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FACTORS AFFECTING GERMINATION AND GROWTH OF SUGARCANE TRANSPLANTS

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FACTORS AFFECTING GERMINATION AND GROWTH OF SUGARCANE TRANSPLANTS

by

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ABSTRACT

Transplants are produced and sold in South Africa for the planting of seedcane supply plots (nurseries), commercial fields, and for gap filling. The most important factor constraining the use of transplants is the low germination of single-budded setts (SBS) planted in polystyrene trays. The main aims of this project were to develop practical methods for optimising germination and to control pathogens without adversely affecting germination.

Seedcane quality, cane age, storage and treatments using heat, chemicals and fungicides affected germination and growth. Germination of SBS from old seedcane was significantly higher when taken from the top than from the middle and bottom of the stalk. Storage of seedcane for three and eight days after harvest adversely affected germination and growth. Topping of stalks three days before harvest increased germination potential, but results were variable, depending on cane age, cane quality and further treatments. Treatment of SBS at both 50°C for 120 minutes and 52°C for 30 minutes controlled *Clavibacter xyli* subsp. *xyli* (*C. x. xyli)* (the causal organism of ratoon stunting disease) more effectively than treatment of whole setts. After treatment of SBS at 52°C for 30 minutes, germination was greater than that after treatment at 50°C for 120 minutes, and *C. x. xyli* was eliminated from stalks of six out of seven varieties. Treatment of SBS at 52°C for 10 minutes significantly improved both germination and plant growth. Treatment of SBS for 10 minutes after addition of ethephon to the hot water significantly increased germination compared with the untreated control, but not compared with treatment with hot water alone. After treatment of SBS with fungicides, germination was highest after treatment with Eria® (Novartis), a chemical with two active ingredients, namely carbendazim and difenoconazole. Compared with no treatment and the short hot water treatment, treatment with Eria® in hot water (52°C) significantly improved germination and plant growth in both unsterilised and sterilised medium. Treatment of SBS and drenching of trays with a solution of propamocarb-HCl and benomyl had no effect on germination or growth, indicating the limited role of systemic infections and soilborne pathogens in germination failure. However, germination and growth were significantly increased

when the same SBS were also treated with Eria®, suggesting that germination was predominantly increased by the plant growth regulator activities of its active ingredients. When used separately, both difenoconazole and carbendazim significantly increased germination, and difenoconazole significantly increased plant growth. The conclusion drawn from these results is that germination failure of SBS in trays is mainly due to the inappropriate hormonal balance for germination within the SBS, rather than systemic infections or infection by soilborne pathogens. Therefore, germination and growth can be optimised by using mature, good quality seedcane, and by treatment of SBS with chemicals that adjust the hormonal balance in the bud region to one appropriate for germination.

PREFACE

The experimental work described in this dissertation was carried out at the South African Sugar Association Experiment Station Pathology Department from January 1995 to December 1996, under the supervision of Dr Mark Laing and Mr Roger Bailey.

These investigations represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is clearly acknowledged in the text.

Signed .. 

J.L. Goodall

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CONTENTS

LIST OF ABBREVIATIONS

AFP	air-filled porosity
ANOVA	analysis of variance
C+D	carbendazim and difenoconazole
C+F	carbendazim and flusilazole
CB	composted bagasse
CEC	cation exchange capacity
CPB	composted pine bark
C. x. xyli	*Clavibacter xyli* subsp. *xyli*
df	degrees of freedom
FC	filtercake
GA_3	gibberellic acid
HWT	hot water treatment
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
IFM	immunofluorescence microscopy
LSD	least significant difference
NAA	naphthalene acetic acid
PBS	½-phosphate buffered saline
PCM	phase-contrast microscopy
PDA	potato-dextrose-agar
PRS	poor root syndrome
RSD	ratoon stunting disease
SASEX	South African Sugar Association Experiment Station
SBS	single-budded sett
SHWT	short hot water treatment
WHC	water-holding capacity
WS	whole sett

INTRODUCTION

The South African sugar industry is one of the world's leading producers of high quality sugar. In the 1995/96 season, 404 143 hectares of land were under sugarcane, 288 980 hectares of which were harvested for milling. In this and the previous season, 1,667 million tons of sugar were produced, generating a direct income of approximately R2, 95 million (Anon., 1996).

Over 20 million tons of sugarcane is produced by 47 000 sugarcane growers in 16 cane producing areas, extending from Northern Pondoland in the Eastern Cape to the Mpumalanga Lowveld (Figure 1). Approximately 13% of the total crop is produced by more than 45 000 small-scale growers with an average plot size of 2 to 25 hectares, and more than 70% is produced by approximately 2 000 large-scale growers with an average farm size of 165 hectares. The remainder of the sugar is produced by milling companies, principally Illovo Sugar Ltd. and Tongaat-Hulett Sugar Ltd., who own 12 of the 16 sugar mills (Anon., 1996).

Sugarcane was first planted in South Africa on the KwaZulu-Natal North Coast in 1847. The South African Sugar Association Experiment Station (SASEX) was established in 1925, its chief function being to introduce, quarantine, screen and release new varieties. SASEX now serves as the centre for basic research, sugarcane breeding and advisory, development, extension, education and training services.

The commercial sugarcane varieties grown today are complex hybrids derived from crosses between two or more *Saccharum* species, including the noble canes *Saccharum officinarum* L. and the wild canes *S. spontaneum* L. (Daniels & Roach, 1987). *S. officinarum* is high in sucrose, but often susceptible to serious diseases such as mosaic, smut and red rot; whereas *S. spontaneum* displays high resistance to moisture stress, low temperatures and many sugarcane pests and diseases (Rao, 1989). In the past, many varieties were introduced to South Africa, but lost favour because of their susceptibility to pests and diseases, or their low sucrose yields (McMartin, 1948, 1958). Presently, varieties derived from local breeding programs are

screened and only released to growers if they have a high measure of general resistance to diseases and pests, as well as a high sucrose content and good agronomic qualities.

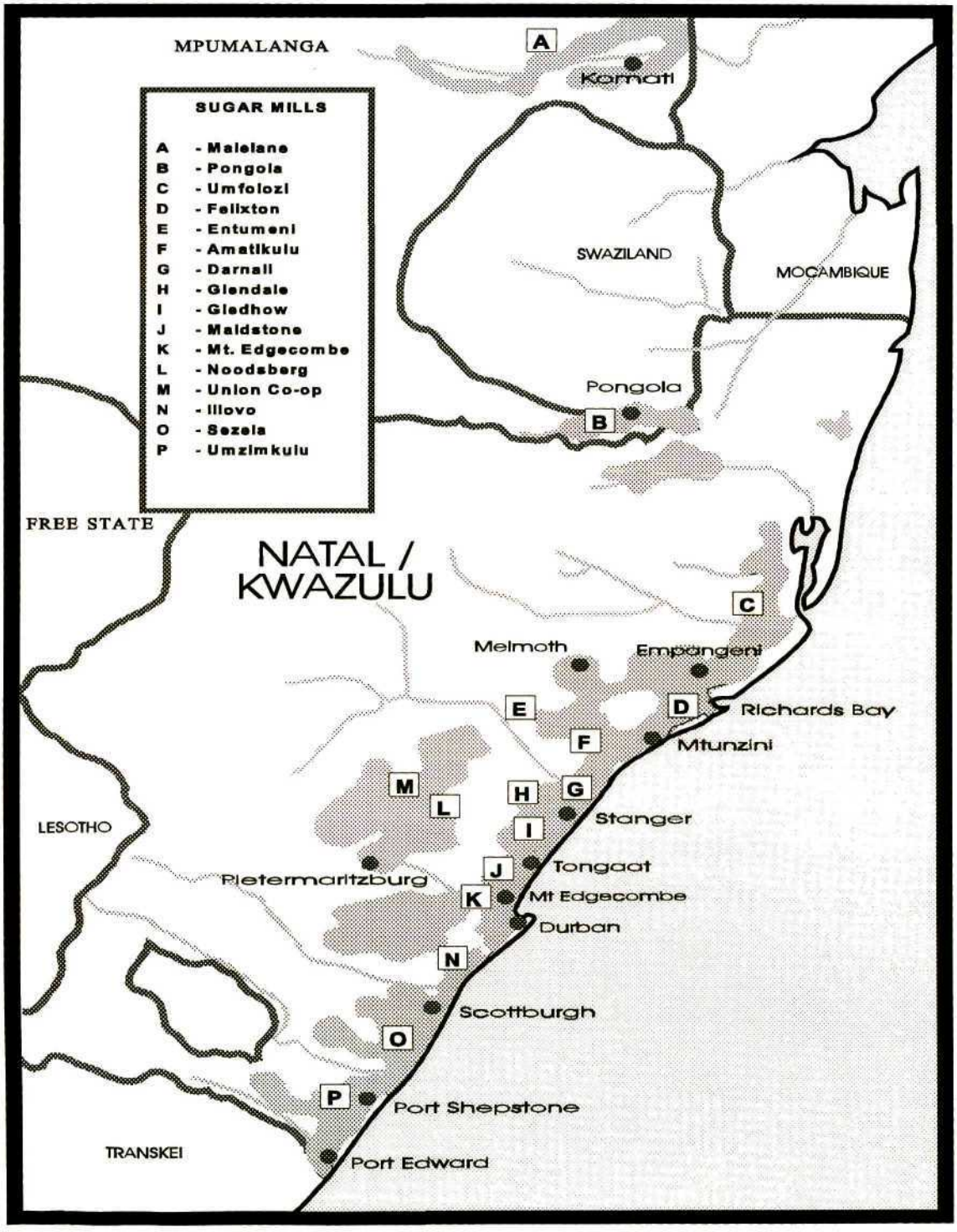

Figure 1 Sugar producing regions and mills in South Africa.

Commercially, sugarcane is vegetatively propagated using setts, which are often referred to as 'seed', 'seedcane' or 'seed material'. These are sections of stalk containing three to six lateral buds and are traditionally planted into furrows in the field. The buds on the stalk germinate to produce primary shoots that form stools after development of secondary shoots from basal buds (Julien *et al.*, 1989).

A new planting technique using transplants was introduced into South Africa in the early 1980s. This involves the cutting of stalks into single-budded setts (SBS) which are planted into polystyrene trays. Each bud and band of root initials is capable of producing a new plant that can be planted into the field. A transplant nursery was established at SASEX in 1986 to provide transplants for pathology trials, some plant breeding propagation plots and for bulking-up of new varieties (Anon., 1988). Currently, in the South African sugar industry, approximately ten million transplants are produced and sold annually by nurseries at four Illovo Sugar mills at Pongola, Sezela, Umfolozi and Gledhow, and several private nurseries.

Seedcane and transplants used for commercial planting must be of the highest quality to ensure profitable crop production. Ratoon stunting disease (RSD) is caused by the bacterium *Clavibacter xyli* subsp. *xyli (C. x. xyli).* This is the most economically important and widely distributed disease of sugarcane in South Africa, causing substantial losses in yields. Since this disease is systemic, it is important that healthy seedcane or transplants are planted into commercial fields to prevent further spread. Heat treatment of setts at 50°C for 120 minutes has been used as part of an integrated plan to reduce the levels of *C. x. xyli* in the South African sugar industry. Farmers are encouraged to establish their own seedcane nurseries with transplants grown from heat-treated setts to provide healthy seed material for commercial planting. In the Pongola mill area, transplants are occasionally used for planting of irrigated commercial fields, but where commercial fields are rainfed and irrigation systems are not used, the planting of transplants is not recommended because a drought period would inhibit or delay growth.

Other advantages associated with the use of nursery-grown transplants are that they can be used for rapid bulking up of healthy true-to-type seed material to provide good quality seedcane for nurseries, and for filling gaps in rows of cane grown from setts. Since far fewer transplants are required for field planting than seedcane required for the conventional planting method, a smaller truck capable of carrying a one ton load and less labour is required for the planting operation. Additionally, inspection and roguing of transplants are quicker and easier than that of conventionally planted seedcane (Thomas, 1984; Tucker, 1992; Mtshali, 1995).

The use of transplants is currently constrained by several factors. Firstly, SBS germination in most nurseries is poor, particularly when setts are heat treated to control *C. x. xyli*. Low germination rates increase production costs, discouraging sugarcane growers from buying and planting sugarcane transplants. In addition, production of transplants is not always cost effective because procedures such as tray preparation and hand placement of SBS into individual cells are labour intensive (Anon., 1988; Ingamells, 1989). Furthermore, cane from transplants may be initially less productive than cane grown from conventional sett planting, although yields in subsequent ratoon crops are comparable, particularly under irrigated conditions (McIntyre, 1993).

The aim of this project was to investigate factors influencing the successful production of sugarcane transplants. The emphasis was on developing practical methods of optimising germination of SBS in trays on a commercial scale, to make production quicker, easier and more economically viable, and to increase plant vigour so that stronger transplants can be planted into the field. A further aim was to find treatments that control RSD without adversely affecting germination. Transplant growers might then be more willing to heat treat setts because production costs would not be adversely affected by low germination rates.

A number of factors affecting transplants were examined in this study. These included seedcane quality to determine the effect of original bud position, cane storage before planting, and removal of the apical meristem a few days before harvesting, on germination of SBS in trays and subsequent growth of the transplants. Hot water treatment of SBS and whole setts at

50°, 52° and 54°C was examined to determine the effect of various treatments on germination, plant growth and control of *C. x. xyli*. In addition, short heat treatments and plant growth regulators were tested to determine which treatments could be used on a commercial scale to stimulate germination of SBS. Furthermore, various fungicides were tested to determine their control of sett and root pathogens, and their effect on germination and growth. Finally, various planting media were tested to determine their effect on plant growth.

Since some of these factors affected germination and growth more than others, a more in depth study was made in these areas. In most experiments, due to the large quantities of seedcane required, the preliminary experiments consisted of several treatments tested against two commercial sugarcane varieties. Since several commercial varieties are grown in transplant nurseries it is important that germination and growth of all are positively affected by treatment. Therefore, the best treatments from these experiments were subsequently tested against four to six commercial varieties to determine whether results were repeatable, and to examine the interaction between treatments and several commercial varieties.

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1. LITERATURE REVIEW

This literature review deals with all aspects of sugarcane transplant production. Sugarcane anatomy and physiology are discussed because they are crucial to the germination process. Pathogens inhibiting germination of sugarcane are discussed because they may adversely affect germination and growth of transplants in the nursery. Procedures used to improve germination of conventionally planted cane and ratoon cane are also discussed because they can also be applied to single-budded setts (SBS) for the production of transplants.

1.1 Anatomy of Germination and Growth

The sugarcane plant shows sympodial vegetative growth and survival is ensured by replacement adventitious buds emerging from the base of the plant (Rees, 1980). The stalk consists of nodes, internodes and leaves attached to the node. A lateral (axillary) bud, a band of root initials, a leaf scar and a wax band are present in the region of the node. Each lateral bud is an embryonic shoot which has the potential for development, and the outgrowth of which is referred to as 'germination'. Once the bud has germinated to produce a primary shoot, secondary shoots then develop from basal buds that in turn give rise to tertiary shoots to form the stool. Sett roots develop from primordia in the root band region under the influence of auxins that are present in the setts. After two to three months, the sett roots are completely replaced by shoot roots that develop from primordia on the lower portion of the developing shoots (Barnes, 1964 & 1974; Julien *et al.*, 1989). Buds may not germinate because of inhibition by apical dominance, physical injury, drying of the stem, excess water, poor nutritional status of the seedcane and infection of the setts by organisms that cause sett and root decay (Barnes, 1974).

