



**PROCEDURES FOR THE ESTABLISHMENT AND
OPERATION OF APPROVED-SEED PLOTS
FOURTH EDITION 2013**

by

**BJ CROFT and MC COX
MN07002**

Contact:

Barry Croft
Biosecurity Manager
Sugar Research Australia
90 Old Cove Road
Woodford Q 4514
Telephone: +61 7 54345901 Facsimile: +61 7 54345909
Email: bcroft@sugarresearch.org.au

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

October 2013

© Copyright by Sugar Research Australia Ltd. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior permission of SRA.

Disclaimer In this disclaimer a reference to “we”, “us”, “our” or “SRA” means Sugar Research Australia Limited and our directors, officers, agents and employees. Although we do our best to present information that is correct and accurate, we make no warranties, guarantees or representations about the suitability, reliability, currency or accuracy of the information we present in this report for any purposes. Subject to any terms implied by law and which cannot be excluded, we accept no responsibility for any loss, damage, cost or expense incurred by you as a result of the use of, or reliance on, any materials and information appearing in this report. You, the user, accept sole responsibility and risk associated with the use and results of the information appearing in this report and you agree that we will not be liable for any loss or damage whatsoever (including through negligence) arising out of, or in connection with the use of this report. We recommend that you contact our staff before acting on any information provided in this report.

Warning Our tests, inspections and recommendations should not be relied on without further, independent inquiries. They may not be accurate, complete or applicable for your particular needs for many reasons, including (for example) SRA being unaware of other matters relevant to individual crops, the analysis of unrepresentative samples or the influence of environmental, managerial or other factors on production.

CONTENTS

	Page No
1. INTRODUCTION.....	1
2. RECORDS.....	2
3. MOTHER PLOTS	3
3.1 Definition	3
3.2 Site selection.....	3
3.3 Establishment and entry of new varieties.....	3
3.4 Procedures for heat treatment and disinfection	5
3.4.1 Procedure for CS-LHWT	5
3.4.2 Procedure for LHWT	5
3.4.3 Procedure for SHWT (Short-hot-water treatments)	5
3.4.4 Procedures for fungicide treatment to reduce sugarcane smut infection.....	6
3.4.5 Procedure for disinfecting implements	6
3.5 Planting within the plot	7
3.6 Cutting planting material and harvest	7
3.7 Inspections	8
3.7.1 Diseases with external symptoms	8
3.7.2 RSD inspections	8
3.7.3 Variety purity and identity.....	8
3.8 Crop cycle	9
4. APPROVED-SEED PLOTS	9
4.1 Definition	9
4.2 Site selection.....	9
4.3 Establishment and entry of new varieties.....	10
4.4 Planting within the plot	11
4.5 Inspections	11

4.5.1	Diseases with external symptoms	11
4.5.2	RSD inspections	11
4.5.3	Variety purity and identity.....	12
4.5.4	Weeds	12
4.6	Distribution of approved seed	12
4.6.1	Classes of approved seed.....	12
4.6.2	Distribution	12
4.7	Crop cycle	13
4.8	Harvest of excess cane	13
APPENDIX 1 –	Certification of mother plots and approved-seed plots	37
APPENDIX 2 –	Hot-water treatment of sugarcane to control diseases and pests	42
APPENDIX 3 -	RSD sampling procedures	50
APPENDIX 4 -	Guidelines for leaf sampling for DNA fingerprinting	54

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 sugar 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 multiple 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 License 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 and the procedures are based on world's best practice for disease management in sugarcane. Quality-control measures are built into the procedures to ensure that, as far as possible, the disease-free status of the plots is maintained. This involves regular visual inspection and sampling for RSD. Variety integrity is also ensured by sampling leaves of varieties for DNA fingerprinting to ensure that variety identification is correct.

Hot-water treatment plays an important role in the production of approved seed. BSES research has shown that hot-water treatment can control RSD, leaf scald and chlorotic streak, and overseas research has shown that smut can be controlled by hot-water treatment. Hot-water treatment can affect germination, and in many regions cane is first hot-water treated and planted into a mother plot. Cane from the mother plot is used to establish the approved-seed plot from which cane is supplied to growers. The use of the mother-plot system reduces the risk of large areas of germination failure after hot-water treatment. In some districts where insect borne or aerially borne diseases are present, the mother plots are established in a remote area to reduce the risk of spread of these diseases from commercial crops.

The following procedures set the minimum standards for operation of approved-seed plots under agreements between SRA and distribution agents.

2. RECORDS

Records of the source of planting material used in the seed plots, treatments applied, sampling for RSD and DNA fingerprinting and visual inspections are essential for quality control. ***All distribution agents should record all plantings, treatments, sampling for RSD and DNA fingerprinting and visual inspections carried out on their seed plots.*** A form to assist in keeping records is shown in Appendix 1.

If one of the following diseases is recorded during an inspection, the distribution agent must immediately advise the Chief Executive Officer of SRA in writing.

