is recommended that all HWT cane be treated with a registered smut fungicide.

Two fungicides are registered for smut control; these are: -

• propiconazole (Throttle®, Tyrant®);

• flutriafol (Sinker®)

The fungicides should be applied as per the label. Propiconazole must be applied as a dip for at least 5 min.; Sinker® can be applied as a dip or as a spray through a planting machine.

CHLOROTIC STREAK AND INSECT PESTS

The agent which causes chlorotic streak (*Phytocercomonas venanatans*) is particularly sensitive to heat and can be eliminated completely by treatment at 50^{o}C for 30 minutes. This short HWT (SWHT) does not control RSD or leaf scald; it does however improve cane germination.

Short HWT of 50-52^{o}C for 20-30 minutes is widely used to kill insect pests when cane is being moved from one area or country to another.

APPENDIX 3 - RSD sampling procedures

ASSAY

Selection of stalks

Stalks should be sampled at random throughout the plot. The largest stalks in poorly-grown stools (possibly poorly-grown due to RSD) should be selected.

Number of stalks to sample

The probability of detecting RSD in a field that is showing no obvious stunting depends on the number of stalks examined and the sensitivity of the diagnostic technique. The probability of a correct diagnosis is greatly increased as the sample number increases. For example, to have 95% probability of detecting disease that is randomly distributed and at a 10% incidence, 29 samples are needed; at 1% infected stools, 298 samples; and at 0.1% infected stools, 2,996 samples (Figure 1). Obviously, the practicality of handling the cane and the labour available will limit the number of samples able to be collected. Approved-seed and experimental plots will require much more rigorous sampling than routine farm plant sources. In approved-seed plots, 50-100 stalks should be sampled from each variety. In small plots < 200 m long, 10-50 samples should be collected.

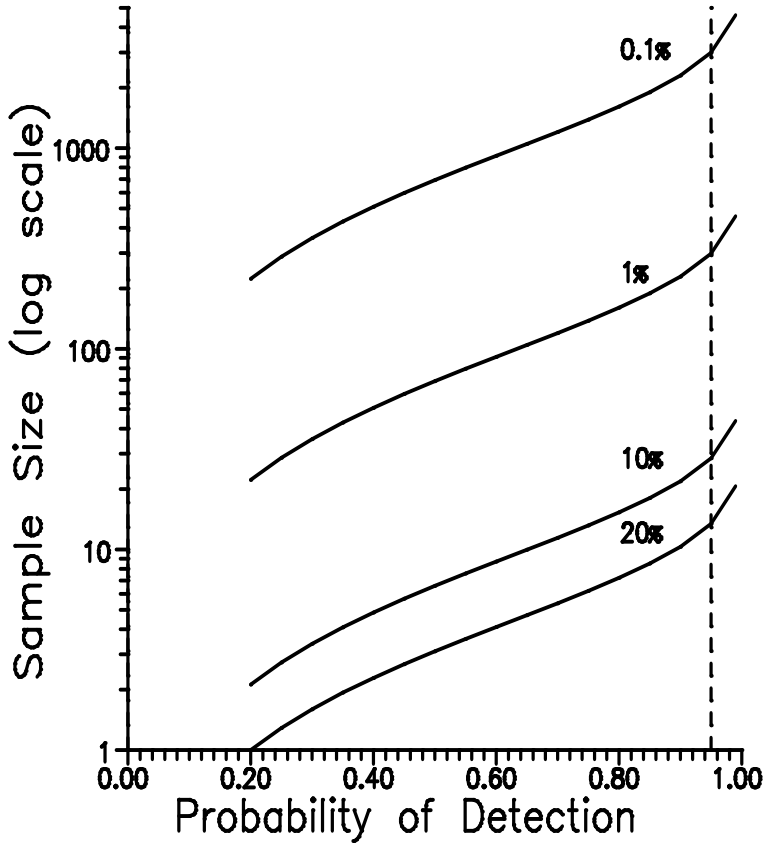

Figure 1. Probability of detecting a positive with different sample sizes

Section of the stalk to sample

Extracts for RSD diagnosis should be taken from the base of the stalk, since bacteria are generally more concentrated in these nodes, particularly early in the season. In mature cane, the first node of reasonable length, 7.5-15 cm, can be sampled for ease of extract collection.

Bulking of samples

Extracts can be bulked to reduce the number of samples to be tested. However, this may reduce the chance of detecting the disease. The current recommendation is to combine 16 xylem extracts into each sample tube.

Equipment required

1. 5 mL titre-tubes and caps.
2. Rubber milking machine inflation boot.
3. Air compressor - either 240 volt air compressor or a compressor that can be operated from a vehicle's 12 volt cigarette lighter. High-pressure compressors for car tyres are suitable.
4. Secateurs or long handled, beak blade lopping shears, and these should be sharp.
5. Esky with cooler block.
6. Methylated spirits and cleaning rags.