1.2 Physiology of Germination and Growth

1.2.1 Apical dominance

The apical meristem and the recently formed stem and leaf tissues above the uppermost unfolded leaf, constitutes the apical bud. Meristems are also found in the axils of the leaf primordia giving rise to lateral buds, similar to the apical bud (Hillman, 1990). On an intact sugarcane plant, the meristems of lateral buds remain inactive due to complete or partial retardation of mitotic activity by the apical bud (Phillips, 1969). This is indirectly due to auxin, which is produced at the apex of a growing shoot and is transported basipetally. Secondary messengers such as ethylene and abscisic acid (ABA) are considered to transfer the inhibiting message to the lateral buds (Cline, 1991). The degree of apical dominance is determined by genetic and environmental factors, and by the physiological age of the plant (Phillips, 1969). Apical dominance in sugarcane is strong, allowing the shoot to develop as a single main axis (Moore, 1969, Julien *et al,* 1989).

Increased cell divisions have been detected in lateral buds that are relieved of inhibition by removing the potential source of auxin in the shoot apex (Rubinstein & Nagao, 1976). An increase in cell division was noted from about 24 hours after shoot decapitation in soybean (*Glycine max* L. Merr.) (Ali & Fletcher, 1970), and one hour after decapitation of *Cicer* (Guern & Usciati, 1972). In sugarcane, a complex sequence of physiological and biochemical events is initiated, resulting in bud break. The complexity of this event is characterised by changes in food metabolites and the activity of appropriate enzymes and plant hormones (Anon, 1984).

1.2.2 Plant growth regulators

The five classes of hormones that control plant growth are naturally occurring in sugarcane. There is a balance in the levels of the hormones in the apical tissues to control germination of

lateral buds, cell division, elongation and maturation (Vlitos, 1974). Identifying the role of individual hormones gives an indication of which plant growth regulators can be applied to increase bud germination and subsequent growth. Experiments were carried out at the SASEX to determine the factors limiting germination by studying the changes in concentration of IAA, abscisic acid (ABA), gibberellins and cytokinins during germination and early growth (Anon., 1981, 1982, 1983 & 1984). Single-budded setts were stored at unfavourable (20°C) and favourable (30°C) temperatures for germination and harvested every second day to be analysed for the presence of plant hormones. At both temperatures the buds were still dormant at day three. However, when conditions were favourable for germination, bud burst and initial shoot growth occurred at day five and shoot growth continued at day nine. Results obtained from these experiments (discussed in the following sections) indicated that the activation of lateral bud germination is directly related to the hormonal balance in the bud region (Anon., 1984).

Plant growth regulators have been applied to sugarcane setts to improve germination, to stubble in the field after harvesting to improve sugar yields by stimulating even sprouting, and to standing, intact stalks as a ripener to increase sucrose percentage and juice purity (Bhale & Hunsigi, 1994).

1.2.2.1 Auxins

Auxins induce elongation in shoot cells, stimulate cell division and initiate root formation in many plant species. Indole-3-acetic acid is the most important hormonal auxin produced by plants (Weaver, 1972; Preece & Read, 1993). This hormone is synthesised in relatively large amounts from the precursor tryptophan by enzymes which predominantly occur in regions of intense metabolic activity, particularly the meristems (Moore, 1979).

Auxins have been isolated from the stem apical tissue and from roots of sugarcane (Brandes & Van Overbeek, 1948; Cutler & Vlitos, 1962). In the experiments performed at SASEX, the concentration of IAA was higher in the buds than in the nodes or internodes. When setts were

kept at a low temperature (20°C), this level was maintained. However, at a favourable temperature (30°C), the IAA was present at lower concentrations at bud swell, bud break and growth. On the thirteenth day there was a considerable increase in IAA level, possibly preventing the formation of tillers from lateral buds at a later stage. These results suggested that levels of IAA must be lowered in the bud region to enhance germination and early growth (Anon., 1984). However, Castro *et al.* (1975) observed that pretreating setts in 250 ppm IAA increased germination and, in Brazil, soaking of setts in the synthetic auxin, indole-3-butyric acid (IBA), at 10 ppm for one hour enhanced sprouting and root development (Verri *et al.*, 1983).

1.2.2.2 Gibberellins

The function of gibberellins is in stimulating cell division and cell elongation (Moore, 1979). Gibberellins have been detected in roots, stem apical tissue and in the inhibited and developing lateral buds of sugarcane. The major gibberellin present in sugarcane, gibberellic acid (GA_3), occurs in large quantities in the sett in comparison with IAA, and takes precedence over IAA in the control of cell expansion and stalk elongation (Most, 1967). High endogenous concentrations of GA_3 are required to activate lateral bud germination by stimulating the production of enzymes necessary for the germination process (Preece & Read, 1993).

Exogenous applications of GA_3 have been used commercially to promote internode elongation in sugarcane to increase the concentration of sugar in the stalk (Weaver, 1972; Anon., 1980; Preece & Read, 1993). Treatment of decapitated shoots or isolated nodal stem segments of sugarcane with exogenous GA_3 has promoted lateral bud growth (Kato, 1953; Wickson & Thimann, 1958; Chang & Lin, 1962; Shiah & Pao, 1963; Bendigeri *et al.*, 1986). In the experiments at SASEX, the amount of free gibberellins increased considerably from day zero to day three at both temperatures. The gibberellin concentration decreased in non-germinating SBS at day five and decreased in the germinating SBS after initial shoot elongation. This

decrease in concentration suggested that it was only required for bud swell and bud break and not required in large quantities for the subsequent growth of the shoot and roots (Anon., 1982).

1.2.2.3 Cytokinins

Cytokinins are associated with rapidly growing tissue, causing cell division and differentiation (Weaver, 1972). They are also involved in the movement of metabolites and other growth substances (Anon., 1984). Kinetin and zeatin are naturally occurring cytokinins synthesised in the roots and are transported acropetally in the vascular tissue to the shoots. They promote the differentiation of the vascular tissues in the bud traces, releasing lateral buds from apical dominance (Sachs & Thimann, 1964; Moore, 1969).

Cytokinins occur in dormant lateral buds of sugarcane (Most, 1969) and in higher concentrations in the actively growing bud (Anon., 1984). Bendigeri *et al.* (1986) observed that treatment of sugarcane setts with 100 ppm cytozyme for five minutes stimulated germination and tillering, and Bull (1969) reported that kinetin induced rapid germination.

1.2.2.4 Ethylene

Ethylene is a simple hydrocarbon (C_2H_4) that releases buds from dormancy. It is also involved in the induction of adventitious roots by inhibiting polar transport of IAA (Morgan & Gausman, 1966; Weaver, 1972; Preece & Read, 1993) and stimulating metabolism of IAA (Beyer & Morgan, 1970). The effect of ethylene on lateral buds resembles that of decapitation. Ethylene stimulates peroxidase activity that destroys auxin (Hall & Morgan, 1964). As a result, main shoot growth is restricted (Prasad & Cline, 1986) and side shoots, tillers and strut roots develop (Hall & Morgan, 1964).

Ethephon (2-chloroethanephophonic acid; Ethrel®, Rhône-Poulenc) is a liquid, ethylene-releasing compound that became available in South Africa in 1969 (Sterry, 1969). The main use of ethephon in sugarcane production is as a ripener to increasing sucrose percentage and juice purity by causing elongation of the internodes (Anon., 1980).

In South Africa, dipping of the setts in 2400 ppm ethephon improved bud germination of N17 in greenhouse trials (Anon., 1988b). In Cuba, Diaz *et al.* (1995) reported that ethephon applied at 120, 240, 360 and 480 ppm for three minutes significantly improved germination of four sugarcane varieties. In addition, stalk population and cane sugar yields in two poor-germinating varieties were significantly increased by dipping the setts in 120 ppm ethephon solution before planting into the field. Manoharan *et al.* (1992) also obtained high cane yields when setts were soaked in ethephon, and in Louisiana, ethephon (250 ppm) applied as a whole-stalk treatment for 30 minutes before planting increased the rate of sprouting of stalk buds and usually increased tiller populations (Millhollon & Legendre, 1995).

Ethephon can also be sprayed onto the stubble in the field, resulting in sprouting (Yang & Ho, 1980). Ethephon (500 ppm) increased the number of tillers (Burg & Burg, 1968; Peng, 1984; Bhale & Hunsigi, 1994) and significantly increased the cane yield of the ratoon crop compared with the control by increasing weight of cane and number of internodes (Peng, 1984; Bhale & Hunsigi, 1994). Additionally, Diaz *et al.* (1995) reported that germination, stalk population and cane yield were significantly higher than that of the control when ethephon was sprayed onto the leaves in the seedcane nursery 20 days before cutting.

1.2.2.5 Inhibitors

Inhibitors retard cell division and cell elongation in shoot tissues and thus physiologically regulate plant height without causing malformation of leaves and stems (Weaver, 1972). Abscisic acid (ABA) has a common intermediate with gibberellins, farnesyl pyrophosphate, and inhibits lateral bud growth of sugarcane at high concentrations (Moore, 1969).

Chlormequat chloride is an anti-auxin that restricts growth of apical buds, enhancing sprouting of lower buds (Bhale & Hunsigi, 1994). Treatment of ratoon stubble of sugarcane with chlormequat chloride significantly increased the number of tillers and cane yield of the ratoon crop compared to the control (Peng & Twu, 1978; Bhale & Hunsigi, 1994).

Many triazoles shorten internodes and increase root development. Triazoles block the gibberellin biosynthesis pathway by inhibiting the oxidation of *ent*-kaurene to *ent*-kaurenoic acid. They have been used to prevent lodging in rape, to inhibit growth of ornamental plants and lawns, to induce resistance to drought and chilling, and to stimulate generative growth (Lürssen, 1987). Propiconazole (Tilt®, Novartis), when used simultaneously with a hot-water treatment for 20 minutes at 52°C, has been reported to stimulate germination and growth of lateral buds and root growth of sugarcane (Comstock *et al.*, 1984). Paclobutrazol has been used to enhance the production of numerous small shoots and shoot meristems at the basal part of *Spathiphyllum floribundum* Schott 'Petite' and *Anthurium andreanum* Schott, by promoting the shoot-inducing effect of exogenous cytokinins and inhibiting gibberellic acid biosynthesis (Werbrouck & Debergh, 1996; Werbrouck *et al.*, 1996).

In summary, the activation of lateral bud germination is directly related to the hormonal balance within the buds. Increased concentrations of auxins and ABA suppress germination and growth, whereas increased concentrations of gibberellins, cytokinins and ethylene stimulate germination and growth.

1.3 Ratoon Stunting Disease

Ratoon stunting disease (RSD) is caused by the xylem-limited bacterium, *Clavibacter xyli* subsp. *xyli* Davis *et al.* (*C. x. xyli)* (Davis *et al.,* 1980, 1984). This disease is the most widely distributed disease of sugarcane in South Africa, causing substantial losses in yields due to shorter, thinner stalks and reduced stalk populations (Bailey & Bechet, 1986; Anon., 1996). In a first ratoon crop of a trial conducted under rainfed conditions at Mount Edgecombe, RSD infection caused severe yield reductions in varieties N17 (-36%), N14 (-24%), NCo376 (-24%) and N21 (-20%), and intermediate yield reductions occurred in N12 (-14%) and N19 (-13%). Large reductions in yields also occurred in an infected plant crop of a trial conducted under irrigation at Pongola (Anon., 1996).

In field tests in 1996, *C. x. xyli* was most prevalent in the commercial (31%) and seedcane (21%) fields in the Pongola and Umfolozi areas. The pathogen was also detected in fields along the South Coast (6%), Mpumalanga (12%), KwaZulu-Natal Midlands (2%), the lower South Coast (5%) and the along the North Coast (5%) (RA Bailey, 1997, pers. comm.[1]). The low level of *C. x. xyli* in the latter areas was due to a SASEX programme initiated to promote the consistent testing of seedcane for *C. x. xyli* and rejection of infected seedcane. The result is that, in general, the industry is now planting healthy seedcane and the incidence of *C. x. xyli*-infection in commercial cane fields and intended seedcane sources in most areas in South Africa has declined significantly (Bailey & Tough, 1991).

Symptoms of *C. x. xyli*-infection are inconspicuous (Bailey & Bechet, 1986) and the pathogen is detected at SASEX using direct immunofluorescence microscopy (IFM) or phase-contrast microscopy (PCM). The first technique is more accurate, requiring only 3.1×10^3 cells.ml^{-1} in naturally-infected sugarcane sap for detection, compared with the concentration 2×10^6 cells.ml^{-1} required by the method of PCM (Guzman & Victoria, 1993).

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Since none of the commercial varieties possess total resistance or tolerance to *C. x. xyli*, its control depends on methods other than varietal resistance. The main spread of *C. x. xyli* is on cutting tools that become contaminated by harvesting infected volunteers. Therefore, it is important that *C. x. xyli* is prevented from entering nursery and commercial fields by planting seedcane that is free of *C. x. xyli*. Thermotherapy is the only method used to eliminate *C. x. xyli* from infected setts. All sett tissues must reach the required threshold of time and temperature combination for the death of the pathogen (Benda & Ricaud, 1977; Anon., 1979). Cane can be treated with hot water (HWT), hot air, moist air or aerated steam to eliminate *C. x. xyli* from infected setts. Hot-water treatment at 50°C for 120-180 minutes is commonly used worldwide (Steindl, 1961) and treatment at 50°C for 120 minutes is the only method used in South Africa (Anon., 1994). The use of HWT will be discussed in Section 1.5.2.1.

1.4 Root and Sett Rots

The major causes of germination failure of sugarcane in the field are sett rots caused by *Fusarium* spp. and *Gibberella fujikuroi* (Sawada) Wollenweber, red rot caused by *Glomerella tucumanensis* (Speg.) V. Arx & E. Müller (Imp. *Colletotrichum falcatum* Went.), pineapple disease caused by *Ceratocystis paradoxa* (Dade) C. Moreau, and root rots caused by *Pythium* spp., *Rhizoctonia* spp. and *Pachymetra chaunorhiza* Croft & Dick (Barnes, 1974; Autrey *et al.*, 1995) (Table 1.1).

1.4.1 Ceratocystis paradoxa

Pineapple disease is an important rot of sugarcane setts (Wismer & Bailey, 1989) caused by the ascomycetous, soilborne fungus, *Ceratocystis paradoxa*. Conidia and chlamydospores infect the cut ends of the setts within the first week after planting in infested soil (Mitchell-Innes & Thomson, 1973; Wismer & Bailey, 1989). The fungus spreads rapidly through the parenchyma, reddening the infected tissue as the fungus produces toxic materials that produce

an odour of overripe pineapples. Infected setts decay, becoming hollow and blackened and the toxins inhibit or retard bud growth, kill young shoots shortly after emergence, and inhibit root growth (Barnes, 1974). This may result in substantial losses in sugar yields (Mitchell-Innes & Thomson, 1973; Wismer & Bailey, 1989).

Pineapple disease is rarely a problem when setts are planted under conditions that favour rapid germination. However, setts become susceptible when germination is delayed due to the use of old seedcane or heat-treated setts, and the planting of setts when temperatures are low in soil that is too wet or too dry. In South Africa, pineapple disease is most severe in crops planted in autumn and winter, particularly when low temperatures coincide with seasonally low rainfall (Wismer & Bailey, 1989).

Pineapple disease infection can be prevented using young seedcane, optimising conditions for germination and by treating the seedcane with a registered fungicide.