Serious diseases requiring immediate notification to SRA (diseases in this list will be referred to as serious diseases in this document):

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

If inspections or the results of a DNA fingerprint show that there has been a mislabelling or mixing of a variety in the mother plot or approved-seed plot, the distribution agent must immediately advise the Chief Executive Officer of SRA in writing.

3. MOTHER PLOTS

3.1 Definition

A plot that provides planting material free of serious diseases for planting of approved-seed plots.

3.2 Site selection

1. 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.
2. Inspect surrounding cane to establish that no serious diseases are present. This includes adequate sampling of all surrounding blocks for RSD.
3. Separate the plot by at least a 10 m headland from any.
4. Isolate the plot where insect-transmitted diseases (eg Fiji leaf gall and sugarcane mosaic) are present, unless disease incidence is at low levels. Separate mother plots geographically from the nearest known infected field.
5. Locate the plot 500 m from any blocks with a recent history of leaf scald.
6. Select sites suitable for reasonable cane growth, with irrigation available if required, and which are secured as far as possible from damage by animal pests.
7. Signpost the plot to notify that entry is restricted to authorised personnel only.
8. Fallow the site for at least 6 months and ensure that there are no volunteer sugarcane plants before establishing the plot.

3.3 Establishment and entry of new varieties

1. No smut susceptible varieties (rating 8-9) should be planted in the mother plot.
2. Select planting material from a plot that has been derived from cane that has received a long-hot-water treatment (LHWT) or cold-soak long-hot-water treatment (CS-LHWT) (LHWT = 50°C for 3 hours, CS-LHWT = 40-hour soak at ambient temperature followed by 50°C for 3 hours) and fungicide treatment for smut in two consecutive years.
3. Inspect the plant source for RSD (see notes on RSD sampling) and other systemic diseases particularly smut, Fiji leaf gall, leaf scald, sugarcane mosaic, striate mosaic, chlorotic streak, sclerophthora stunt, bacterial mottle and dwarf diseases, and genetic abnormalities. 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 that there is no mixing of varieties. A sample for DNA fingerprinting is recommended, and should be taken early enough so that results will be obtained before the actual planting of the cane. SRA Limited 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 (see 3.7.3).
5. If the operator of the approved-seed plot does not have control of the plots used to supply cane for planting in the mother plot, they should ensure that they are supplied with a signed statement verifying that the treatments and inspections listed in points 2-4 are met.
6. The source of plant material used to establish tissue-culture plantlets to be planted into a mother plot should meet all of the conditions listed in points 2-4 and should have originated from the region in which the mother plot is located or from a location with a similar incidence of serious disease. The plantlets must be hardened in a nursery facility in a location with a similar incidence of serious disease to the region in which the mother plot is located. Plantlets should be inspected for serious diseases by an experienced inspector during the hardening stage.
7. Cold-soak long-hot-water treat (CS-LHWT) all cane, as described in section 3.4.1, followed by a fungicide treatment with a chemical registered for control of sugarcane smut as per label of the fungicide (see section 3.4.4), before planting into the mother plot (excludes tissue cultured plantlets).
8. Clean and disinfect the planter as described in section 3.4.5.
9. Thoroughly disinfect all cane knives or use a separate set of cane knives kept for use in the mother plot only.

3.4 Procedures for heat treatment and disinfection

Detailed instructions for hot-water treatment and operation of hot-water treatment plants are given in Appendix 2. The following instructions are for hot-water treatment of cane for use in mother plots.

3.4.1 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.
2. Treat whole stalks or 2-eye setts in baskets.
3. Stack whole stalks loosely in layers approximately three stalks deep with 50 mm spacers between the layers (eg pieces of timber).
4. Hot-water treat 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 that there is adequate circulation and check temperature control regularly. The temperature should remain at 50 ± 0.2 °C.

3.4.2 Procedure for LHWT

Hot-water treat the cane for 3 hours at 50°C in bundles of whole stalks. Ensure that there is adequate circulation and check temperature control regularly. The temperature should remain at 50 ± 0.2 °C.

3.4.3 Procedure for SHWT (Short-hot-water treatments)

Various short-hot-water treatments can be used to control smut or chlorotic streak.

Smut

For maximum control of smut, hot-water treat bundles of cane at 52°C for 45 min. This treatment is recommended when moving cane from a smut-infested area to a non-infested area.

For general smut control within a smut-infested area, hot-water treat bundles of cane at 52°C for 30 min.

The long-hot-water treatments used for RSD and leaf scald are also effective at controlling smut.

Chlorotic streak

For control of chlorotic streak, hot-water treat bundles of cane at 50°C for 30 min.

3.4.4 Procedures for fungicide treatment to reduce sugarcane smut infection

After hot-water treatment, smut can re-infect treated cane if the cane is planted in soil that is infested with smut spores or smut spores land on the cane buds before they are planted or spores in the soil can infect young tillers in the plant crop. Some intermediate-to-resistant varieties become more susceptible to smut immediately after hot-water treatment because the bud scales are softened by the hot water allowing entry of the smut fungus. Fungicides can be used to reduce re-infection for a number of months after planting, and it is recommended that all hot-water treated cane be treated with a fungicide registered for the control of sugarcane smut.

