REFEREED PAPER

EFFICACY OF MECHANICAL HARVESTER DECONTAMINATION PROCEDURES TO REDUCE THE RISK OF RATOON STUNT (RSD) SPREAD

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Abstract

Ratoon stunt (RSD - *Leifsonia xyli* subsp. *xyli* - *Lxx*) is considered to be a manageable disease, provided the recommended integrated management strategy is followed. Once a field has been planted, the main risk of spread is at harvest. While it is possible to easily and effectively decontaminate cane knives during the harvesting operation, mechanical harvesters pose more of a challenge. An effective disinfectant and a suitable method are required to decontaminate mechanical harvesters and to minimise the risk of RSD spread, particularly between fields and farms. A foaming quaternary ammonium compound (QAC), containing benzalkonium chloride and didecyl dimethyl ammonium chloride, was found to be effective against *Clavibacter michiganensis* (used as an indicator bacterium) and *Lxx* in *in vitro* and *in vivo* experiments, respectively. This disinfectant was identified as a suitable alternative to the current industry standards, Jeyes Fluid and methylated spirits, for minimising the risk of RSD spread on farm implements. Three field trials confirmed that mechanical harvesters transmit RSD from infected to healthy stools at harvest. The procedure used to decontaminate the harvesters took two people up to 40 minutes to complete, and while it reduced spread into healthy cane in the trials, it did not eliminate the risk completely. Management strategies to accommodate this risk will need to be implemented.

Keywords: ratoon stunt, RSD, *Leifsonia xyli* subsp. *xyli*, mechanical harvester, decontamination, disinfectant

Introduction

Ratoon stunt (RSD), caused by *Leifsonia xyli* subsp. *xyli* (*Lxx*), is widespread in South Africa and is estimated to cause annual losses of over R50 million (McFarlane *et al*., 2018). It is an insidious disease with no obvious external symptoms and can be inadvertently spread by planting infected seedcane (Steindl, 1961; Egan, 1980; Roach, 1987) or from infected to healthy plants on contaminated farm implements and machinery, such as cane knives (Steindl, 1950; Bailey and Tough, 1992; Comstock *et al*., 1996), planters, and mechanical harvesters (Taylor *et al*., 1988; Damann, 1992; Hoy *et al.,* 1999). Bacterial populations in xylem sap increase as the cane matures (Gillaspie and Teakle, 1989; Grisham, 1991), so the most important periods of spread occur at planting and harvest (Hoy *et al*., 1999). Disease incidence within fields increases with each harvest (Grisham, 1991; Bailey and Tough, 1992).

Ratoon stunt is considered to be a manageable disease, provided the recommended integrated management strategy is followed: primarily planting healthy seedcane from a certified or approved source, decontaminating farm implements, and fallow periods that allow sufficient time to identify and remove volunteers from the previous planting. Volunteers can be an important source of inoculum in newly-planted fields (Gillaspie and Teakle, 1989). It is relatively easy to effectively decontaminate cane knives (Bailey and Tough, 1992) and, provided recommendations are followed, the risk of spread on cane knives between fields is low. Unlike diseases such as smut and mosaic, RSD is not spread by wind, rain or insect vectors (Gillaspie and Teakle, 1989) and should therefore not pose a risk to neighbouring farms. While the use of cane cutters through contractors increases the risk of farm-to-farm spread, this is minimal if time is taken to decontaminate their cane knives. Jeyes Fluid [carbolic acid, 10% (v/v)] and methylated spirits (75% v/v) are currently recommended for this purpose in South Africa (Bailey and Tough, 1992).