Table 1.1. Sugarcane sett, stem and root diseases.

Causal agent	Disease	Countries
Ceratocystis paradoxa (Dade) C. Moreau	Pineapple disease	59*
Glomerella tucumanensis Went	Red rot	74*
Fusarium moniliforme (Sheldon)	Fusarium sett or stem rot	82*
F. tricinctum Cda. Sacc.	Fusarium sett or stem rot	1
Gibberella fujikuroi (Sawada) Wollenweber	Fusarium sett or stem rot	31*
Pachymetra chaunorhiza Croft & Dick	Pachymetra root rot	1
Pythium aphanidermatum (Edson) Fitzp.	Root rot	-
P. arrhenomanes Drechsler	Root rot	20*
P. graminicola Subram.	Root rot	-
P. tardicrescens Van.	Root rot	1
Rhizoctonia spp.	Root rot	11*

(* indicates that the disease occurs in South Africa) (after Autrey *et al.*, 1995)

1.4.2 Pythium spp. and Pachymetra chaunorhiza

Pythium spp. are the causal agents of a root rot of sugarcane and produce small, smooth walled oogonia and lobulate sporangia on infected roots (Croft & Magarey, 1990). *Pythium* spp. are involved in Poor Root Syndrome (PRS) in Australia (Egan *et al.*, 1984), stubble decline in Louisiana (Edgerton *et al.*, 1929) and caused the failure of the variety Lahaina in Hawaii (Carpenter, 1920).

During the 1920s, a combination of *Pythium* root rot, seed piece rots and sugarcane mosaic virus caused severe damage to the sugarcane industry in Louisiana. The introduction of interspecific hybrids increased resistance to these diseases (Edgerton, 1939; Lee & Hoy, 1992). However, *Pythium* root rot still causes significant reductions in growth and yields of hybrid cultivars in Louisiana (Hoy & Schneider, 1988). Here, cane is harvested at the onset of winter and the stubble buds are inactive over the winter months when temperatures are low and soil aeration is poor. When temperatures are again favourable for germination, many buds fail to germinate, resulting in few vigorous shoots (Edgerton, 1939; Hoy & Schneider, 1988).

In Louisiana, many *Pythium* spp. have been isolated from sugarcane roots and their pathogenicity to local varieties has been tested. *P. arrhenomanes* (Edson) Fitzp. is the most common species causing severe root rot symptoms and significant reductions in shoot and root number and weight (Hoy & Schneider, 1988; Lee & Hoy, 1990, 1992). *P. spinosum* Sawada, *P. dissotocum* Drechsler and *P. graminicolum* Subram. also cause root rot, but are not as severe (Rands & Abbott, 1939; Hoy & Schneider, 1988; Lee & Hoy, 1992).

In Queensland, *P. graminicola* affects seedlings up to three months of age and incidence can reach 30-70%, depending on cultivar. It causes reddening of roots and lesion development, accompanied by a flaccid rot of the tertiary roots, a reduction in fine root growth, and a small amount of primary root rot (Magarey, 1986; Croft & Magarey, 1984, 1990). Root development has also been reduced by infection with *P. myriotylum* (Croft, 1988).

P. arrhenomanes (Croft & Magarey, 1984; Magarey, 1986) and *Pachymetra chaunorhiza* (Croft & Magarey, 1984; Dick *et al.*, 1989) are the main pathogens, along with nematodes and symphylids, in the disease complex known as PRS. They cause serious yield losses in the tropical rainfall areas of northern Queensland in Australia (Croft & Magarey, 1984; Egan *et al.*, 1984; Croft & Magarey, 1990). In 1979 and 1980 there was a high incidence of root rot and approximately 3 500 hectares showed moderate to heavy root rotting (Egan *et al.*, 1984). The pathogens reduce vigour and cause water stress, leaf yellowing, poor tillering, uneven stalk height and lodging (Croft & Magarey, 1984; Egan *et al.*, 1984; Reghenzani, 1984). Temperature affects the prevalence of these pathogens, with severe rotting of primary roots occurring at low temperatures (15-20°C) and lateral pruning at high temperatures (26-30°C) (Rands & Dopp, 1938).

P. chaunorhiza is only known to occur in the Queensland and was initially referred to as the 'root rot fungus' (Croft & Magarey, 1984, Dick *et al.*, 1989). *P. chaunorhiza* is an Oomycete fungus which is distinguished from *Pythium* root rot by producing larger verrucose oogonia and no sporangia on rotted roots (Croft & Magarey, 1984). It causes a soft and flaccid rot of the primary and secondary roots, particularly at the root tips and does not affect fine root growth or cause red root lesions (Magarey, 1986; Croft & Magarey, 1990).

The use of fungicides, soil fumigation and solarisation are not economically practical for large scale control of root rots because they are expensive, may eliminate beneficial microorganisms and have adverse effects on the environment. However, they have been useful in investigations to determine the causes and effects of root and sett rots. In experiments in Louisiana and Queensland, these treatments increased sugarcane growth and decreased the severity of root rot (Croft *et al.*, 1984; Egan *et al.*, 1984; Reghenzani, 1984, 1988; Hoy & Schneider, 1990).

In Queensland, pasteurisation of PRS-soil increased the top and root growth of plants by 100-215% compared with the untreated controls, and almost eliminated root rot symptoms. In another experiment, re-inoculation of sterilised soils with soil from an PRS affected area

restricted top and root growth, PRS symptoms were visible and *P. arrhenomanes* was isolated from the soil (Croft *et al.*, 1984).

Fumigation of sugarcane field soil in pots with methyl bromide caused large increases in plant growth compared with plants grown in either untreated field soil or metalaxyl-treated soil (Hoy & Schneider, 1988, 1990). In two field plots in Australia, fumigation of soil with methyl bromide increased shoot growth by 700% and 450%, root growth by 250% and 120%, and eliminated PRS symptoms (Egan *et al.*, 1984). Croft *et al.* (1984) also reported large increases in top and root growth and complete pathogen control in field trials, and Muchow *et al.* (1995) reported increases of stalk numbers by 24% and cane yields by 6-12%. Fumigation of field soils with methyl bromide has also increased sugar yields of plant crops in South Africa (Thompson, 1985).

Reghenzani (1988) investigated the effect of soil solarisation on sugarcane yields in North Queensland and its effects on the two pathogens responsible for PRS using clear 150 μm thick, polyethylene film to raise soil temperatures. In field trials, there was an improvement in growth of the cane when soil was solarised compared with the control. Yield was significantly increased by 73.5% in the plant cane and by 16.9% in the first ratoon. In pot trials, solarisation eliminated *P. chaunorhiza* but *Pythium* and nematodes recolonised the solarised pots before harvest of the plant crop.

1.4.3 Rhizoctonia spp.

There are few reports of *Rhizoctonia* affecting sugarcane. *R. solani* Kühn and *R. palida* Matz. were isolated and identified as important root pathogens of sugarcane in Barbados (Matz, 1920; Bourne, 1922), causing serious root rotting in the glasshouse. However, these pathogens did not affect roots of plants in the field (Edgerton *et al.*, 1929).

1.4.4 Fusarium spp.

Fusarium moniliforme (Sheldon), *F. tricinctum* Cda. Sacc. and *Gibberella fujikuroi* (Sawada) Wollenweber cause stem and root rot of sugarcane (Autrey *et al.*, 1995). *F. moniliforme* causes a rapid purplish-red discoloration of the vascular bundles, surrounded by reddish-brown discoloration in the parenchyma cells developing from the cut end of the sett inwards. The young roots redden, turn purple and decay, or development is prohibited. Buds swell slightly but fail to germinate causing reduced yield and quality of cane (Bourne, 1961; Mansour & Hamdi, 1983; Ahmad & Malik, 1994).

1.4.5 Glomerella tucumanensis

Red rot caused by *Glomerella tucumanensis* (Speg.) Arx & Mueller affects all parts of the sugarcane plant, particularly standing stalks and planted seed pieces. The infected stalk tissues are red, interrupted by whitish patches. Mycelium develops in the pith cavities and germination is inhibited, particularly in sub-tropical countries during cool or wet weather. Infection of setts occurs from the use of diseased stalks and soilborne infection. Fungicides have not been effective in the control of red rot but heat therapy of seedcane controls systemic infection (Singh & Singh, 1989).

1.5 Methods of Optimising Germination Potential

Vigorous germination of cane setts and rapid early growth of the plant increases cane and sugar yields. Environmental factors, variety, cane quality, cane age, original bud position on the stalk, length of setts and treatments such as thermotherapy and fungicides affect germination of SBS and subsequent growth of the plants. Assessing these factors to determine where improvements can be made is necessary to improve the viability and vigour of the setts and resultant plants.

1.5.1 Quality of seedcane

Seedcane should be healthy, vigorously growing cane, preferably from a field established with cane that has been heat treated to control *C. x. xyli* (Barnes, 1974). Sugarcane varieties differ in the time they take to germinate and the ability of the setts to germinate, so the variety must be adapted to the area in which it will be planted (Benda & Ricaud, 1977).

The youngest buds are those at the top of the sugarcane stalk and the oldest buds are those at the bottom. Clements (1940) and Bellamy & Chinnery (1988) reported that germination time of noble canes increased with bud age, whereas germination time of widely cultivated interspecific hybrids in Barbados was not affected (Bellamy & Chinnery, 1988). Bellamy & Chinnery (1988) also reported that the bud age of both commercial varieties and noble canes did not affect the possibility of germination. However, Abayomi *et al.* (1990) and Sheets (1988) reported that the top section of matured cane gave the fastest and highest germination, compared with buds from the middle or basal parts of the stalk. Therefore, discarding the lower nodes to achieve optimum germination might be necessary.

In Taiwan, stalks are often topped in spring to remove apical dominance so that lateral buds on the stalk begin to germinate while still in the field. After one to two months, stalks containing the plantlets are cut and planted (Peng, 1984).

A small volume of tissue and a single root primordium adhering to the bud (budchip) is enough to ensure germination. However, in an experiment investigating the effect of thermotherapy at 50°C for 30 and 120 minutes on sett size (30 mm, 25 mm, 20 mm and 'bud chips'), germination was higher and plant growth was more vigorous as sett size increased (De Thezy, 1986).

1.5.2 Treatment of setts

1.5.2.1 Long hot water treatment

Hot water treatment (HWT) at 50°C for 120 minutes is the only method currently used in South Africa for the treatment of SBS and whole setts (WS) to eliminate *C. x. xyli* (Anon., 1994). Varieties differ in their ability to withstand heat stress and in the minimum time required to cure RSD. For a successful cure, all sett tissues must reach the required temperature (Benda & Ricaud, 1977), but the rate of heat transfer depends on the length, diameter and the heterogeneity in structure of the sett (Antoine, 1957; Benda, 1972). The longer the treatment, the greater the mortality because fewer plant cells survive to produce shoots and roots (Benda, 1972). The temperature and time combination required to kill RSD is nearly lethal to cane. In South Africa, the varieties N12, N17, N19 and N21 are known to be particularly sensitive to the standard heat treatment of 50°C for 120 minutes (Anon., 1990).

An experiment was carried out at SASEX to determine the efficacy of heat treatment of SBS at 50°C in controlling *C. x. xyli*. It was found that treatments for 30 and 60 minutes provided little control of *C. x. xyli*, and infection still occurred after treatment for 90 minutes. Only treatment for 120 minutes completely controlled *C. x. xyli* (Anon., 1988a).

In Hawaii, treatment of infected seed-pieces at 52°C for 30 minutes resulted in 20.6% infection remaining, whereas treatment at 50°C for 120 minutes completely controlled *C. x. xyli*. Benda (1972) reported that treatments of setts at 52°C for 30 minutes for three consecutive days provided adequate control of *C. x. xyli* (1.2% infection remaining) and better growth than treatment of 50°C for 120 minutes.

Serial heat treatments are recommended when stalks of heat-sensitive varieties cannot be treated at the maximum temperatures applicable. This involves the pre-treatment of cane at the required temperature for a short period to adapt the cane to the high temperature. The cane is then more resistant to subsequent treatments and can withstand longer treatments and higher

temperatures than cane that has not been pre-treated (Benda & Ricaud, 1977). Young heat-sensitive cane is often pre-treated at 50°C for 10-20 minutes 1-5 days before treatment at 50°C for 120 minutes (Benda & Ricaud, 1977; Gillaspie & Teakle, 1989).

1.5.2.2 Short heat treatment

Short hot water treatments have been used to improve germination and early growth. When heat doses are not lethal, the rate of germination of roots and shoots may increase, as well as the proportion of buds that germinate (Benda & Ricaud, 1977). A short heat treatment causes a number of physiological changes in sugarcane, including increased secretion of sugar (Benda & Irvine, 1974) and loss of apical dominance by the establishment of an appropriate hormonal balance in the bud region (Brandes & van Overbeek, 1948; Anon., 1984). In experiments at SASEX, much of the ABA and IAA leached out of the setts into the water when two varieties, NCo376 and J59/3, were heat treated for a short period of time, stimulating germination of the setts (Anon., 1984).

Experiments in Pakistan and Taiwan showed that the soaking of sugarcane setts in water at 52°C for 20 minutes caused rapid development of buds and growth of young cane stools (Peng, 1984) and significantly increased the numbers of shoots at three months after planting (Farid, 1990). Benda (1972) and Comstock *et al.* (1981) also reported an increased rate of germination when SBS were treated at 52°C for 15 and 20 minutes. Treatment of seed-pieces for 10 and 20 minutes in benomyl (Benlate®, Du Pont de Nemours) in hot water (52°C) improved germination by 10-90% compared with benomyl in cold water (Anon., 1975; Comstock *et al.*, 1981). When Sheets (1988) treated setts at temperatures from 35-60°C for 5, 10, 15 and 20 minutes, treatment at both 40°C and 45°C for five minutes provided highest germination of three varieties.

1.5.2.3 Chemical treatments

Many attempts have been made to improve germination by treating setts with plant growth regulators and chemicals. In general, the use of chemicals, except the plant growth regulators GA_3 and ethylene, has not been beneficial and they are rarely used commercially.

Good germination, high plant populations and high sugar yields were obtained in a field trial in India when setts were dipped in a solution containing 250 ppm carbendazim (Bavistin®, BASF) and 0.05% urea (Jayabal & Chockalingam, 1991). Hardy (1973) also reported that the addition of 0.3% urea to the hot water tank significantly improved the germination of NCo376 but did not affect germination of NCo334 and NCo310.

Germination of setts has also been increased, though often not by great margins of difference, by soaking setts in ascorbic, ferulic, vanillic and caffeic acids (Mohandas & Naidu, 1984), chlorogenic acid (Solomon & Srivastava, 1990), lime (Mohandas *et al.*, 1983), magnesium sulphate, saturated lime and calcium chloride (Mohandas *et al.*, 1983; Peng, 1984), potassium chloride (Jayabal & Chockalingam, 1990) and diammonium phosphate (Kathiresan, 1995).

In India, some sugarcane growers dip setts in a cow-dung slurry to act as an anti-dehydration agent (Thirunavukkarasu & Narayanan, 1988), and the dipping of SBS and bud-chips in a 50% solution of cows' urine has also increased sprouting and plant vigour (Kathiresan, 1995).

1.5.2.4 Fungicides

When temperatures are low or setts have been heat treated, fungicides must be applied to protect setts. Otherwise, when adverse conditions prevail and germination is delayed, disease microorganisms could infect the sett, resulting in root and sett rots and high bud mortality (Barnes, 1974).