Fungicides can also be used to reduce infection in healthy planting material of intermediate varieties.

Registrations are available for two fungicide for the treatment of cane stalks for control of smut:

- propiconazole (NUFARM THROTTLE);
- 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 spray through a planting machine.

3.4.5 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 implement 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 with Cane Knife Steriliser® or Steri-Max® at 1 in 100 dilution and leave stand for 5 minutes. Renew disinfectant solution daily, or whenever it becomes dirty.

Alternatively, implements can be disinfected with 70% methylated spirits. Methylated spirits is faster acting and implements can be used after 1 minute. However, methylated spirits is flammable and care should be taken when using it near harvesters.

3.5 Planting within the plot

1. Fallow all land to be planted in the mother plot for at least 6 months and eliminate volunteers early in the fallow period.
2. Use cane varieties already in the mother plot as planting material for future planting in the mother plot. Treat the cane as follows:
 - (a) LHWT at 50°C for 3 hours is sufficient if leaf scald resistance rating of the variety is less than 4.
 - (b) CS-LHWT if leaf scald resistance rating of the variety is 4 or greater.

Check with a SRA Variety Officer for the latest ratings for varieties.

3. After hot-water treatment, apply a fungicide registered for control of sugarcane smut as per the label of the fungicide (see section 3.4.4).
4. Wash and thoroughly disinfect planting and cutting machinery.

3.6 Cutting planting material and harvest

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

3.7 Inspections

3.7.1 Diseases with external symptoms

1. Inspect mother plots by walking every row at least three times during a season. Inspect at young plant cane, advanced tillering to out-of-hand, and at 6-12 months of age.
2. Record the percent smut infection (assume two stools/m of row, count the number of infected stools and divide by 2 times the length of row inspected and multiple by 100). If a smut-infected stool is found 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 a plot of a variety. The cumulative number of infected plants per plot during a crop should be reported (i.e. the total number of infected stools at all inspections).
3. 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 when contacting a pathologist.
4. Notify the Chief Executive Officer of SRA immediately if any serious diseases are found.

3.7.2 RSD inspections

1. Sample all varieties in the mother plot for RSD.
2. Sample at least 50-100 stalks per variety and bulk extracts into 12-25 samples. Varieties in plots < 200 m long require 10-50 samples per plot.
3. Collect extracts in tubes and send to the SRA RSD laboratory at Indooroopilly for ELISA assay. Detailed sampling and sample handling procedures are given Appendix 3.

3.7.3 Variety purity and identity

1. Carefully inspect the mother plot to ensure that no mixing of varieties has occurred at planting.
2. Check new varieties by comparing the cane in the mother plot with a stalk sample supplied by SRA. Collect a sample for DNA fingerprinting of all new varieties for distribution by the 1 December each year. Send the sample to the laboratory nominated by SRA by the 1 December each year (protocol for sample collection is given in Appendix 4).

3. 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 digging out the plants.
4. Notify the Chief Executive Officer of SRA immediately if any mislabelling or mixing of varieties is suspected.

3.8 Crop cycle

1. Do not allow cane to remain in the mother plot past first ratoon if the plot is to continue at the same site.

4. APPROVED-SEED PLOTS

4.1 Definition

Plots from which sugarcane varieties that are true-to-type and free of important diseases are distributed to commercial producers for establishment of their own on-farm seed propagation, or for commercial planting.

4.2 Site selection

1. 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.
2. Select sites suitable for reasonable cane growth, with irrigation available if required, and which are secured as far as possible from damage by animal pests.
3. Inspect surrounding cane to establish that no serious diseases are present. This includes adequate testing for RSD.
4. Separate the plot by at least a 10 m headland from any commercial. If there is no headland between the plot and commercial crops, plant six rows of approved seed of a highly smut-resistant variety between the plot and commercial cane.
5. Isolate the plot where insect-transmitted diseases (eg Fiji leaf gall and sugarcane mosaic) are present, unless disease incidence is at low levels.
6. Locate the plot 500 m from any blocks known to be infected with leaf scald.
7. Signpost the plot to notify farmers that they must report to staff before entering the plot.

8. Fallow the site for at least 6 months and ensure that there are no volunteer sugarcane plants before establishing the plot.