Mechanical harvesting is being practised in some areas in South Africa. Research in Australia and the USA indicated that RSD spread by mechanical harvesters is rapid (Taylor *et al*., 1988; Damann, 1992; Hoy *et al*., 1999). There is therefore increased risk of field-to-field spread on growers' farms in South Africa. Of more concern, is the increased risk of spread into Certified and Approved Seedcane nurseries and farm-to-farm spread. In Australia, spread was minimised by decontaminating the harvester after cutting infected cane ([1]unpublished data, PWJ Taylor). A similar approach was followed to that which was effective for the decontamination of mechanical planters (Croft and Kaupilla, 1989). The procedure involved decontaminating all accessible parts of the harvester that came into direct contact with freshly cut stubble, or where infected juice could accumulate and drip onto the cane row. These parts identified as being a source of contamination were washed with tap water under high pressure before applying a quaternary ammonium compound (QAC) containing benzalkonium chloride (0.1% v/v) with a knapsack. These compounds are effective against Gram positive bacteria, such as *Lxx,* and benzalkonium chloride is recommended for the decontamination of farm implements in other sugar industries (Gillaspie and Teakle, 1989).

This study investigated the efficacy of the harvester decontamination procedure recommended in the Australian sugar industry. Alternatives to the disinfectants that are currently being used in the South African industry (Jeyes Fluid and methylated spirits) were also tested.

Materials and Methods

Disinfectants - laboratory assays

Three formulations containing quaternary ammonium compounds (QAC) with their constituent surfactants, and that are registered for use in the food and agricultural industries in South Africa, were tested *in vitro* (Table 1). A new disinfectant, with a proprietary blend of various sulfate salts and bioflavonoids, was included in the study. Since *Lxx* is not suitable for *in vitro* assays due to its poor growth in culture, closely related *Clavibacter michiganensis* was used as an indicator bacterium.

Table 1. Registered quaternary ammonium compounds tested against *Clavibacter michiganensis* in use-dilution assays

Code	Active ingredient	Proportion of formulation (%)	Recommended rate (label)	LD 50 (concentrate)
QAC 1	Benzalkonium chloride (C10-16-Alkyl dimethyl benzyl ammonium chloride) Didecyl dimethyl ammonium chloride	6.4	3%	>2000mg/kg
QAC 2	Didecyl dimethyl ammonium chloride	12.6	0.15%	>3000mg/kg
QAC 3	Didecyldimethyl ammonium chloride	12.0	1%	>4000mg/kg

A use-dilution test was used to assess the efficacy of the disinfectants (Risser, DD https://scholarlycommons.pacific.edu/open-textbooks/5). Nutrient broth was inoculated with *C. michiganensis* and incubated at 30ºC for 3 days or when turbidity (OD_{600}) exceeded 0.5 nm using a spectrophotometer (Biotek, Model Synergy HT). Sterile glass beads were aseptically dipped into the culture and dried on sterile filter paper in a laminar flow for approximately 40 minutes. The selected QAC disinfectants were tested at four concentrations [0.06, 0.1, 0.3 and 0.6% by mass of active ingredient/s in the formulation) using sterile deionised water, and 1 mL aliquots were transferred to sterile 1.5 mL microfuge tubes. The proprietary mix was used at the recommended concentration of 0.55% (w/v). A single coated bead was transferred to a tube with disinfectant and left for 5 minutes before transferring to a fresh tube of sterile nutrient broth. This was repeated for all disinfectants at all concentrations. Two standards, Jeyes Fluid (carbolic acid – 10% v/v) and ethanol (70% v/v) were included, along with a water control with no disinfectant. The treatments were replicated five times. The tubes containing the beads were incubated at 30ºC and turbidity (OD_{600}) was measured after 5 days. A sample (20 µL) was taken from each tube and streaked onto nutrient agar to confirm the presence/absence of *C. michiganensis*. The experiment was repeated. To determine the contact time required for effective disinfection, the experiment was repeated using two concentrations of each QAC disinfectant (0.06 and 0.6% by mass of active ingredient/s in the formulation) and three specified soak times (dip, 1 min and 5 mins). The inoculated beads were soaked in the proprietary mix for 5 minutes only.