Many fungicides have been reported to be effective against pineapple disease, including benomyl (Wismer, 1968), guazatine (Steiner & Byther, 1973), carbendazim (Mitchell-Innes & Thomson, 1974), thiophanate (Bechet, 1977), triadimefon (Comstock & Ferreira, 1978) and propiconazole (Comstock *et al.*, 1984).

In South Africa, setts are usually treated with benomyl (250 ppm; Benlate®, du Pont de Nemours) or guazatine (800 ppm; Panoctine®, Rhône Poulenc) before planting (Anon., 1993a) and both fungicides can also be added to the heat treatment tank (Anon., 1994) (Table 1.2). Benomyl, the first non-mercurial fungicide found to be effective when used as a sett treatment (Wismer, 1968; Mitchell-Innes & Thomson, 1973 & 1974), has been shown to stimulate germination (Eastwood, 1972) and to control *C. paradoxa* in *in vitro* tests and in inoculated seed pieces (Liu *et al.*, 1972).

Table 1.2 Fungicides registered in South Africa for treatment of cane setts and SBS. (WP=wettable powder, SC=suspension concentrate, DS=powder for dry seed treatment).

Active ingredient	Trade name	Company	Fungicide group	Form.	Active ingred. (g)	Dosage
benomyl	Benlate® Spotless® Fundazol®	Du Pont Unisun Sanachem	benzimidazole	WP	500 g/kg	0.5 $g.l^{-1}$
guazatine	Panoctine®	Rhône Poulenc	quanidine	SC	400 g/l	2.0 $ml.l^{-1}$
triadimefon	Bayleton®	Bayer	conazole, SDI	DS	250 g/kg	1.0 $g.l^{-1}$

(from Krause *et al.*, 1996)

Carbendazim, which is closely related to benomyl, (375 ppm; Bavistin®, BASF; 153 ppm; Derosal®, AgrEvo) has also been found to be effective against both *C. paradoxa* (Bechet, 1977) and *G. tucumanensis* (Saharan & Satyavir, 1994).

Propiconazole (12.5, 25 and 50 ppm; Tilt®, Novartis) is effective in controlling pineapple disease *in vitro* and in setts (Comstock & Ferreira, 1981; Taylor & Ryan, 1984; Anon., 1985; Raid, 1990; Raid *et al.*, 1991). It has increased germination and stimulated growth when used at concentrations of 6-125 ppm (Comstock, 1981; Comstock & Ferreira, 1981, 1982, 1983; Weibelzahl, 1990) but this stimulation gradually decreased when the concentration was greater than 12 ppm (Comstock & Ferreira, 1983). Propiconazole is phytotoxic at 100 ppm in hot water and phytotoxic at 500 ppm in cold water (Comstock & Ferreira, 1981).

Fungicides shown to be effective against *Pythium* and *Pachymetra* root rots include thiram, captan (Surendra & Kumar, 1989), mancozeb (Magarey & Bull, 1994; Magarey *et al.*, 1995), maneb, zineb (Magarey & Bull, 1994), captafol, fenaminosulf, etridiazole, propamocarb-HCl, pyroxyfur (Croft *et al.*, 1984), methoxyethylmercury chloride, quintozene, carboxin and fosetyl-Al (Peshney *et al.*, 1994).

Croft & Magarey (1984) and Magarey *et al.* (1995) reported that drenching the soil with fungicides active against Oomycetes (metalaxyl, fosetyl-Al and propamocarb) did not affect top and root growth. However, Hoy & Schneider (1988, 1990) reported that drenching of the soil with metalaxyl significantly increased the number and weight of shoots and roots in greenhouse and field experiments, and reduced root rot severity in *Pythium*-infested soil in pots.

1.6 Factors Affecting Growth

1.6.1 Growing medium and fertigation

Growth of transplants partly depends on the quality of the growing medium and fertigation. There should be a balance between drainage, air-filled porosity (AFP) and water-holding capacity (WHC) as well as adequate nutrient elements. The optimum AFP levels for plants grown in media are usually 15-25%. A coarse media has a high AFP and poor WHC, and a medium with a high proportion of fine particles has a low AFP and high WHC. A medium is

often waterlogged when the AFP is 5-10% causing oxygen deficiency and algal growth (Handreck & Black, 1984). Oxygen deficiency seriously impairs the root and nutrient uptake of sugarcane, causing a sharp decline in root weight when the level at the soil surface drops to 3% (Banath & Monteith, 1966).

Composted bagasse, composted pine bark and filtercake are commonly used growing media for transplant production. In experiments at SASEX, the overall germination and shoot development was good when SBS were planted in composted bagasse mixtures and a sand and filtercake mixture (Anon., 1992). Bagasse is the residue obtained by crushing cane stalks for sucrose extraction. It consists mainly of cellulose with some mineral matter, sugars and various other substances (pentosans, lignin, gums and ash). Bagasse initially exhibits good physical and chemical properties for the propagation of transplants (Anon., 1993b). However, it decomposes over time and its physical structure is degraded, with the result that AFP decreases and WHC increases. Composted pine bark has a stable physical structure because although the tannins and cellulose are degraded, the lignin is resistant to enzymatic degradation (Handreck & Black, 1984).

Filtercake is particulate matter separated from the juice by filtration during the clarification process. It is a soft, spongy, amorphous, dark brown material and contains sugars, fibre and coagulated proteins including wax, albuminoids and inorganic salts (Kale & Shinde, 1986).

In South Africa, application of commercial liquid fertilisers or granular formulations is recommended twice weekly during summer and weekly during winter (Anon., 1993a). In Hawaii, no fertiliser is applied for the first two weeks until the root system is active. Thereafter, a weekly spray application is made of a 1N:1P:1K formulation (Ingamells, 1989).

1.6.2 Germination temperature

Ingamells (1989) reported that the optimum temperature range for germination, growth and root development to prevent pathogen infection of the sett is 30°-35°C. In an investigation carried out at SASEX to determine the effect of various temperatures (20°, 24° and 28°C) on the germination and growth of transplants, the highest germination and growth of NCo376 and N12 was at 28°C and lowest germination was at 20°C (De Thezy, 1986).

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2. SURVEY OF FOUR TRANSPLANT NURSERIES

Abstract

Problems were encountered when production systems of four sugarcane transplant nurseries were compared. Germination of setts in trays was often low, increasing production costs. There was a number of contributing factors reducing germination, including the use of old and poor quality seedcane. Pre-germination of setts and heat treatment of whole setts at 50°C for 120 minutes weakened buds that were often subsequently damaged by handling. High temperatures and humidity in the germination rooms increased yeast and bacterial growth on setts, inhibiting germination. Root and shoot growth of transplants was also adversely affected by the use of poor quality media with high water-holding capacities. It was concluded that the greatest problem was low germination, which can be overcome by using good quality seedcane and by improving heat-treatment procedures, sett treatments and growing medium. This would result in the requirement of less seedcane, labour and preparation time.

2.1 Introduction

Four sugarcane transplant nurseries were visited early in 1995 to compare their production systems and to determine where improvements might be made. A table listing the factors of Table 2.1 was drawn up beforehand and was used to evaluate and discuss the production methods, including seedcane source and age, heat treatment temperature and duration, growing medium, irrigation and fertilisation. The nurseries were located at Sezela (South Coast), Malelane (Mpumalanga), Pongola and Gledhow (North Coast).

2.2 Results and Discussion

There was apparently little collaboration between the nurseries, as reflected by the differences in the procedures and materials used. Table 2.1 summarises the production methods employed at the four nurseries. By comparing the systems of each nursery, it was possible to determine which were the most economical and effective procedures currently used for the production of transplants.

The growing media commonly used in the nurseries included bagasse, composted bagasse, filtercake and composted pinebark. Composted bagasse was a popular medium that was readily available to be composted on the site at minimal cost. It is spongy, ensuring easy

placement of the setts in the trays, and had sufficient air-filled porosity for vigorous plant growth. Media containing raw uncomposted bagasse soon composted in the trays. This resulted in nitrogen depletion by microorganisms that are involved in the decomposition process. Additionally, the roots were fully exposed when plants were pulled out of the trays, resulting in the supply of little organic matter and moisture when planted into the field, delaying growth. Composted pine bark (CPB) was also a popular medium, but relatively expensive because it had to be bought from commercial factories.

Frequent problems were encountered with composted filtercake. It had a high water-holding capacity resulting in poor root growth and abundant algal growth on the surface of the medium. It was sometimes mixed with sand to improve drainage, making the trays heavy to transport. Furthermore, particles of composted filtercake were small, often falling through the drainage holes at the bottom of the trays. Additionally, the wettability of filtercake was low when dry, causing the water to run off the trays instead of penetrating the medium.

The optimum age of seedcane for good germination and growth was considered to be between eight and ten months. When seedcane was older than 12-months, major germination problems were encountered. It was also noted that germination was far better when seedcane was cut and treated soon after harvesting than when these procedures were delayed.

Three of the nurseries hot water treated whole setts (WS) which were approximately 60 to 100 cm long. Treatment at 50°C for 120 minutes was considered less damaging to WS than to single-budded setts (SBS). However, the buds that were weakened by the treatment were subsequently damaged, resulting in poor germination. Heat treatment of WS also required a large heat treatment tank that is expensive to run because it holds a larger quantity of water and uses more electricity than a smaller tank required for treatment of SBS. Therefore, in the experiments described in this dissertation, the emphasis was placed on the treatment of SBS to improve germination.

Three of the nurseries pre-germinated the SBS or WS in a germination room before planting into trays. However, this step was time consuming and growth did not always continue after

planting. Buds were also often damaged when pre-germinated WS were cut into SBS, resulting in reduced germination rates.

Most of the nurseries irrigated the trays excessively, resulting in waterlogging and slow plant growth, particularly when filtercake was used. Vigorous plant growth was obtained when trays were irrigated for seven minutes four times a day.

The temperatures in the germination rooms of all nurseries were extremely high, resulting in germination problems. Additionally, the geyser elements in water baths created vast quantities of steam, resulting in yeast and bacteria growing on the walls of the germination room and on the setts, particularly when setts were placed in open trays. It was concluded that a temperature of 28°-32°C was ample for good germination, and the use of heater-fans and watering the setts daily to maintain high humidity may be effective in reducing such problems.

The average germination in the nurseries was 55-70%, with lower germination during winter months. This resulted in the need for more seedcane, labour, preparation time, electricity, space and growing medium. As a result, the selling price of the transplants was high (12-25 cents per transplant). The greatest potential for reducing production costs was evidently to improve germination.

A number of problems were encountered at the nurseries surveyed, including poor medium, over-watering, poor quality seedcane, and time-consuming procedures that could be avoided. The ideal situation would be to use good quality seedcane as soon as it is harvested and to cut the stalks into SBS that would be subsequently heat-treated or treated with a chemical or fungicide to stimulate germination. The SBS should be planted directly into trays to avoid bud damage due to unnecessary handling. An increase in germination in the commercial nurseries would result in less time spent on bulking-up of trays, efficient use of nursery area and less seedcane, growing medium, labour and time required for transplant production, thus reducing production costs.

Table 2.1 Production systems used at four commercial sugarcane transplant nurseries.

Factors	Sezela	Malelane	Pongola	Gledhow
Trays	98-cell polystyrene	98-cell polystyrene	98-cell polystyrene	98-cell polystyrene
Growing medium	Raw bagasse and composted pine bark	Composted bagasse and filtercake	Bagasse and composted pine bark mixture	50% composted bagasse 50% filtercake
Root pruning agent	Styroseal®	Everdip®	Styroseal®	None
Varieties commonly used	N12, N16, N21	N14, N19, N24, N22, N25, N17	N19, N22, N25, N14, N24	NCo376
Age of seedcane	9-month	N19 8- or 9-months other varieties 10-months	8-9 month	14-month
Preparation of setts	a) WS treated at 50°C for 2 hr b) WS cut into SBS c) SBS planted into trays	a) WS heat treated at 50°C for 2hr b) WS pre-germinated in lug-trays in germination room c) WS cut into SBS d) SBS planted into trays	a) WS cut into SBS b) SBS treated at 50°C for 2hr c) SBS pre-germinated in lug-trays in germination room d) SBS planted into trays	a) WS treated at 50°C for 2 hr b) WS cut into SBS c) SBS pre-germinated in lug-trays in germination room d) SBS planted into trays
Fungicide	Benlate® sprayed onto trays	Panoctine® dip	Panoctine® dip	Panoctine® dip
Germination room	35°C, 2 fans, steam bath with bore valve in middle of room	32°C, 3 geyser elements	34°C, geyser element	28-40°C, geyser element
Irrigation	2-3 cycles of 1 hour/day	4 cycles of 20 minutes/day	4 cycles of 7 minutes/day	1 cycle of 90 minutes/day
Shade	No shade	Hail netting only	Hail netting only	No shade, use plastic covered tunnels in winter
Time in nursery	8 - 12 weeks	6 - 7 weeks	6-9 weeks	6-7 weeks

3. GENERAL PROCEDURES

3.1 General Propagation Technique

All experiments were conducted in the Pathology Department at SASEX. The initial propagation technique is described here, and the modifications are described in following chapters. Healthy and *Clavibacter xyli* subsp. *xyli* (*C. x. xyli*)-infected seedcane was obtained from variety propagation plots at SASEX. The growing point and leaves were removed before the stalks were cut into SBS (25 mm in length) using a twin-bladed circular saw. The SBS were placed in either cotton bags or wire baskets to be heat treated at 50°C for 120 minutes in a 40 *l* heat treatment tank. After thermotherapy, the SBS were soaked in guazatine (800 ppm; Panoctine®, Rhône Poulenc) for five minutes and planted in a growing medium of composted bagasse in 98-cell polystyrene trays, which had been treated with a copper-based root pruning solution (Plasdip®; Starke-Ayres). Each tray was divided into four sections, each of which was a treatment plot containing 20 SBS. The trays were placed in a hot and humid germination room (28°C, relative humidity 75-95%) for 3-7 days, and were then transferred to benches in the open. Here the trays were irrigated four times a day for ten minutes during summer months and for three minutes a day during the winter months.

Since most experiments were completed 28 days after planting, the nutrients in both the setts and the medium were sufficient to last this period. A hydroponic fertiliser (3:1:3 (38), Gromor®) was applied only when nutrient deficiencies were visible.

All transplants were harvested from each plot for data collection 28 days after planting. Germination was determined by counting the healthy shoots that had emerged in each plot. Dry mass was determined by cutting each transplant at medium level, placing all shoots from each plot into a paper bag and drying them at 60°C until the mass was constant.

The leaves of transplants in experiments described in Sections 5.2 and 5.3 were trimmed two weeks before transplanting into the field to reduce transpiration. The scissors were sterilised with 90% ethanol after trimming the leaves of each transplant to avoid the spread of *C. x. xyli*.

3.2 RSD Testing

3.2.1 Transplants

Transplants were examined for *C. x. xyli* when they were three months old using immunofloresence microscopy (Harris & Gillaspie, 1978). Stems of the transplants were harvested and the outer leaves were removed. A piece of tissue was aseptically cut from each stem, placed into an eppendorf tube containing 35 μl of distilled water and centrifuged at 8000 rpm for ten minutes. The tissue was removed, the pellet resuspended in water and 20 μl of the suspension placed in a on a 10-welled microscope slide to air dry.