4.3 Establishment and entry of new varieties

1. No smut-susceptible varieties (rating 8-9) should be planted in the approved-seed plot.
2. Only plant with cane from the mother plot or from a plot not more than 1 year away from hot-water treatment. The plant source must have been intensively inspected and passed as free of RSD and other systemic diseases, particularly smut, Fiji leaf gall, leaf scald, sugarcane mosaic, chlorotic streak, striate mosaic, sclerophthora stunt, bacterial mottle and dwarf diseases, and genetic abnormalities.
3. Ensure that the variety is correctly identified and there are no volunteer stools of other varieties.
4. If the operator of the approved-seed plot does not have control of the plots used to supply cane for planting in the approved-seed plot, they should ensure that they are supplied with a signed statement verifying that the treatments and inspections listed in point 2 and point 3 have been met.
5. The source of plant material used to establish tissue-culture plantlets to be planted into a mother plot should meet all of the conditions listed in points 2-4 and should have originated from the region in which the mother plot is located or from a location with a similar incidence of serious disease. The plantlets must be hardened in a nursery facility in a location with a similar incidence of serious disease to the region in which the mother plot is located. Plantlets should be inspected for serious diseases by an experienced inspector during the hardening stage.
6. Smutintermediate to susceptible varieties must be treated with Sinker at planting (see section 3.4.4).
7. If smut is present in the mother plot, all varieties in which smut was detected must be given a SHWT for smut (52°C for 30 min, see section 3.4.3) and smut fungicide treatment (see section 3.4.4).
8. Where no mother plot is available, the cane should be LHWT (or CS-LHWT if leaf scald rating is 4 or greater) (see sections 3.4.1 and 3.4.2) followed by a smut fungicide treatment (section 3.4.4) before planting
9. If there is a risk of chlorotic streak infection in the mother plot, the cane should be SHWT (section 3.4.4) followed by a smut fungicide treatment (section 3.4.4) before planting.
10. Disinfect planting and cutting equipment before commencement of operations.

4.4 Planting within the plot

1. Fallow the site for at least 6 months and ensure that there are no volunteer sugarcane plants before establishing the plot.
2. Cane to be planted should be treated as outlined in section 4.3.

4.5 Inspections

4.5.1 Diseases with external symptoms

1. Inspect approved-seed plots by walking every row at least three times during a season. Inspect at young plant cane, advanced tillering to out-of-hand, and at 6-12 months of age.
2. Record the percent smut infection (assume two stools/m of row, count the number of infected stools and divide by 2 times the length of row inspected and multiple by 100). If a smut-infected stool is found 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 a plot of a variety. The cumulative number of infected plants in a plot during a crop should be reported (i.e. the total number of infected stools at all inspections).
3. 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 when contacting a pathologist.
4. Notify the Chief Executive Officer of SRA in writing immediately if serious diseases are found. If smut exceeds 0.5% (1 stool in 100 m) in any variety discuss the distribution of cane from the plot with SRA before distribution commences.

4.5.2 RSD inspections

1. Conduct intensive sampling for RSD in all varieties in the approved seed plot.
2. Sample at least 50-100 stalks per variety and bulk extracts into 12-25 samples. Varieties in plots < 200 m long require 10-50 samples per plot.
3. Collect extracts in tubes and send to the SRA RSD laboratory at Indooroopilly for ELISA assay. Detailed sampling and sample handling procedures are given Appendix 3.
4. Send any doubtful or positive samples to a SRA pathologist for confirmation of diagnosis.

4.5.3 Variety purity and identity

1. Carefully inspect the approved-seed plot to ensure that no mixing of varieties has occurred at planting.
2. Notify the Chief Executive Officer of SRA in writing immediately if any mislabelling or mixing of varieties is suspected.
3. Collect a sample for DNA fingerprinting of all new varieties for distribution by 1 December each year. Send the sample to the laboratory nominated by SRA by 1 December each year (protocol for sample collection is given in Appendix 4).
4. 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 digging out the plants.

4.5.4 Weeds

1. Inspect the plot for weeds and carry out appropriate control measures to ensure that the plot is as weed-free as possible.

4.6 Distribution of approved seed

4.6.1 Classes of approved seed

Approved seed should be sold or distributed under three categories.

- Approved seed: Cane from the plant crop in the approved-seed plot.
- First-ratoon approved seed: Cane from the first-ratoon crop of approved seed. Sold as larger quantities if required, but with a notice stating that this cane has been cut in the previous season.
- Second-ratoon approved seed: If sold, it should be for commercial planting material only, and purchaser must accept that no guarantee of the disease status is given or implied.

4.6.2 Distribution

Different systems are used to distribute approved seed in different regions. The following are descriptions of some methods of distribution. It is important to ensure that the distribution method reduces the risk of contamination of the approved seed by diseases and that varieties are not mixed.

- Farmer cuts cane by hand under supervision of staff. To control entry with this system, open the plot only during specified periods on specified days. Staff either supervise disinfection of knives, disinfect knives for 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.
- 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 is delivered to collection points or to the farm. Careful supervision and training of contract workers should be carried out to ensure that they use the correct disinfection procedures (see section 3.4.3). Disinfect knives and plant cutters between varieties.
- Contractors or farmers cut cane by chopper or whole-stalk machines. Distribution agent must supervise disinfection of these machines and ensure that disinfection is carried out thoroughly and they must supervise the machine while cutting to ensure that it does not contact any adjacent commercial cane or cut the wrong variety (see section 3.4.3). Ideally, an arrangement should be made with a reliable contractor or farmer for the use of their machine for all work on the plot.

4.7 Crop cycle

- A crop cycle of only plant, first ratoon and fallow is recommended for approved-seed plots.

Harvest of excess cane

- A reliable contractor or farmer should be contracted to harvest excess cane.
- Staff should supervise the disinfection of the harvester before it commences cutting on the plot.
- Supervise the harvest and do not let the harvester contact any commercial cane adjacent to the approved-seed plot.
- Harvest plant cane first, followed by first ratoon.