Pot experiment

The efficacy of the QAC disinfectants was tested in a pot experiment. Infected stalks of N14, a variety that supports high populations of *Lxx*, were cut with secateurs to contaminate the blades. The blades of the secateurs were then soaked in the disinfectants (3% v/v) for 5 minutes. Jeyes Fluid (10% v/v) and ethanol (70% v/v) were included as standards and the secateurs were soaked in water for the control treatment. The secateurs were then used to cut single budded setts from stalks of N14 that were confirmed to be *Lxx*-free using immunofluorescence microscopy (IFM) (Harris and Gillaspie, 1978). The treatments were replicated ten times. The setts were transferred to seedling trays and germinated in the glasshouse at 28-30ºC. The plants were transferred to 25 L pots after 2 months and grown in the glasshouse for a further 13 months, before testing for the presence of *Lxx* using IFM.

Mechanical harvester field trials

Three trials were conducted to test the efficacy of the decontamination procedure. For all trials, a similar procedure was followed: The first ~10 m of each selected cane row of an established field was removed and replaced with a spreader section of RSD-infected N14 transplants. A 2 m gap was left between the end of each spreader section and the corresponding cane row. Intensive testing using IFM, confirmed that the rows used for each trial were RSD-free before harvest. For the positive control rows, the harvester cut the RSD-infected spreader section

before moving into the healthy cane with no decontamination (Figure 1). For the decontamination/test rows, the harvester cut the spreader section and was then decontaminated before moving into the healthy cane.

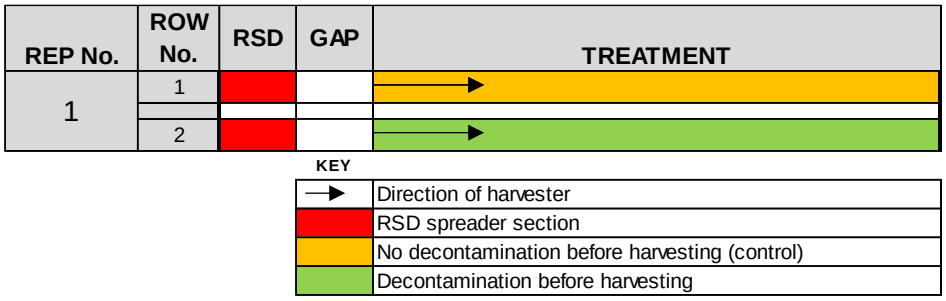

Figure 1. Schematic of the procedure followed for the mechanical harvester trials

The decontamination procedure involved removing, by hand, as much plant and soil debris from the harvester as possible. Focus was placed on all accessible parts of the harvester that were likely to come into contact with the harvested cane row, or where the infected juice could accumulate and drip onto the cane row. This included the base-cutters, crop dividers, throat, rollers, butt-lifter, boot, chopper box, elevator and topper. Finer debris was removed from these parts with water sprayed under high pressure from a 200 L fire cart. QAC1 (benzalkonium chloride and didecyl dimethyl ammonium chloride) was applied at a concentration of 3% (v/v) with a knapsack to all these parts, with a contact time of 5 minutes. When the cane was at least nine months old, one stalk was collected from the first 20 stools of the harvested cane rows. Each stalk was numbered sequentially and tested for RSD using IFM.

Trial 1: Komati Research Station (N49): The tramline spacing did not match the harvester configuration, requiring alternate tramlines to be cut by hand to allow the harvester to move down the rows, reducing the size of the trial. The RSD-infected spreader sections were planted as tramlines (10 transplants per tramline). The trial was harvested on 30 August 2017 with a John Deere CH570 harvester. Three positive control rows and two test rows were harvested.

Trial 2: Komati Research Station (N25, N41): The cane in this trial was planted as single rows. The RSD-infected spreader sections in Trial 1 were used to contaminate the harvester before cutting each row in Trial 2. Treatments were replicated five times. The trial was harvested on 21 August 2018 with a John Deere CH570 harvester.

Trial 3: Bruyns Hill Research Station (N39): The cane in this trial and the RSD-infected spreader sections were planted as single rows. A Claas self-propelled harvester was used, which cut two rows with each pass. The trial was harvested on 25 September 2018. In this trial, the decontamination treatment was replicated three times, while the positive control was not replicated. Strong winds caused the cane to lodge before sampling, and only the first five stools of each row were sampled.