When the drops of suspension were dry, 14 μl of *C. x. xyli*-antiserum (at a dilution of 50 μl in 1000 μl of bicarbonate buffer) was added to each well and the slides were placed in a humid chamber at 30°C for 45 minutes. The slides were washed twice with ½ phosphate buffered saline (½ PBS) and 14 μl FITC-labelled antiserum (20 μl in 1000 μl of bicarbonate buffer) was added to each well. The slides were incubated again for 45 minutes at 30°C at 100% humidity and washed twice with ½ PBS. The slides were blot dried and a small drop of Citifluor was placed in each well and the slide was covered with a large coverslip. The slides were then examined under oil immersion to detect *C. x. xyli* as small fluorescent rods using immunoflorescence microscopy (Appendix 1).

3.2.2 Mature plants

Stalks from transplants in each row were harvested and the lowest, most undamaged internode of cane was cut from the stalk using a sharp knife. Xylem sap was blown through the section using low-pressure compressed air and a moulded resin adapter (Richardson, 1978; Croft & Witherspoon, 1982). A drop of the xylem sap from the end of the stalk piece was transferred by pipette to a microscope slide. The procedures for detecting RSD using immunoflorescence microscopy were the same as in Section 3.2.1.

3.3 Statistical Analysis

All experimental designs used are described in Sokal & Rohlf (1981). The data were analysed using Statgraphics Version 5.0. In every experiment, data were analysed using analysis of variance (ANOVA) to determine the effect of treatment on germination and dry mass. The F-ratio and the significance of the F-ratio were obtained from the ANOVA table to determine if there were significant differences between treatments.

When more than one sugarcane variety was used in an experiment, two- or three-way ANOVA was used to compare treatment means to determine the effect of two or more qualitative factors (eg treatment and variety) on germination or plant growth and the interactions between these factors.

When there were significant F-ratios in the ANOVA tables, the data were analysed using least significant difference tests (LSD) to determine which treatments were significantly different from one another (equation a). The t_{df}-value was substituted by the value obtained from the t-distribution tables using the *error degrees of freedom* taken from the ANOVA table.

$$(a) \quad LSD = t_{df}\sqrt{\frac{2}{n}MS_{within}}$$

To compare all pairs of means, the upper and lower comparison limits were calculated for each mean using equation (b). Two means were significantly different if their limits did not overlap.

$$(b) \quad comparison\ limits = mean \pm \frac{1}{2} LSD$$

3.4 Literature Cited

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3.5 Appendix 1

½ PBS (0.85% NaCl in 0,01 M phosphate) was made up by dissolving 0.96 g sodium di-hydrogen orthophosphate, 1.4 g di-sodium hydrogen orthophosphate, 8.7 g sodium chloride and 0.2 g sodium azide in 1000 m*l* deionized water. The pH was adjusted to 7.0 - 7.4.

0.1 M bicarbonate buffer was made up by dissolving 1.22 g sodium hydrogen carbonate in 146 m*l* deionized water (A). Sodium carbonate (0.57 g) was dissolved in 54 m*l* deionized water (B). Solution A was added to solution B to make a final volume of 200 m*l*. Sodium azide (0.04 g) was added to the final solution and the pH was adjusted to 9.6.

4. SEEDCANE QUALITY

Abstract

Experiments were carried out to determine the effects of original bud position on the stalk on the germination and growth of six commercial varieties. When seedcane was old, the germination of single-budded setts (SBS) from the top of the stalk was significantly higher than germination of those from the middle and bottom of the stalk. Storage of seedcane for three and eight days after harvest adversely affected the germination and growth of varieties N16 and N22, indicating that seedcane must be treated and planted when it is obtained from the field. In experiments to determine the effect of topping seedcane three days before harvest of on the germination of SBS and plant growth, topping did not affect the germination of N11, N12, N16 and N17, and significantly improved the germination of NCo376. In another experiment, topping slightly increased germination of NCo376, and significantly increased the germination of N16. When topped stalks of N16 were subsequently heat treated at 50°C for 120 minutes, germination was the same as that of SBS from untopped cane that had not been treated.

4.1 Bud Position

4.1.1 Introduction

Younger buds at the top of the stalk have a higher germination ability and germinate faster than the older buds at the middle or bottom of the stalk in both noble canes (Clements, 1940; Bellamy & Chinnery, 1988) and commercial varieties in Hawaii and Nigeria (Sheets, 1988; Abayomi *et al.*, 1990). The influence of bud position on germination can be affected by a number of factors. Firstly, the inhibitory effect of the apical bud on the germination of lateral buds decreases once the stalk is cut into single-budded setts (SBS). However, the concentrations of indole-3-acetic acid (IAA) and abscisic acid (ABA) relative to gibberellic acid (GA_3) may remain high in the SBS and delay or inhibit germination. Previous work at SASEX showed that IAA and ABA leached out of SBS during heat treatment, increasing the likelihood of germination (Anon., 1984). Therefore, germination is partly determined by the initial hormone concentration in the bud, which may vary according to the original position of the bud on the stalk.

Secondly, germination of SBS is partly dependent on the health of the buds. In South Africa, larvae of *Eldana saccharina* Walker primarily attack the middle and base of mature stalks. The most common penetration site is at the node, where feeding usually starts and extends into

the internode (Atkinson, 1979). The larvae can survive and increase in numbers in cane stacked for several weeks after harvest, and survive when seedcane is planted at depths of 4 to 10 cm, resulting in poor germination (Carnegie *et al.*, 1976).

Additionally, besides mechanically damaging the stalk, the larvae facilitate infection by the red rot pathogen, *Glomerella tucumanensis* (Edgerton, 1955; Sandhu *et al.*, 1969; Trenor & Bailey, 1989). Trenor & Bailey (1989) found that there was a high correlation between the incidence of red rot and the number of stalks bored by *E. saccharina* larvae, and in Louisiana the borer *Diatreae saccharalis* carried spores of red rot through tunnels it had made in the interior of the stalk. Stalks free of the borer were often free of the disease (Edgerton, 1955). In South Africa, red rot was mainly found in the lower third of the cane stalk (Trenor & Bailey, 1989). *G. tucumanensis* often causes germination failure, particularly during periods of cool and wet wetter (Singh & Singh, 1989).

Since germination can be improved by elimination of damaged buds, or buds with a low germination ability, the aim of this experiment was to determine the effect of original bud position on the germination of six South African commercial sugarcane varieties.

4.1.2 Materials and methods

Seventeen-month old stalks of varieties N12, N16, N17, N19, N22 and NCo376 were divided into top, middle and bottom sections, which were subsequently cut into SBS. Each treatment was represented by a total of 60 SBS, divided into three replications of 20 SBS each. Without sorting to remove damaged buds, the SBS were planted into composted bagasse in trays and germination was recorded at 28 days. Data for the percent germination for all six varieties were pooled and evaluated using a two-way analysis of variance (ANOVA). Mean separation was accomplished using least significant differences.

4.1.3 Results and discussion

The overall germination in this study was low because the seedcane was old, conditions were unfavourable for germination, and many SBS, particularly those from the lower section, were damaged by *E. saccharina*. Statistical analysis of the data indicated that the original position of the bud had a significant effect on germination (Table 4.1). The mean germination of the six varieties was significantly higher when SBS were cut from the top than from the middle of the stalk ($P<0.01$), which in turn was significantly higher than that of SBS taken from the bottom of the stalk ($P<0.01$) (Figure 4.1).

Table 4.1 Two-way ANOVA of the mean germination of varieties N12, N16, N17, N19, N22 and NCo376.

Factor	df	Variance-ratio	P-value
Original bud position	2	68.147	0.0000
Variety	5	6.356	0.0003
Original bud position x variety	10	1.666	0.1298

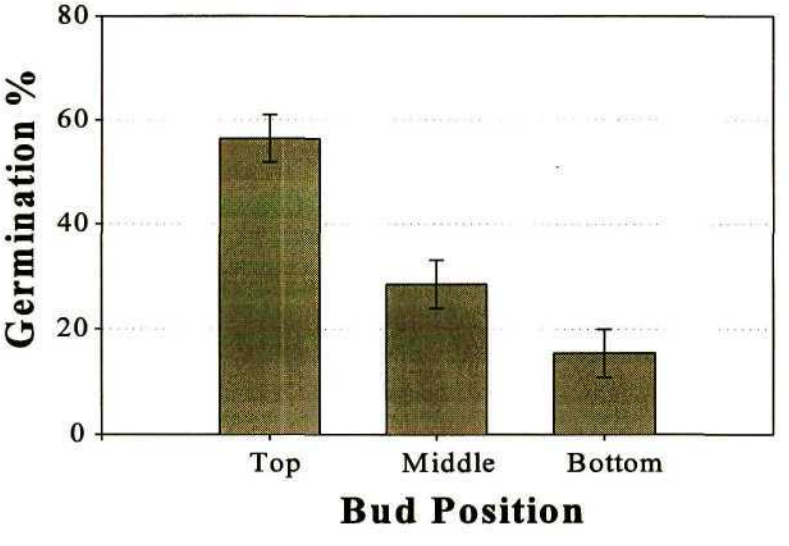


Figure 4.1 Effect of original bud position on the stalk on the mean germination percentages of six sugarcane varieties. Means whose 99% comparison intervals do not overlap are significantly different.

The results were consistent for all varieties as indicated by the lack of a significant interaction between variety and original position of the bud (Table 4.1). Germination of SBS was significantly higher when taken from the top than from the middle of the stalk in varieties N12, N16 and NCo376 ($P<0.01$), and significantly higher than SBS taken from the bottom of the stalk in varieties N12, N16, N17, N19 and NCo376 ($P<0.01$) (Figure 4.2). In all varieties, the germination of the SBS from the bottom of the stalk was lower than germination of those from the middle section of stalk, but not significantly. Germination of N22 also decreased as age of the buds increased, but not significantly.

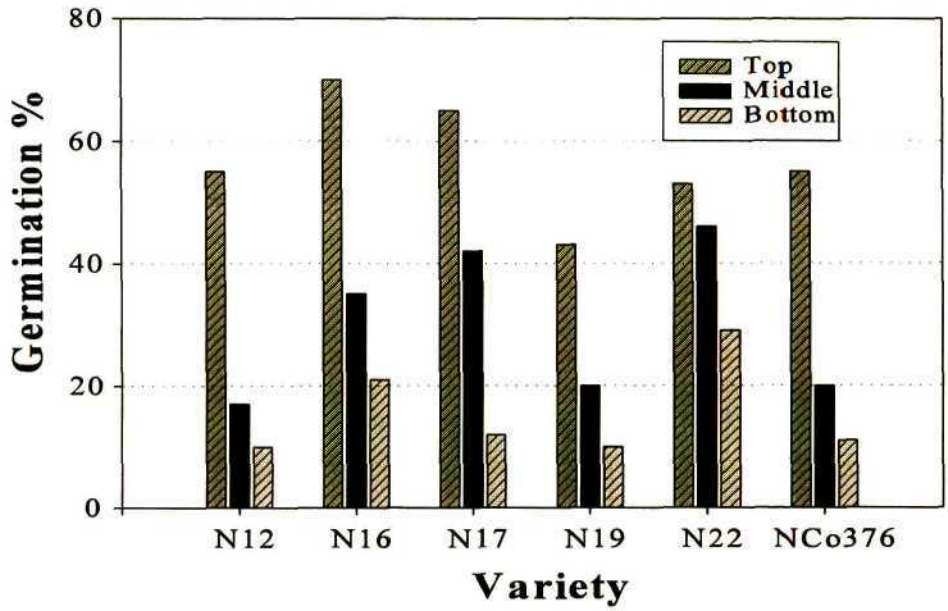


Figure 4.2 Effect of original bud position on the stalk on germination of varieties N12, N16, N17, N19, N22 and NCo376.

Therefore, in this experiment the youngest buds at the top of the stalk had the greatest potential for germination, which could possibly be due to lower levels of IAA and IBA in this region. However, this needs to be substantiated with biochemical studies of hormone levels in the different stalk sections. The low germination of SBS from the older sections may be attributed to the generally higher incidence of *E. saccharina* penetrating the lower section of the stalk (Atkinson, 1979). The results strongly suggested that when the use of old seedcane cannot be avoided, only SBS from the upper parts of the stalk should be used and must be sorted before planting to discard all visibly infected and damaged buds.

4.2 Seedcane Storage

4.2.1 Introduction

Storage of seedcane after harvest and before preparation of transplants can cause dehydration of the stalks, further spread of systemic pathogens, further damage by *E. saccharina* and the fermentation of sugars, resulting in poor germination. Transplant nurseries sometimes store seedcane either outside in the sun or under cover for several days before they are able to plant. When cane is stored at unfavourable conditions, the buds may swell but usually do not germinate and roots do not develop from the primordia. The aim of this experiment was to determine the effect of storage of cane before planting on germination of SBS and the subsequent growth of two commercial sugarcane varieties.

4.2.2 Materials and methods

Stalks of N16 and N22 were collected from the field, one, three and eight days before planting and stored in a well-ventilated shed. After this period the stalks were cut into SBS and planted in composted bagasse in trays. Each treatment was represented by a total of 60 SBS, divided into three replications of 20 SBS each. Germination and dry mass were recorded after 28 days. And the data for both varieties were pooled and evaluated using a two-way analysis of variance (ANOVA). Mean separation was accomplished using least significant differences.

4.2.3 Results and discussion

Statistical analysis of the data indicated that storage of the seedcane had significant effects on both germination and dry mass (Table 4.2). Both mean germination and dry mass of the two varieties were significantly lower when seedcane was stored for three ($P < 0.05$) and eight days ($P < 0.01$) than when stored for one day (Figure 4.3). Although levels of *E. saccharina* were not quantified in this study, it was noted that seedcane stored for more than one day was

severely damaged by *E. saccharina* larvae and *G. tucumanensis*, whereas few SBS were damaged by borings of *E. saccharina* larvae when seedcane was stored for one day. The seedcane stored for eight days was also dry and the sugars were fermenting, indicated by the scent of ethanol.

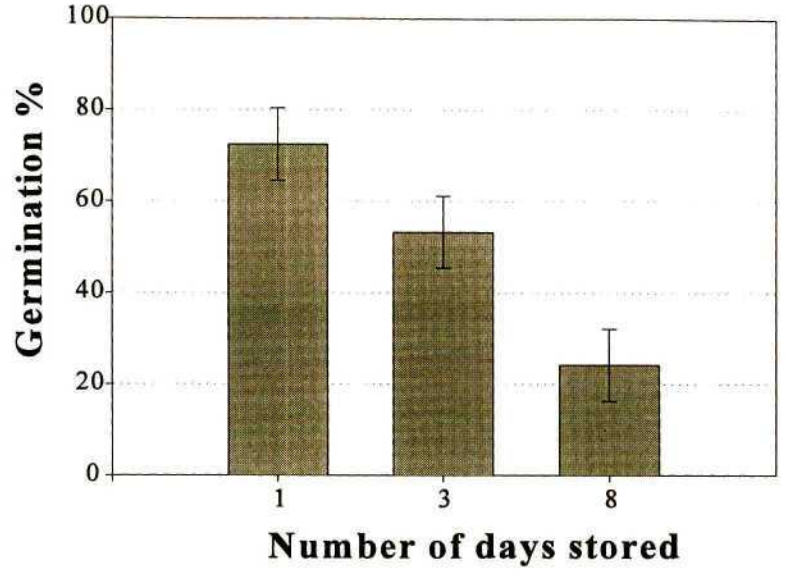
Table 4.2 Two-way ANOVA of the mean germination and dry mass data of varieties N16 and N22 after storage for one, three and eight days before planting.