APPENDIX 1 – Certification of mother plots and approved-seed plots

RECORD OF PLANTING AND INSPECTION OF MOTHER PLOTS AND APPROVED-SEED PLOTS

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 MN07002 “Procedures for the establishment and operation of approved-seed plots: Fourth Edition 2013”.

.....

Signature of staff member authorised to sign by the distribution agent

Location of plot (government map details or GPS coordinates)

Please provide in addition to the completed tables:

- 1. A map of the plot showing blocks, block numbers and varieties.**
- 2. Reports from RSD laboratory of results of RSD tests.**
- 3. Report from DNA fingerprinting laboratory.**

Table 1 Source of planting material and treatments

Treatments are the hot-water treatments applied to the cane at planting in the current planting season (Nil, SHWT, LHWT, CS-LHWT), only required for plant crops.

Category – MP = mother plot, ASP = approved-seed plot.

Source – give 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.

Table 2 Disease inspections

Visual inspections enter date inspected

Smut - calculated the percent smut infection

= (number of infected plants all inspection/2 x the metres of row)x100

RSD show number of samples.

Table 3 Summary of Approved Seed distributed

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

Table 4 DNA fingerprinting (new varieties only)

APPENDIX 2 – Hot-water treatment of sugarcane to control diseases and pests



INTRODUCTION

Hot-water treatment of sugarcane cuttings (stalks or stalk pieces) to control the spread of diseases and pests was pioneered in Australia. Hot-water treatment can reduce the risk of cuttings being infected with ratoon stunting disease, leaf scald, chlorotic streak, sugarcane smut and downy mildew and can free cuttings of insect pests. It does not usually control virus diseases such as sugarcane mosaic and Fiji leaf gall. Hot-water treatment is only a part of an integrated pest management program and must be used in combination with disease inspection, crop management and hygiene to reduce the risks of reinfection (see attached ‘Procedures for the establishment and operation of approved-seed plots’).

Protocols for hot-water treatment vary depending on the disease or pest that is the 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, to obtain effective control of ratoon stunting disease and leaf scald disease, the temperature must not drop below the target temperature or the disease will not be controlled effectively. This means that temperature control must be maintained within narrow tolerances. Circulation of the water is also critical to ensure that all parts of the tank are maintained at the correct temperature and that there are no cold or hot spots. Checking of the temperature 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 hot water treatment facilities and protocols for control of diseases and pests have been developed by SRA from many years of experience.

HOT-WATER TREATMENT 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

Circulation of the water in the tank is critical to prevent cold or hot spots. The general rule is that the circulation pump must have sufficient capacity to circulate the entire volume of water in the tank at least six times per hour.

The inlet pipe should be located at the base of the tank under the false floor or under supports that hold the cane stalks. A suitable screen should be in place to prevent blockages in the circulation system by leaf and stalk material. Water is returned to the tank via pipes located at the top of the tank.

Heating

The most common form of heat supply for the hot-water treatment tanks is steam that is often supplied from a sugar mill. Alternatively, electrical elements or gas or oil furnaces heat some facilities. In all cases, sufficient heating should be supplied to maintain the water at the required temperature, which is usually 50°C. Facilities that rely on steam from the sugar mill are restricted to operation while the mill is operating and, if a mill breakdown occurs during the treatment, the load may be lost.

The heating elements or steam injection pipes can be placed at the bottom of the tank or can be 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 can be used, as long as the structure of this aid does not impede the circulation of the water. Baskets should be made with mesh with holes that are as large as possible to allow water movement, but small enough to prevent cane stalks or cuttings from passing through the holes. Often facilities have a winch to load and remove the sugarcane.

Temperature control

Automatic control of temperature is essential for effective operation of the facilities. The temperature control mechanism should aim to maintain the temperature at 50 ± 0.2 °C. If the temperature is elevated above this range for extended periods, it will cause poor

germination. If the temperature drops below this range for extended periods, disease control will be less effective.

Loss of heat from the top of the tank can be reduced by a lid but in warm climates and with tanks with adequate heating capacity this is not always necessary. Insulation around the sides of the tank can also reduce the loss of heat. Reducing airflow around the tank by enclosing the tank in a shed, or with walls in the direction of the prevailing wind, will help prevent heat loss.

Temperature monitoring

The temperature in the tank should be monitored with a suitable chart recorder throughout all treatments. On a regular basis, the distribution of temperature in various positions in the tank should be monitored to ensure that the circulation is adequate and that there are no hot or cold spots. This can be done with an electronic data logger fitted with suitable thermocouples. The accuracy of all temperature recording devices including thermometers must be checked to ensure that the treatments are made at the correct temperature.

Water quality

The water used to fill the tank 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. If the water is not changed regularly, it will start to ferment from the sugars leached from the cane and the water may have a deleterious effect on germination of the treated cane.

In the past in some countries, fungicides have been added to the water in the tanks to protect the cane when it is planted after treatment. This is not necessary and the disposal of the large volume of fungicide solution in the tank is an environmental problem.