Procedure

1. For extraction at headland or shed, collect stalk pieces with at least 3-4 internodes. Take extracts the same day. It is much more difficult to collect xylem extract if stalks are allowed to dry out. Extracts are easier to collect in the morning.
2. Cut a single-node section of stalk from the stalk base. Cut one end square and the other at a 45° angle. If the stalk is dirty, clean the tip of the angled end and avoid soiling the angled cut surface.
3. Do not select insect-damaged, rat-damaged or rotten stalks. Avoid internodes with growth cracks where possible. If growth cracks are present, cut one end at a node.
4. Turn the air compressor on. Press the flat end of the stalk piece into rubber holder (Figure 2). Allow the fluid that bubbles out of the stalk to run off the angled tip of the stalk directly into a tube. Collect extracts from up to 16 stalks in the one tube. Collect approximately 5.0 mL of extract. Do not completely fill the tubes since freezing will cause the caps to dislodge.

NOTE: Cane juice collected by squeezing stalks or with brix samplers is not suitable for RSD diagnosis.

Figure 2. Equipment used for collecting xylem extracts for RSD diagnosis

5. Label each tube clearly using bar codes. Complete a record sheet to ensure sample details are recorded.
6. Clean and disinfect secateurs or lopping shears and the rubber holder between plots by wiping off organic matter and swabbing or spraying with either household bleach or methylated spirits.
7. Freeze the samples on return to the office.
8. Send samples to the SRA RSD laboratory at Indooroopilly by Air Express or other reliable overnight courier, in an Esky on cooler blocks. Follow the other recommended procedures, as communicated by the RSD laboratory.

The address and contact details are:

Sugar Research Australia Limited
50 Meiers Road
Indooroopilly QLD 4068
Attn. Lucy Gibbs
Email: RSDassaylab@sugarresearch.com.au

Other RSD assay sampling: please consult a SRA pathologist for sampling and dispatch advice.

APPENDIX 4 - Guidelines for leaf sampling for DNA fingerprinting

1. Select the top-most leaf and remove the midrib. The leaf should be free of any disease symptoms.
2. Cut a small length (10-15 mm) and place in the storage tube (Figure 3) supplied.
3. The number of samples to collect will vary depending on the size of the plot, and whether mixing of varieties is suspected. If the latter is suspected, collect samples from throughout the plot and separate the different types, placing them in separate tubes.
4. For details on sampling for DNA fingerprinting, delivery of samples (including storage tube supplies), contact your local SRA Variety Officer

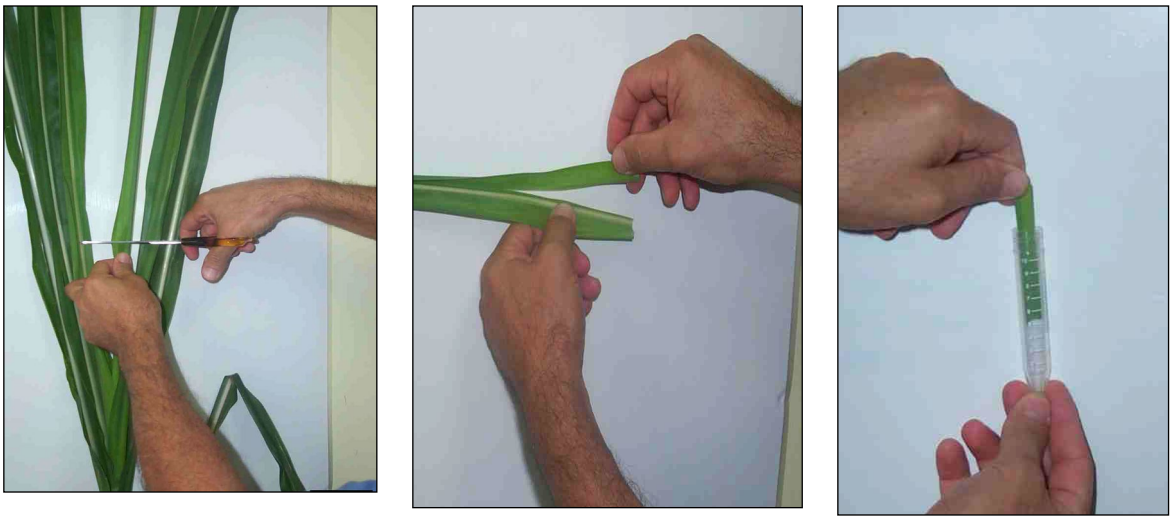

Figure 3. Procedure for collecting leaf samples for DNA fingerprinting