Results and Discussion

Disinfectants – laboratory assays

The three QAC disinfectants were as effective against *C. michiganensis* as the standards at the four concentrations tested *in vitro* (Figure 2a). No growth was detected in the test tubes with the inoculated, disinfected beads after 5 days, or on the agar plate post-test. The proprietary mix was less effective than the QACs and the standards in disinfecting the beads in these experiments, although the OD_{600} was significantly lower than the control (P<0.001) (Figure 2b).

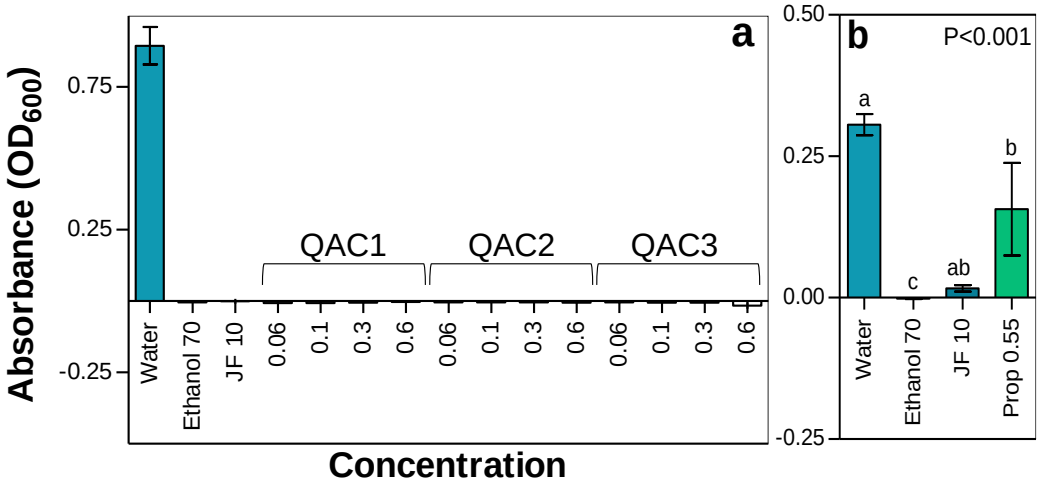

Figure 2. Efficacy of a) QAC disinfectants (QAC 1: Benzalkonium chloride + Didecyl dimethyl ammonium chloride, QAC 2 and QAC 3: Didecyl dimethyl ammonium chloride) used at four concentrations (% mass of active ingredient/s in each formulation), and b) a propriety mix (100% v/v), on the growth of *Clavibacter michiganensis*. Standards: Ethanol (70% v/v) and Jeyes Fluid (10% v/v); Control: Deionised water. Contact time 5 minutes. Different letters above the bars in Figure 2b indicate significant differences between treatments.

The QACs were as effective as the standards when applied as a dip and when the beads were soaked in the disinfectants for 1 and 5 minutes (Figure 3). The standard contact time for QACs is 5 minutes and this would be recommended for the decontamination of farm implements, to ensure their efficacy in a field situation.