HOT-WATER TREATMENT PROTOCOLS FOR DISEASES AND PESTS

RATOON STUNTING DISEASE

Hot-water treatment is most often used to control ratoon stunting disease (RSD). RSD is caused by the bacterium *Leifsonia xyli* subsp. *xyli* and is widely distributed in nearly all sugarcane-producing countries of the world. The disease can cause losses of 5-60% and is spread by planting infected cuttings and by cutting implements such as cane knives, harvesters and planters. Hot-water treatment of infected stalks of sugarcane at 50°C for 3 hours gives greater than 99% control of this disease (LHWT). In some countries, the treatment is reduced to 50°C for 2 hours, but research in Australia suggests that the control of the disease is not as effective at this shorter treatment time.

Selection of cane for treatment

The cane to be treated should be as free of RSD as possible. If the cane is 100% infected with RSD, there is a risk that a small percentage of escapes may occur and the residual disease can spread the disease to other plants when they are cut for further propagation. If there is no alternative to using known diseased cane, it is recommended that the cane be treated in two consecutive years before it is used as a disease-free plant source.

To improve the germination of the hot-water-treated cane the source of the cane should be selected carefully. The cane should be well grown and reasonably mature (preferably close to 12 months of age). The buds and root primordia should be in good condition and cane affected by stem rots or insect damage should be avoided.

Presentation for hot-water treatment

The dead leaves (trash) on stalks to be hot-water treated can be removed, but this is not essential. Dead leaves can cause blockages in the circulation system, so care should be taken to clean screens in the circulation system more regularly if cane is treated with leaves attached.

The most common method of treating cane for control of raton stunting disease 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 ends of the tank.

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

Treatment

The treatment for raton stunting disease is 50°C for 3 hours. Timing commences as soon as the cane is placed in the tank. If the time of treatment is extended past 3 hours, poor germination may be experienced. Maintaining the temperature at 50 ± 0.2 °C is essential for disease control and good germination.

Initially the tank should be heated to 51-52°C and the cane should be lowered into the tank, ensuring that all cane is fully submerged. The temperature will drop when the cane is lowered into the tank and should be returned to 50°C as quickly as possible. The thermostat should be set at exactly 50°C and this must be checked against the reading of the check thermometer in the tank and adjusted until the check thermometer is reading 50°C. The check thermometer should be checked regularly (10-20 minutes) throughout the treatment and adjustments made to the thermostat if necessary.

Post-treatment

When the cane is removed it should be cooled as quickly as possible by spraying the cane with water at ambient temperature. The buds on the cane after removal from the tank are soft and the cane should be handled as little as possible until the cane is cooled. The cane can be planted once it has cooled, but some people 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 should be avoided if possible.

The cane setts should be dipped or sprayed with a fungicide after treatment (eg propiconazole) to protect them from sett rots. Hot-water-treated cane is particularly susceptible to sett rots.

The soil should be in ideal condition for planting sugarcane to maximise the chances of successful germination. Ideally, cane should be hot water treated when the soil temperature and moisture are ideal for germination to provide good germination conditions.

Management of hot-water-treated cane

All implements that could cut the leaves or stalks of the sugarcane must be sterilised before entering the hot-water treatment plot. To sterilise implements they should be thoroughly washed and sprayed with 70% methylated spirits or 1% quaternary ammonium disinfectant. Quaternary ammonium disinfectants require a 5-minute contact time to give 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 tank to 51-52°C.**
- 4. Load cane and treat for 3 hours at 50°C, commence timing when crate is loaded into the tank.**
- 5. Cool cane quickly at the end of the treatment by spraying with cool water.**
- 6. Handle cane as little as possible until it is thoroughly cooled; cane should be left for 1-2 days before planting.**
- 7. Spray or dip cuttings with a suitable 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 the plot of hot water treated cane.**

LEAF SCALD

Leaf scald is a disease cause by the bacterium, *Xanthomonas albilineans*. It can cause complete death of susceptible cultivars, and is spread by planting infected cuttings, strong wind-blown rain and cutting implements. Research has shown that hot-water treatment alone does not control this disease. For effective control, the cane must first be soaked in water at ambient temperature for 40-48 hours and followed immediately after by hot-water treatment for 3 hours at 50°C (CS-LHWT). Leaf scald is difficult to control by hot-water treatment and to improve the control care must be taken to maximise circulation in the tank by carefully stacking the cane in layers separated by spacers. Treated cane should be planted in an area that is unlikely to be reinfected from infected sugarcane or infected alternative hosts.

Selection of cane for treatment

The cane to be treated should be as free of leaf scald as possible. If there is no alternative to using known diseased cane, it is recommended that the cane be treated in two consecutive years before it is used as a disease-free plant source.

To improve the germination of the hot-water-treated cane, the source of the cane should be selected carefully. The cane should be well grown, reasonably mature (preferably close to 12 months of age). The buds and root primordia should be in good condition and cane affected by stem rots or insect damage should be avoided.