Factor	df	Germination %		Dry mass	
		Variance-ratio	P-value	Variance-ratio	P-value
Storage period	2	22.646	0.0001	55.605	0.0000
Variety	1	5.0970	0.0434	2.632	0.1307
Storage period x Variety	2	1.301	0.3081	30.816	0.0000

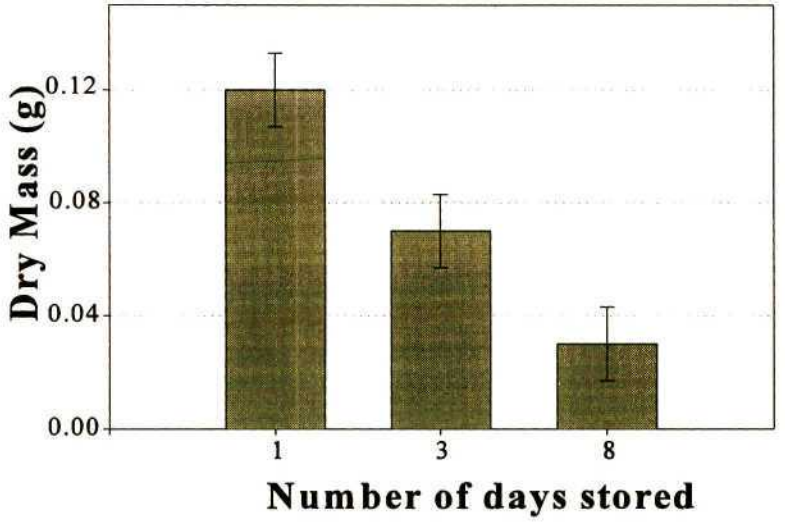
The germination results were consistent for both varieties as indicated by the lack of a significant interaction between variety and storage period (Table 4.2). Storage of the seedcane before planting had a significant effect on the germination of both N16 and N22. Storage for three days decreased germination, particularly of N22, and storage for eight days significantly decreased germination of both varieties ($P<0.01$) (Figure 4.4a). The adverse effects that storage had on these two varieties may be due to the fact that N16 is susceptible to *E. saccharina* and N22 is of intermediate resistance (MG Keeping, 1997, pers. comm.[1]). The use of a variety such as N12 that is resistant to *E. saccharina* may result in less adverse effects on germination, due to lower initial numbers of *E. saccharina* in the stalks.

Germination of both varieties stored for one day would be acceptable in a commercial nursery (72 & 73% germination). However, the low germination rates obtained when seedcane was stored for three days or longer (16-60%) would be unsatisfactory, increasing production costs.

[1] Dr MG Keeping. SASEX, Private Bag X02, Mount Edgecombe, KwaZulu-Natal.



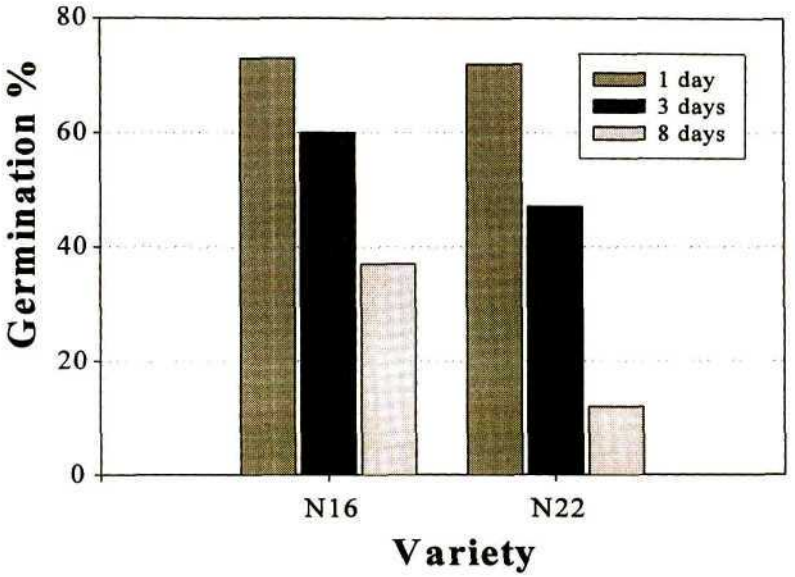
(a)



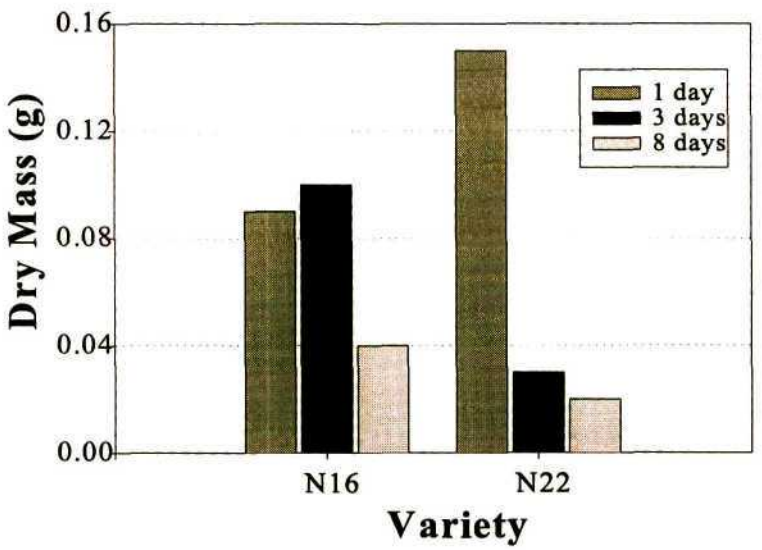
(b)

Figure 4.3 Effect of seedcane storage on mean germination (a) and dry mass (b) of varieties N16 and N22.

The dry mass results were not consistent for both varieties as showed by the significant interaction between storage period and variety ($P < 0.01$) (Table 4.2). Storage of seedcane for three days had no effect on dry mass of N16 but significantly decreased dry mass of N22 (Figure 4.4b). Storage for eight days significantly decreased dry mass of both varieties.



(a)



(b)

Figure 4.4 Effect of seedcane storage on the germination (a) and dry mass (b) of varieties N16 and N22.

It can be concluded that storage of seedcane for a prolonged period causes significant decreases in germination and plant growth. Plant growth of N22 was more severely affected by storage than that of N16, suggesting that the response to storage may depend on variety. However, the different responses were possibly due to the initial levels of systemic infection by *E.*

saccharina and red rot, and this can depend on the resistance of varieties to these. These results strongly indicate that it is essential for growers to cut, treat and plant seedcane as soon as it is obtained from the field. Any delay in planting may result in dehydration of the stalk, spread of pests and pathogens and fermentation of sugars, all of which adversely affect germination and subsequent growth.

4.3 Effect of Topping on Germination and Dry Mass

4.3.1 Introduction

Removal of the shoot apex ('topping') promotes germination of the lateral buds by removing the source of apical dominance (Rubinstein & Nagao, 1976; Anon, 1984). This technique has been successfully used in Taiwan where seedcane is topped one to two months before harvesting to allow the lateral buds to germinate on the standing seedcane before they are harvested for planting into the field (Peng, 1984). The aim of the following two experiments was to determine the effect of topping the seedcane stalks three days before seedcane collection on germination of SBS and plant growth. The effect of topping stalks of two commercial varieties on germination of heat-treated and untreated SBS was determined in Experiment A, and the effect of topping seedcane stalks of five sugarcane varieties on germination and plant growth was determined in Experiment B.

4.3.2 Materials and methods

In Experiment A, 12 standing stalks of N16 and NCo376 were topped in the field by removing the youngest stem and leaf tissues at the natural breaking point. These stalks were marked with tape so that they could be easily identified in the field. Three days later the topped stalks and 12 untopped stalks of each variety were collected, and each tied into a bundle. The stalks of each bundle were cut into SBS (approximately six cm), half of which were heat treated at 50°C for 120 minutes. All SBS were then placed into cotton bags which were then placed in open trays in a germination room (28°C). The number of swollen buds and shoots was recorded after seven days, the total of which will be referred to as 'germination'.

In Experiment B, six stalks each of varieties NCo376, N11, N12, N16 and N17 were topped in the field. Three days later the topped stalks and six untopped stalks (control) of each variety were collected from the field and cut into SBS which were planted into composted bagasse in polystyrene trays. The varieties NCo376, N11 and N12 were planted during the middle of

winter (19 July 1996) and N16 and N17 were planted while temperatures were still low (29 August 1996). Germination and dry mass were recorded 28 days after planting and the data for all varieties in each experiment were pooled and evaluated using two- or three-way analysis of variance (ANOVA). Mean separation was accomplished using least significant differences.

4.3.3 Results and discussion

Experiment A

Statistical analysis of the data indicated that both topping and heat treatment had a significant effect on germination (Table 4.3). Topping significantly increased the mean germination (+24%, $P<0.01$) (Figure 4.5a), and hot water treatment (HWT) significantly decreased the mean germination of the two varieties (-24%, $P<0.01$) (Figure 4.5b).

Table 4.3 Three-way ANOVA of the mean germination of varieties N16 and NCo376 after topping and heat treatment (HWT) of single-budded setts.

Factor	df	Variance-ratio	P-value
Topping	1	9.011	0.0062
HWT	1	9.011	0.0062
Variety	1	16.315	0.0005
Topping x HWT	1	1.873	0.1838
Topping x variety	1	4.397	0.0467
HWT x variety	1	2.596	0.1202
Topping x HWT x variety	1	3.356	0.0794

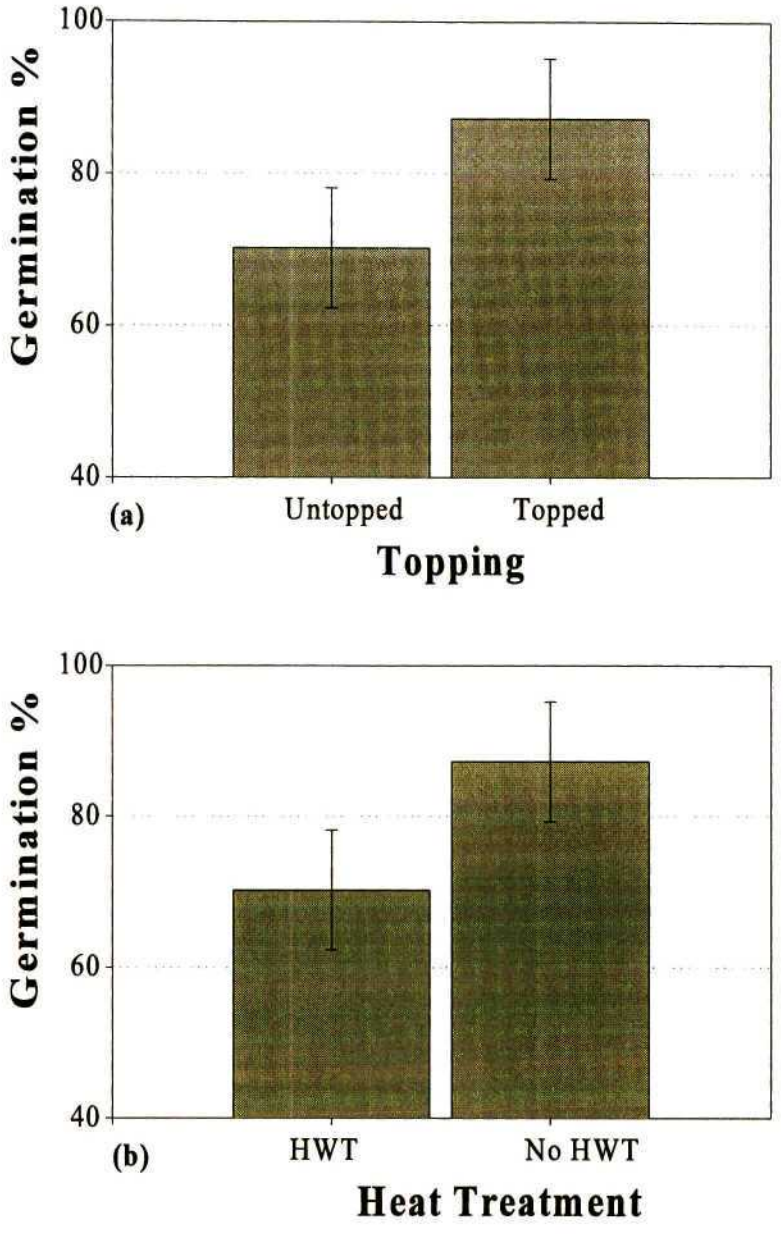


Figure 4.5 Effect of topping (a) and heat treatment (b) on the mean germination of varieties N16 and NCo376.

The effect of topping on germination was not consistent for both varieties as indicated by the significant interaction between variety and topping ($P<0.05$; Table 4.3). Topping increased germination of NCo376, but not significantly so, and significantly increased germination of N16 ($P<0.01$) (Figure 4.6a).

The interactions between HWT and both topping and variety was not significant (Table 4.3). Heat treatment at 50°C for 120 minutes slightly reduced germination of NCo376 and significantly reduced germination of N16 ($P<0.01$) (Figure 4.6b). Topping increased germination of both untreated and heat treated SBS. Germination of heat treated SBS from untopped stalks was low. However, germination of heat treated SBS from topped stalks was similar to that of untreated SBS from untopped stalks, indicating that topping possibly increased the tolerance of SBS to heat treatment (Figure 4.6c).

To conclude, topping the seedcane stalks three days before harvest increased germination of both varieties, particularly when seedcane was subsequently heat treated. The lack of a significant improvement in germination of NCo376 after topping was probably due to the already high germination of the untopped control, even after heat treatment. Therefore, little improvement in germination could be expected.