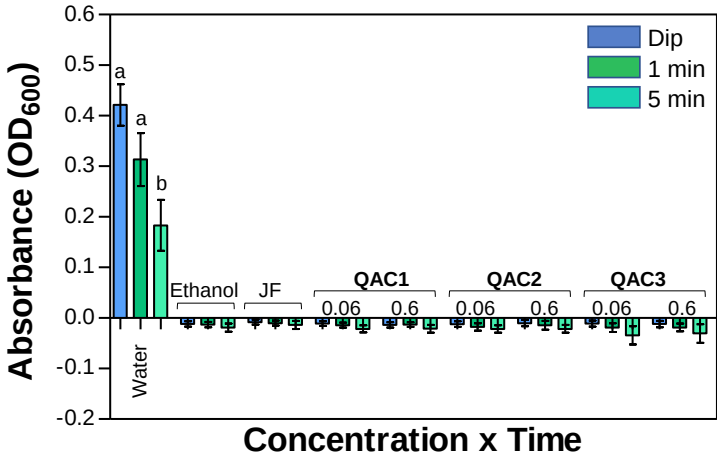

Figure 3. Efficacy of QAC disinfectants (QAC 1: Benzalkonium chloride + Didecyl dimethyl ammonium chloride, QAC 2 and QAC 3: Didecyl dimethyl ammonium chloride) used at two concentrations (% mass of active ingredient/s in each formulation) on the growth of *Clavibacter michiganensis*. The disinfectants were applied to inoculated beads at three specified soak times (dip, 1 min and 5 mins). Different letters above the bars indicate significant differences between treatments.

Disinfectants – pot trial

In the pot trial, *Lxx* was detected in 70% of the plants in the water control treatment and in 10% of the plants in the Jeyes Fluid treatment. Plants in the ethanol and QAC 1 treatments tested negative. *Leifsonia xyli* subsp*. xyli* was detected in both the QAC 2 and QAC 3 treatments, despite the concentration of the disinfectants being higher than the recommended rate. Benzalkonium chloride is used effectively in other sugarcane industries to manage RSD (Croft, 1989; Gillaspie and Teakle, 1989). The formulation containing a combination of benzalkonium chloride and didecyl dimethyl ammonium chloride (QAC1) will be recommended as an alternative to Jeyes Fluid and methylated spirits. CropLife has confirmed that the sugar industry does not require specific registration of this chemical for use as a disinfectant on farm implements and machinery.

Harvester field trials

In Trial 1, RSD was detected in all positive control rows and in one stool in one test row (Figure 4). The results were similar to the reports from Australia, where infection ranged from 0 to 67% of the first seven stools in the positive control rows (Taylor *et al*., 1988). The transmission of *Lxx* extended as far as ten stools along the row in those trials.