Presentation for hot water treatment

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

Treatment

The treatment for leaf scald is to soak the cane for 40-48 hours in water at ambient temperature followed by hot water treatment at 50°C for 3 hours. During the cold-soak period, the water should be slowly replaced by slowly running fresh water to prevent stagnation of the water, which can affect germination. Extending the cold-soak period beyond 48 hours should not affect the treatment, but cane left in water for more than 48 hours will begin to shoot and produce roots. The hot-water treatment should ideally commence immediately after the cane is removed from the cold-soak tank, but a short delay of a few hours should not affect the treatment. Timing of the hot-water treatment commences as soon as the cane is placed in the tank. If the time of treatment is extended past 3 hours, poor germination may be experienced. Maintaining the temperature at $50 \pm 0.2^\circ\text{C}$ is essential for disease control and good germination.

Initially the tank should be heated to 51-52°C and the cane should be lowered into the tank ensuring that all cane is fully submerged. The temperature will drop when the cane is lowered into the tank and should be returned to 50°C as quickly as possible. The thermostat should be set at exactly 50°C and this must be checked against the reading of the check thermometer in the tank and adjusted until the check thermometer is reading 50°C. The check thermometer should be checked regularly (10-20 minutes) throughout the treatment and adjustments made to the thermostat if necessary.

Post-treatment

When the cane is removed, it should be cooled as quickly as possible by spraying the cane with water at ambient temperature. The buds on the cane after removal from the tank are soft and the cane should be handled as little as possible until the cane is cooled. The cane can be planted once it has cooled, but some people 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 should be avoided if possible.

The cane setts should be dipped or sprayed with a fungicide after treatment (eg propiconazole) to protect them from sett rots. Hot-water treated cane is particularly susceptible to sett rots.

The soil should be in ideal condition for planting sugarcane to maximise the chances of successful germination. Ideally, cane should be hot-water treated when the soil temperature and moisture are ideal for germination to provide good germination conditions.

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

Management of hot water treated cane

All implements that could cut the leaves or stalks of the sugarcane must be sterilised before entering the hot-water treatment plot. To sterilise implements, they should be thoroughly washed and sprayed with 70% methylated spirits or 1% quaternary ammonium disinfectant. Quaternary ammonium disinfectants require a 5-minute contact time to give effective control.

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 (eg 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 tank to 51-52°C.**
- 5. Load cane and treat for 3 hours at 50°C, commence timing when crate is loaded into the tank.**
- 6. Cool cane quickly at the end of the treatment by spraying with cool water.**
- 7. Handle cane as little as possible until it is thoroughly cooled; cane should be left for 1-2 days before planting.**
- 8. Spray or dip cuttings with a suitable fungicide.**
- 9. Plant in an area well separated from known sources of leaf scald disease.**
- 10. Plant cane when conditions are ideal for germination. Good soil tilth, ideal soil moisture and temperature.**
- 11. Protect cane from reinfection by sterilising all cutting implements used in the plot of hot-water-treated cane.**

SMUT

Smut, caused by the fungus Sporisorium scitamineum, is a serious disease of sugarcane that was recorded for the first time in Australia in 1998 in the Ord River Irrigation Area and was subsequently found in Queensland in 2006. The disease can cause complete crop loss in susceptible varieties. It can be spread by wind-borne spores that are produced in fruiting bodies known as whips. The spores can travel long distances on the wind and can infect buds on standing stalks, buds on stalks planted into infested soil and young developing shoots or tillers in plant and ratoon cane.

For maximum control of smut, cane should be hot-water treated in bundles at 52°C for 45 min. This treatment is recommended when moving cane from a smut-infested area to a non-infested area.

For general smut control within a smut-infested area, cane should be hot-water treated in bundles 52°C for 30 min.

The long-hot-water treatments used for RSD and leaf scald are also effective at controlling smut.

After hot-water treatment, smut can re-infect treated cane if the cane is planted in soil that is infested with smut spores or smut spores land on the cane buds before they are planted or young tillers in the plant crop. Some intermediate-to-resistant varieties actually become more susceptible to smut after hot-water treatment. Fungicides can be used to reduce re-infection for a number of months after planting, and it is recommended that all hot-water treated cane be treated with a fungicide registered for the control of sugarcane smut.

Fungicides can also be used to reduce infection in healthy planting material of intermediate varieties.

Registrations are available for two fungicides for the treatment of cane stalks for control of smut:

- propiconazole (NUFARM THROTTLE);
- 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 spray through a planting machine.

CHLOROTIC STREAK, DOWNY MILDEW AND INSECT PESTS

The agent which causes chlorotic streak is particularly sensitive to heat and can be eliminated completely by treatment at 50°C for 30 minutes. The cause of this disease is unknown. This short hot-water treatment (SWHT) does not control ratoon stunting disease or leaf scald. This treatment improves the germination of sugarcane.

Short-hot-water treatments of 50-52°C for 20-30 minutes are widely used to kill insect pests when cane is being moved from one area or country to another.

Downy mildew, which is caused by a fungus, can be almost completely eliminated by hot-water treatment at 50-52°C for 30 minutes with little effect on germination.

The requirements for treatment of these diseases are the same as those given for ratoon stunting disease above, except that the treatment time is reduced to 20-30 minutes. Temperatures of 50-52°C can be used to control these diseases.