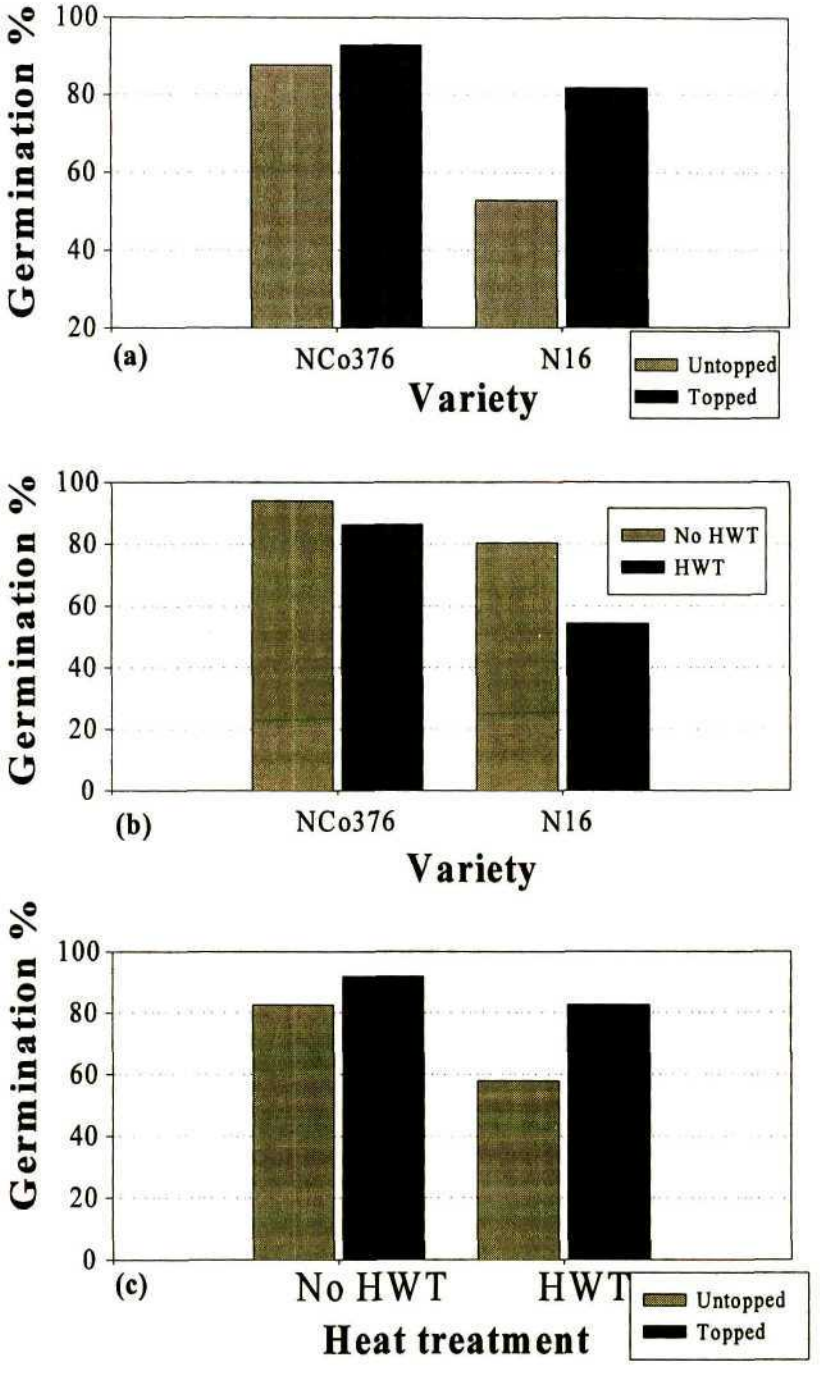


Figure 4.6 Effect of topping on the germination of heat-treated and untreated single-budded setts of varieties NCo376 and N16. The interaction between topping and variety was significant (a), but the interactions between heat treatment and variety (b) and heat treatment and topping (c) were non-significant.

Experiment B

Statistical analysis of the data indicated that topping increased the mean germination of the five varieties (+16%), but not significantly so (P=0.05; Table 4.4; Figure 4.7). The effect of topping on germination was consistent for all varieties, as indicated by the lack of a significant interaction between topping and variety (Table 4.4). In contrast to the previous experiment, topping significantly increased germination of NCo376 (Figure 4.8). This was probably because germination of NCo376 in this experiment was low (57%) due to lower temperatures and the use of older seedcane. Therefore, the effect of topping on germination depends on factors such as seedcane quality and germination conditions, rather than on variety. Topping decreased germination of N11 and increased germination of N12, N16 and N17.

Table 4.4 Two-way ANOVA of the mean germination and dry mass data of varieties N11, N12, N16, N17 and NCo376 after topping.

Factor	df	Germination %		Dry weight (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Topping	1	3.752	0.0670	15.037	0.0011
Variety	4	1.911	0.1480	32.301	0.0000
Topping x variety	4	1.437	0.2584	11.148	0.0001

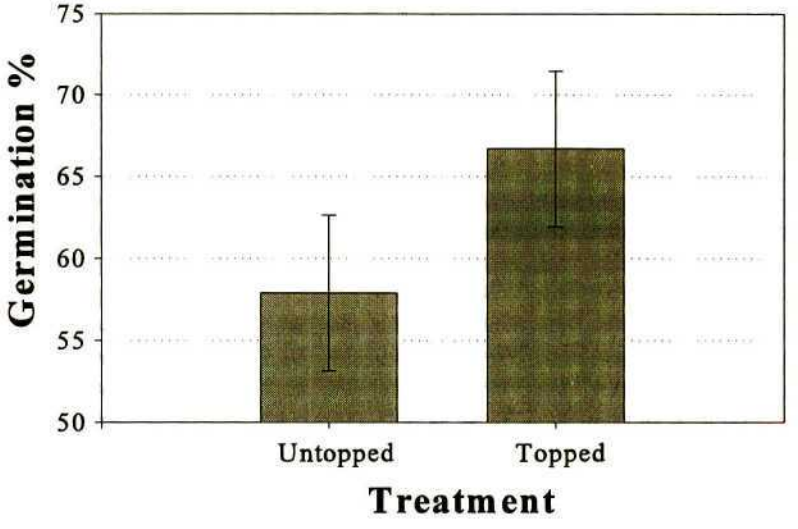


Figure 4.7 Effect of topping on the mean germination of varieties NCo376, N11, N12, N16 and N17.

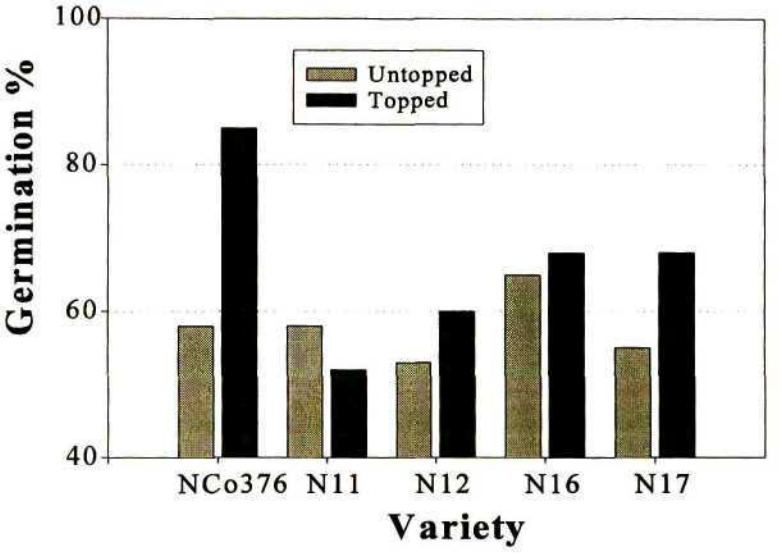


Figure 4.8 Effect of topping on the germination of five sugarcane varieties.

Statistical analysis of the data indicated that topping significantly increased the mean dry mass of the five varieties (+43%, $P<0.01$) (Table 4.4; Figure 4.9). The results were not consistent for all varieties as indicated by the significant interaction between variety and topping (Table 4.4). Topping significantly increased dry mass of NCo376, increased dry mass of N12, and had little effect on dry mass of N11, N16 and N17 (Figure 4.10).

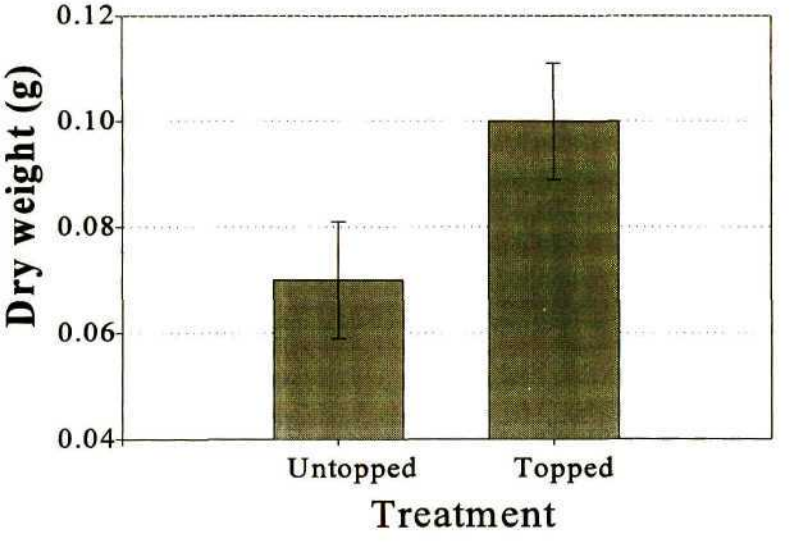


Figure 4.9 Effect of topping on the mean dry mass of varieties NCo376, N11, N12, N16 and N17. Means whose 95% comparison intervals do not overlap are significantly different.

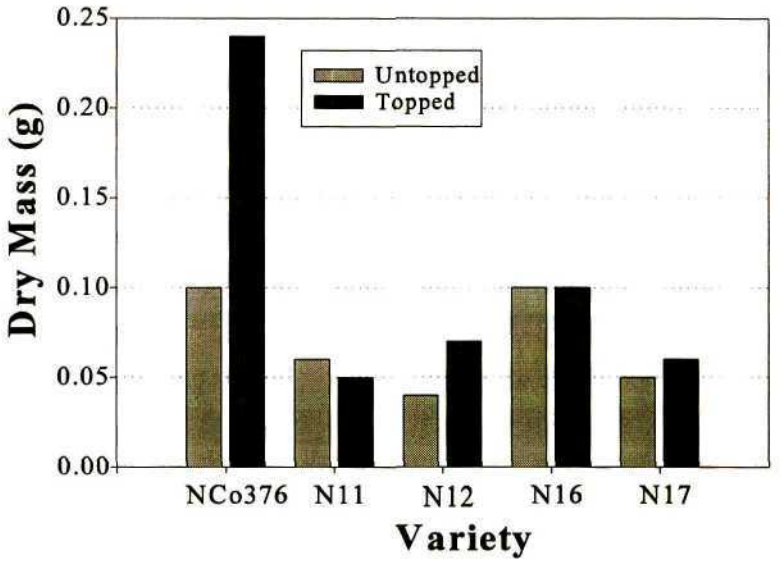


Figure 4.10 Effect of topping on the dry mass of five sugarcane varieties.

To summarise, topping of seedcane stalks three days before collection significantly improved germination and dry mass of NCo376 but had no significant effect on germination and dry mass of varieties N11, N12, N16 and N17. Therefore, topping three days before collection is not recommended when SBS are not subsequently heat treated. Since topping prevented the adverse effects of heat treatment on germination in Experiment A, further research is necessary in this area.

To summarise this chapter, it is important that good quality seedcane is used for transplant production to obtain good germination and plant growth. The age of the seedcane should be 8-10 months because the germination of SBS from senescent or immature seedcane is often poor. All visibly damaged SBS and older nodes should be discarded. Additionally, topping of seedcane in the field had no significant effect on germination of untreated SBS of N11, N12, N16 and N17, but significantly improved germination of NCo376 and heat-treated N16. Therefore, topping of seedcane more than three days before collection, particularly before heat treatment at 50°C for 120 minutes, may increase germination and warrants further research.

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5. HOT WATER TREATMENT

Abstract

Three experiments were carried out to determine the effect of various hot-water treatments of single-budded setts (SBS) and whole setts (WS) on germination of buds and control of *Clavibacter xyli* subsp. *xyli* (*C. x. xyli*). In the preliminary experiment, treatment of SBS at 52°C for 30 minutes controlled *C. x. xyli* in 15-week-old transplants and significantly increased germination of N19 but not N14. The standard treatment of 50°C for 120 minutes reduced germination of both N14 and N19. In the subsequent experiments, treatment of SBS of varieties N12, N14, N16, N17, N19, N24 and N25 at 52°C for 30 minutes significantly decreased germination, but not to the same extent as the standard treatment. Treatment of SBS at 52°C for 30 minutes controlled *C. x. xyli* in varieties N12, N16, N17, N19, N24 and N25, but not in N14.

5.1 Preliminary Experiment

5.1.1. Introduction

Hot water treatment (HWT) of 50°C for 120 minutes is the method commonly used in South Africa for the treatment of whole setts (WS) and single-budded setts (SBS) to control *Clavibacter xyli* subsp. *xyli* (*C. x. xyli*), the causal organism of ratoon stunting disease (RSD) (Anon., 1994). Although this treatment does not achieve total elimination of *C. x. xyli* from setts, a high level of control is achieved (Anon., 1979; Anon., 1988) and the use of this practice has reduced levels of the pathogen in areas in South Africa (Bailey & Tough, 1991, Bailey *et al.*, 1994).

Varieties differ in their minimum treatment time required to kill *C. x. xyli*, depending on the thickness of the stalk, growing conditions, cane quality and cane age. Treatment at 50°C for 120 minutes has adverse effects on the germination of the varieties N12, N17, N19 and N21, whereas the varieties NCo376 and N14 are relatively tolerant (Anon., 1990). The common practice in transplant nurseries is to heat treat whole stalks or WS that are approximately 60-100 cm. However, subsequent handling can damage many buds. Due to high water and electricity costs, and the expense of a large tank, heat treating SBS would be more economical than that of WS. However, SBS are more sensitive to HWT than WS because the tissues of SBS reach the required temperature quicker than those in the centre of WS, resulting in tissue damage after prolonged treatment. Therefore, shortening the treatment time may be possible

so that *C. x. xyli* is eliminated and germination is not unduly adversely affected. In previous experiments at SASEX to determine the efficacy of various periods of HWT at 50°C in controlling *C. x. xyli*, treatment of SBS for less than 120 minutes did not completely control *C. x. xyli* (Anon., 1988). In Hawaii, low levels of infection still occurred after WS were treated at 52°C for 30 minute. However, treatment of infected SBS at 52°C for 30 minutes may be effective in eliminating *C. x. xyli*.

The aim of this experiment was to determine the effect of HWT of SBS and WS at various temperatures and periods on germination of SBS in trays and their control of *C. x. xyli*.

5.1.2 Materials and methods

Seventeen-month-old stalks of varieties N14, N19, N17 and NCo376 were obtained from a *C. x. xyli*-infected variety collection at SASEX. Single-budded setts of N14 and N19 were cut using a twin-bladed circular saw and placed into cotton bags, and WS (approximately 50 cm) of N17 and NCo376 were tied into bundles. The setts were then immersed in the water tank at the required temperature for the required period (Table 5.1).

Table 5.1 Hot-water treatments used to treat single-budded setts (SBS) of the varieties N14 and N19 and whole setts (WS) of the varieties N17 and NCo376.

Temperature	**Time (minutes)**	
	SBS	**WS**
50°C	60, 90, 105, 120 & 135	60, 90, 120 & 135
52°C	30, 60, 90 & 120	30, 60, 90 & 120
54°C	15, 30, 45 & 60	30, 60, 90 & 120

The WS were subsequently cut into SBS. All SBS were then soaked in guazatine (800 ppm; Panoctine®, Rhône Poulenc) for five minutes and pre-germinated in cotton bags in the germination room. Each treatment was represented by a total of 50 SBS divided into two

replications of 25 SBS each. After five days, SBS with swollen buds or shoots were planted into composted bagasse in polystyrene trays. Germination was recorded 28 days after planting and transplants of N14 and N17 were tested for the presence of *C. x. xyli* using immunoflorescence microscopy (IFM), as described in Section 3.2.1 after 15 weeks. Data for percent germination and plant dry mass for both SBS and WS were pooled and evaluated using a two-way analysis of variance (ANOVA). Mean separation was accomplished using least significant differences.

5.1.3 Results and discussion

5.1.3.1 Heat treatment of SBS

Examination of the xylem sap of N14 using IFM showed that all transplants grown from untreated SBS were infected with *C. x. xyli*. Cells of *C. x. xyli* were also present in transplants grown from SBS treated at both 50°C for 60 and 90 minutes and 54°C for 15 minutes. Cells of *C. x. xyli* were not detected in transplants grown from SBS treated at 50°C (105, 120 and 135 minutes), 52°C (30-120 minutes), and 54°C (30-60 minutes). Since the presence of *C. x. xyli* was reported in transplants treated at 50°C for 105 minutes in previous work at SASEX (Anon., 1988), but not in this experiment, these results were not regarded as conclusive. There remains a possibility that *C. x. xyli* was present in the young transplants, but at low concentrations that could not be detected using IFM.