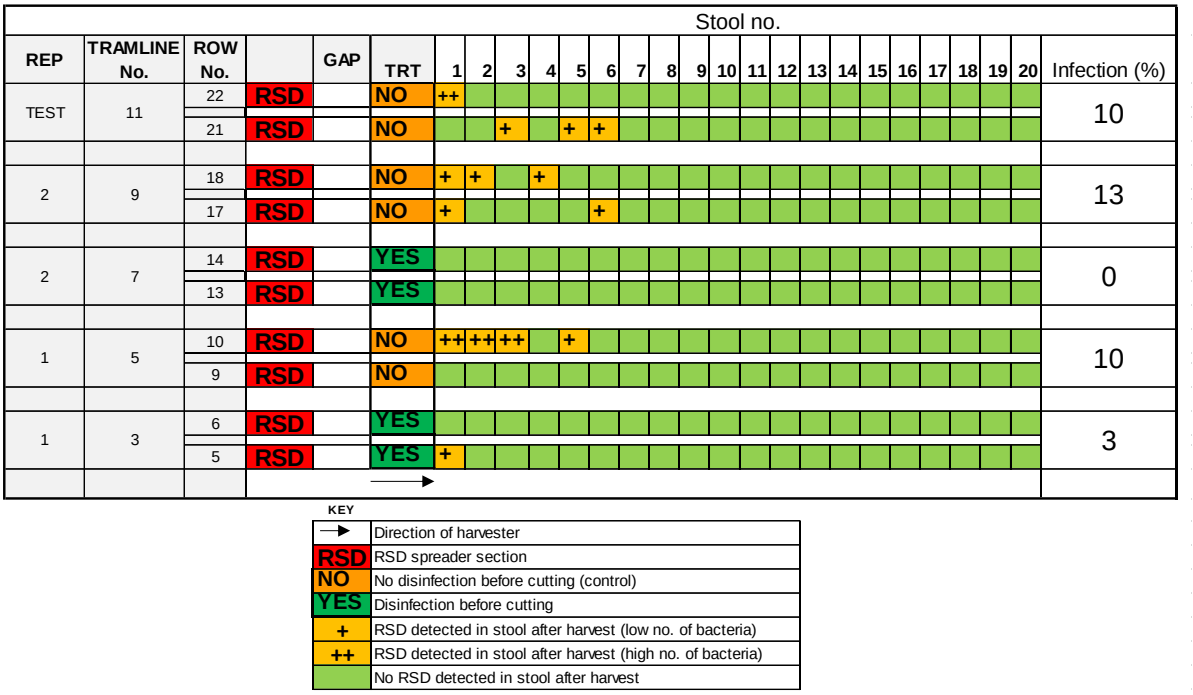

Figure 4. RSD incidence in previously healthy rows of N49, with or without the decontamination of the mechanical harvester, after harvesting RSD-infected cane.

Transmission was limited in Trial 2, with RSD being detected in only two stools in one of the five positive control rows (Figure 5), which made it difficult to assess the efficacy of the decontamination process.

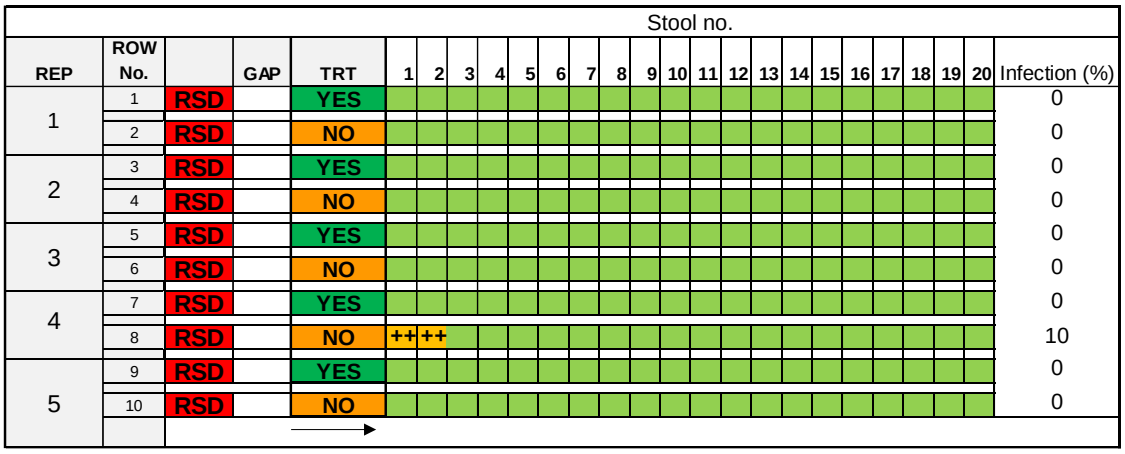

Figure 5. RSD incidence in previously healthy rows of N25 and N41, with or without the decontamination of the mechanical harvester, after harvesting RSD-infected cane (see key in Figure 2).

A similar case of limited transmission was reported in one trial conducted in Australia, where no RSD was detected in some positive control rows (unpublished data, PWJ Taylor). This was attributed to low (undetectable) bacterial populations in the selected stalks. Researchers in the USA reported variability between sites, years and variety (Hoy *et al*., 1999). In those trials, the distance of RSD transmission ranged from 0.2 to 14 m from the inoculation source. Varietal susceptibility is known to affect the rate of RSD transmission on cane knives (Gillaspie and

Teakle, 1989; Bailey and Tough, 1992) and harvesters (Damann, 1992). However, both N25 and N41 included in this trial are susceptible to RSD and support high numbers of bacteria (unpublished data, SA McFarlane). Spread into these varieties was therefore expected. Hoy *et al.* (1999) suggested that environmental conditions may affect the rate of transmission after the extent of spread in one of the eight susceptible varieties included in the trial was high in two trials, and low in the third.

Severe lodging after strong winds affected sampling in Trial 3, and only the first five stools in each row were sampled. RSD was detected in all the stools sampled in the two positive control rows. The decontamination procedure reduced, but did not eliminate, RSD spread in the test rows, with up to 50% of the sampled stools testing positive (Figure 6).