APPENDIX 3 - RSD sampling procedures

(a) Selection of stalks

Stalks should be sampled at random from throughout the plot. The largest stalks in poorly grown stools (possibly poorly grown due to RSD) should be selected where there is uneven crop growth.

(b) 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 at 10% infected stools, you would need 29 samples; 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. Approved-seed plots 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.

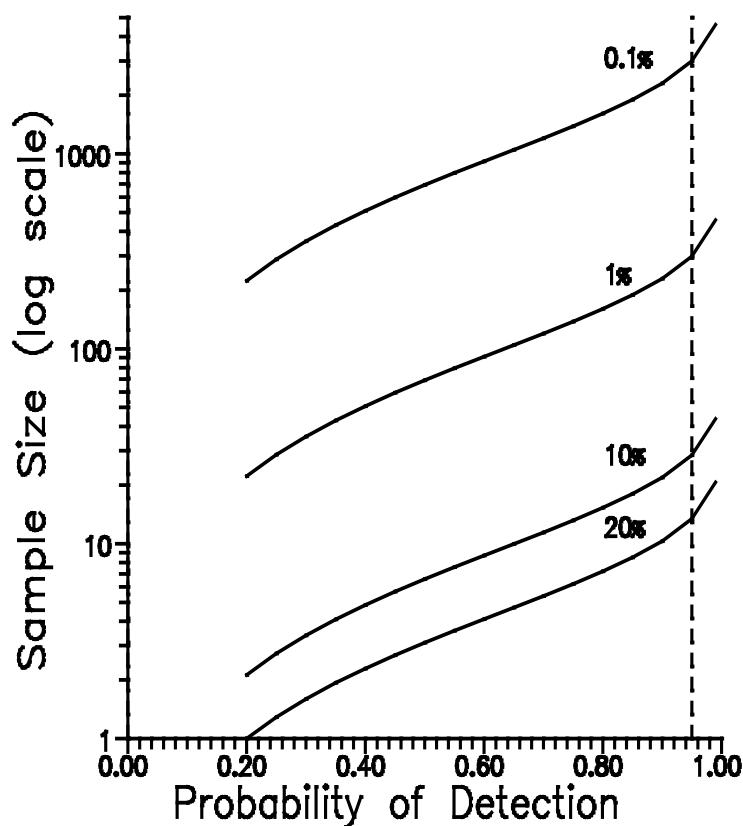


Figure 1 Probability of detecting a positive with different sample sizes

(c) Section of the stalk to sample

Extracts for RSD diagnosis should be taken from towards 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 collection of extract.

(d) Bulking of samples

Extracts can be combined together to reduce the number of samples to be tested. However, this may reduce the chance of detecting the disease. For example, if 1 diseased extract is mixed with 9 healthy extracts, the bacterial concentration is 1/10 of that which was present in the diseased sample. It is possible that the diagnostic test may not be sensitive enough to detect the lower concentration of bacteria in the mixed sample. As a general rule, it is suggested that no more than 4 extracts from individual stalks should be mixed together.

(e) Equipment required

1. 1 mL titertubes and caps and 96-place storage boxes.
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, must be sharp, or similar.
5. Esky with cooler block.
6. Methylated spirits and cleaning rags.

(f) 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 section of stalk with one node from towards the base of the stalk. Cut one end square and one end at a 45° angle. If the stalk is dirty, clean the tip of the angled end and avoid getting dirt on 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 flat end of 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 four stalks in the one tube. Collect approximately 0.6-0.8 mL of extract (minimum acceptable is 0.3 mL). Do not completely fill tubes, since when frozen the caps will be dislodged. Add one drop of Savlon Antiseptic solution or 30% formaldehyde as a preservative and firmly attach a cap to the tube.

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 tube clearly with sample number and place in a 96-well storage box. Complete a record sheet to show position in the box and the block number, farm and district.
6. Clean and disinfect secateurs or lopping shears and rubber holder between plots by wiping off organic matter and swabbing or spraying with methylated spirits.
7. Freeze samples on return to 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. Include a copy of the record sheet that shows the layout of the samples in the box. Notify the laboratory that samples have been dispatched. The address and contact details are:

Sugar Research Australia
50 Meiers Road
Indooroopilly QLD 4068

Attn. Amanda Johnson

Phone : +61 7 3331 3333

Email: ajohnson@sugarresearch.com.au

APPENDIX 4 - Guidelines for leaf sampling for DNA fingerprinting

- Select the top most leaf and remove the midrib. The leaf should be free of any disease symptoms.
- Cut a small length (10-15 mm) and place in the storage tube supplied by the laboratory nominated by SRA Limited to conduct the test.
- The number of samples to collect will vary depending on the size of the plot, and whether mixing of varieties is suspected. If mixing of varieties is suspected, collect samples from throughout the plot and collect different types in separate tubes.
- Send the samples by overnight courier to the address of the laboratory nominated by SRA Limited to conduct the test.
- For details on sampling for DNA fingerprinting including storage tubes, and the address of the nominated laboratory contact the local SRA Variety Officer.