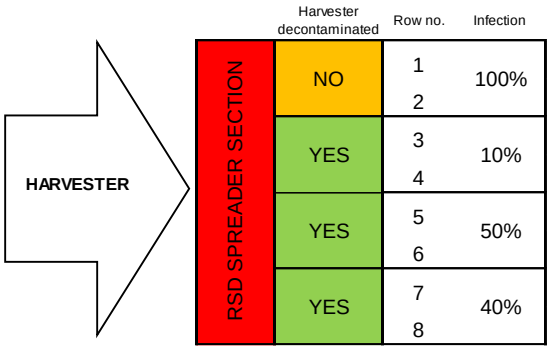

Figure 6. RSD incidence in previously healthy rows of N39, with or without decontamination of the mechanical harvester, after harvesting RSD-infected cane.

For all three trials, the decontamination procedure took two people between 28 and 40 minutes to remove as much plant and soil debris from the harvester as possible, to wash all critical and accessible parts with water and apply the disinfectant. This time included the required 5 minute contact period for the disinfectant. The fire cart, with the pump/delivery hose pressure set as high as possible, was fairly effective in washing the harvester, but it was not possible to remove all the plant and soil debris at the field edge. Once the harvester was back at the depot, a more thorough cleaning was possible. To improve the efficacy of the procedure, the decontamination of the harvester should be conducted at the depot or farm workshop, preferably while the harvester is raised on a trailer or in a designated area that allows easy access to the undercarriage. The use of a disinfection bath (a concrete sump), or high pressure decontamination sprays located at the depot or fitted to the trailer should be considered.

The trials confirmed that RSD is transmitted on mechanical harvesters. While RSD levels were lower in the test rows, indicating that it is possible to reduce spread by following the decontamination procedure recommended in Australia, the risk was not completely eliminated. Although this decontamination procedure is recommended in Australia, it is seldom practised due to the time it takes to complete, and the harvester operators are reluctant to lose this time between fields or farms (personal communication, BJ Croft). However, fields are replanted less frequently in South Africa than Australia. With more harvests, substantial RSD spread and increase is likely over time and the risk of significant yield reductions associated with severe infections is increased. For this reason, the decontamination of harvesters that are operating in the South African industry is recommended. Harvesters should not move between farms

without going through this process. The procedure should also ideally be followed before entering different fields on a farm. Based on the results of these trials, in-field decontamination of mechanical harvesters during the harvesting operation (i.e. stopping the harvester in the middle of a field to decontaminate) is unlikely to be effective. If done thoroughly, the process it time-consuming and access to some of the parts of the harvester will be more restricted in the field. The in-field decontamination of mechanical harvesters is therefore not recommended.

Studies in the USA indicated that the greatest risk of RSD spread and increase is at planting (Hoy *et al.,* 1999). Seedcane cut with mechanical harvesters has multiple points of entry for *Lxx,* and the risk of infection is high. The results from our trials, as well as those in the USA and Australia, highlight the risk of infecting seedcane sources during the harvesting process and introducing infection when planting new fields. Mechanical harvesters that are also assigned to harvest commercial fields during the season must therefore not be used to harvest Certified Seedcane, or ideally any other category of planting material.

Conclusions

The risk of RSD transmission by mechanical harvesters was confirmed. While the decontamination procedure reduced RSD spread, the risk was not completely eliminated. To improve the efficacy of the procedure, decontamination of the harvester should be conducted at the depot or farm workshop, preferably while the harvester is raised on a trailer or in a designated area that allows easy access to the undercarriage.

The decontamination procedure took two people between 28 and 40 minutes to remove as much plant and soil debris from the harvester as possible, to wash all the critical and accessible parts with water and to apply the disinfectant. This included the required 5 minute contact time.

A foaming quaternary ammonium compound containing benzalkonium chloride and didecyl dimethyl ammonium chloride, with a surfactant 2-butoxy-ethanol at a concentration of 3% (v/v), is proposed as a suitable alternative to Jeyes Fluid and methylated spirits.

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