Statistical analysis of the data of N14 and N19 revealed that heat treatment had a significant effect on germination (Table 5.2). The standard treatment decreased the mean germination compared with the control. Treatment at 52°C for 30 minutes increased mean germination compared with the standard treatment ($P < 0.05$) and the control (Figure 5.1). This treatment possibly improved germination by controlling systemic fungal infections and by adjusting the hormonal balance to one favourable for germination. Due to the uncertainty of control of *C. x. xyli* using this treatment, further experiments were necessary to test for the presence of *C. x. xyli* in xylem sap from mature plants. Treatment of SBS at 52°C for 60 minutes had no

effect on mean germination compared with the standard treatment and decreased germination compared with the control. The mean germination was also improved by treatment at 54°C for 15 minutes, but this treatment did not control *C. x. xyli* (Figure 5.1).

Table 5.2 Two-way ANOVA of the mean germination of varieties N14 and N19 after heat treatment of single-budded setts.

Factor	df	Variance-ratio	P-value
Heat treatment	13	17.424	0.0000
Variety	1	24.933	0.0000
Heat treatment x variety	13	1.747	0.1054

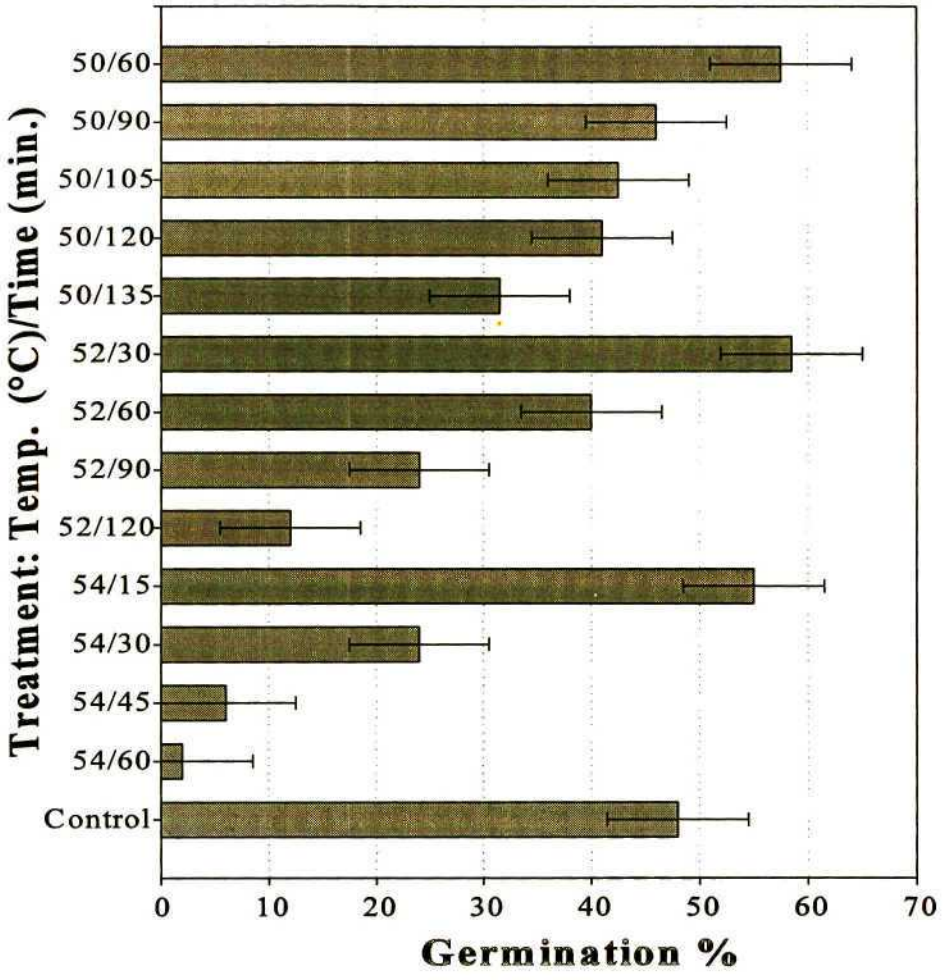

Figure 5.1 Effect of heat treatment of single-budded setts on the mean germination of varieties N14 and N19. Means whose 95% comparison intervals do not overlap are significantly different.

Although N19 is considered to be more sensitive to HWT than N14, the results were consistent for both varieties as indicated by the lack of a significant interaction between variety and HWT (Table 5.2). There were highly significant differences between treatments of N14 and N19 (Table 5.3). Treatment of both varieties at 50°C for 135 minutes, 52°C for 90 and 120 minutes, and 54°C for 30-60 minutes adversely affected germination compared with the control. The standard treatment of 50°C for 120 minutes controlled *C. x. xyli* and decreased germination of N14 (-14%) and N19 (-16%). Treatment at 52°C for 30 minutes significantly improved germination of N19 (P=0.05) and had little effect on the germination of N14 compared with the control and standard treatment. Treatment of SBS at 52°C for 60 had no significant effect on germination of both N14 and N19 compared with the standard treatment.

Table 5.3 Effect of heat treatment of single-budded setts on germination of N14 and N19.

Treatment		Germination (%)		*C. x. xyli* control
Temperature	Time	N14	N19	
50°C	60	55 a	60 a	NO
	90	56 a	36 cd	NO
	105	49 abc	36 cd	YES
	120	50 ab	32 cde	YES
	135	49 abc	14 ef	YES
52°C	30	57 a	60 a	YES
	60	42 abc	38 bc	YES
	90	32 cd	16 def	YES
	120	20 de	4 f	YES
54°C	15	52 ab	58 ab	NO
	30	36 bcd	12 ef	YES
	45	12 e	0 f	YES
	60	4 e	0 f	YES
Control		58 a	38 bc	
LSD (P=0.05) LSD (P=0.01)		17.8 24.7	20.7 28.7	

Means in a column with a letter in common are not significantly different at the 5% level

5.1.3.2 Heat treatment of WS

Examination of the xylem sap of N17 using IFM showed that *C. x. xyli* was present in plants grown from both untreated WS and WS treated at 50°C for 60 and 90 minutes. Cells of *C. x. xyli* were not detected in plants grown from WS treated at 50°C (120 and 135 minutes), 52°C (30-120 minutes) and 54°C (30-120 minutes). As in the previous section, there is a possibility that *C. x. xyli* was present, but was not detected due to the low concentrations of the cells in the young plants.

Statistical analysis of the data indicated that heat treatment significantly affected the mean germination of N17 and NCo376 (Table 5.4). The standard treatment of 50°C for 120 minutes controlled *C. x. xyli* but decreased the mean germination compared with the control. Treatment at 52°C for 30 minutes significantly increased mean germination compared with the standard treatment and the control (P=0.05) and appeared to control *C. x. xyli*. Treatment of 52°C for 60 minutes had no effect on germination compared with the standard treatment (Figure 5.2).

Table 5.4 Two-way ANOVA of the mean germination of varieties N17 and NCo376 after heat treatment of whole setts.

Factor	df	Variance-ratio	P-value
Heat treatment	12	20.477	0.0000
Variety	1	47.200	0.0000
Heat treatment x variety	12	2.133	0.0515

Although N17 is considered to be more heat sensitive than NCo376, the results were consistent for both varieties as indicated by the lack of a significant interaction between HWT and variety (Table 5.4). Prolonged treatment of both varieties at all temperatures (50°C for 135 minutes, 52°C for 90 and 120 minutes, and 54°C for 30-120 minutes) adversely affected germination (Table 5.5). The standard treatment (50°C for 120 minutes) controlled *C. x. xyli* and decreased germination of N17 (-5%) and NCo376 (-14%). Treatment of 52°C for 30 minutes

significantly improved germination compared with both the control (N17; P=0.01) and the standard treatment (N17; P=0.01 and NCo376; P=0.05). Treatment of WS at 52°C for 60 minutes slightly improved germination of both varieties compared with the standard treatment.

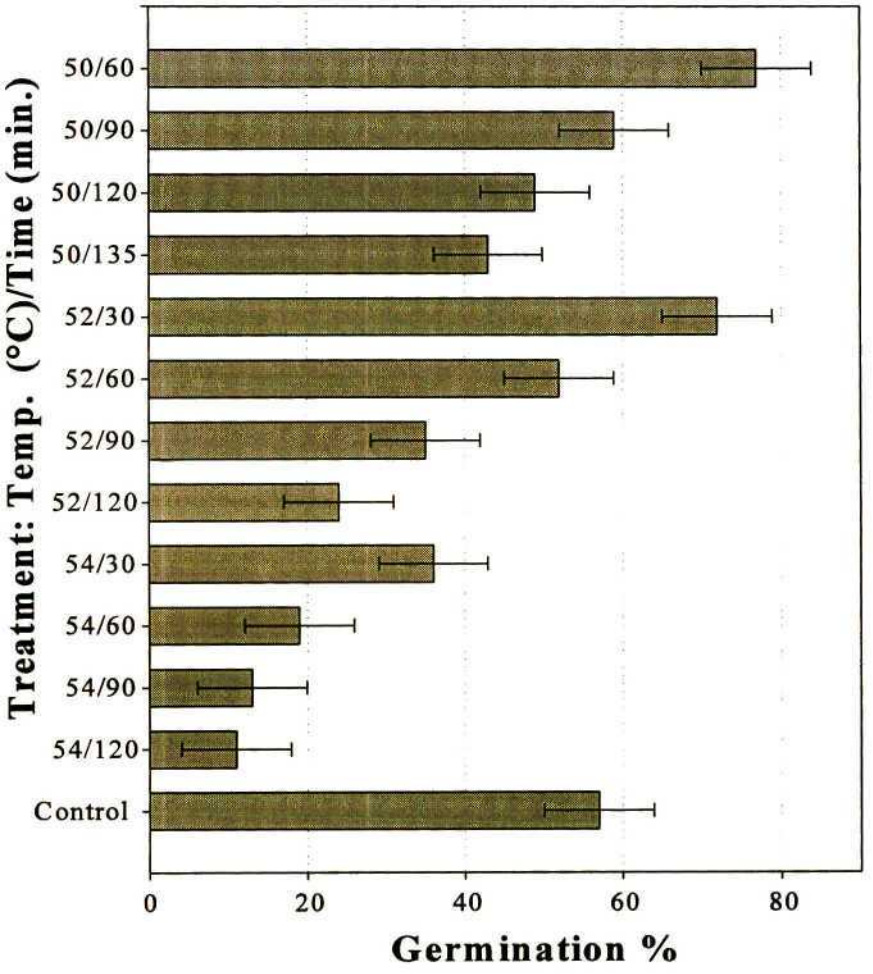

Figure 5.2 Effect of heat treatment of whole setts on the mean germination of varieties N17 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different.

To conclude, WS cannot be treated at 50°C for periods less than 120 minutes because *C. x. xyli* is not eliminated. Treatment at 52°C for 30 minutes was the only treatment that appeared to control *C. x. xyli* and did not adversely affect germination to the same extent as the standard treatment of WS.

Table 5.5 Effect of heat treatment of whole setts on germination percentage of the varieties N17 and NCo376.

Treatment		Germination (%)		*C. x. xyli* control
Temperature	Time	N17	NCo376	
50°C	60	60 b	94 a	NO
	90	60 b	59 bcd	NO
	120	42 cd	49 bcd	YES
	135	34 de	43 bcde	YES
52°C	30	70 a	72 ab	YES
	60	54 b	52 bcde	YES
	90	16 f	35 bcd	YES
	120	6 gh	24 def	YES
54°C	30	28 e	36 cdef	YES
	60	14 fg	18 f	YES
	90	0 h	13 ef	YES
	120	0 h	11 f	YES
Control		44 c	57 abc	
LSD (P=0.05) LSD (P=0.01)		9.9 13.8	27.4 38.2	

Means in a column with a letter in common are not significantly different at the 5% level

5.2 Heat Treatment of Single-Budded Setts and Whole Setts

5.2.1 Introduction

In the preliminary HWT experiment cells of *C. x. xyli* were not detected in 15-week old transplants grown from SBS treated at 52°C for 30 minutes. Compared with the control, this treatment significantly increased germination of N19 and had no effect on germination of N14. Treatment of WS at 52°C for 30 minutes increased germination of N17 and NCo376. The aim of this experiment was to determine the effect of various heat treatments of both SBS and WS on the germination of N12 (a variety considered to be highly sensitive to HWT), and to test for the presence of *C. x. xyli* in mature plants grown from the heat-treated setts.

5.2.2 Materials and methods

Single-budded setts and WS of the heat-sensitive variety N12 were heat treated according to the methods described in Section 5.1.2 using the treatments listed in Table 5.6. Each treatment was represented by a total of 100 SBS, divided into five replications of 20 SBS. Germination was recorded 28 days after planting into trays. The plants were then transplanted into the field to permit accurate testing for *C. x. xyli* in mature plants. Since germination was poor, each row contained transplants from two replicates. Each of the two blocks in the field consisted of seven rows spaced 1.2 m apart, each containing 15 transplants spaced 0.5 m apart. Ten months after planting, one stalk from each transplant was measured for height and the xylem sap was extracted from the lowest undamaged internode to test for the presence of *C. x. xyli* using IFM. Data for the germination of N12 were pooled and evaluated using analysis of variance (ANOVA). Mean separation was accomplished using least significant differences.

Table 5.6. Heat treatments of single-budded setts and whole setts of the variety N12.

Single-budded setts	Whole setts
50°C/105 minutes 50°C/120 minutes 52°C/30 minutes	50°C/120 minutes 52°C/30 minutes 52°C/60 minutes

5.2.3 Results and discussion

Examination of the xylem sap of N12 using IFM showed that 29 of the 30 stalks sampled from the rows planted with untreated transplants were infected with *C. x. xyli* (Table 5.7). None of the stalks were found to be infected after treatment of SBS at temperatures of both 50°C (105 and 120 minutes) and 52°C (30 minutes). Two of the 30 stalks sampled were infected after treatment of WS at both 50°C for 120 minutes and 52°C for 60 minutes. Over half the stalks were infected after treatment of WS at 52°C for 30 minutes.

Therefore, none of the treatments of WS completely eliminated *C. x. xyli*, including the standard treatment of 50°C for 120 minutes. This result confirms previous experiments at SASEX where *C. x. xyli* was detected in 1% of plants grown from WS given this treatment (Anon., 1979). Control of *C. x. xyli* was more effective when SBS were heat treated because heat penetrated the cut ends and reached the centre of the SBS sooner than the centre of the WS. As a result, the treatment time was not sufficient for complete pathogen control in WS and the pathogen probably spread from infected stalks to healthy stalks during cutting.

Table 5.7 Incidence of *C. x. xyli* in plants after heat treatment of infected setts of N12.

Treatment	**Number of stalks with *C. x. xyli* (30 stalks tested)**
Untreated control	29
SBS at 50°C for 105 min	0
SBS at 50°C for 120 min	0
SBS at 52°C for 30 min	0
WS at 50°C for 120 min	2
WS at 52°C for 30 min	18
WS at 52°C for 60 min	2

Statistical analysis of the data indicated that HWT had a significant effect on germination and plant height. Heat treatment of SBS at 50°C (105 and 120 minutes) and WS at 50°C (120 minutes) and 52°C (60 minutes) significantly decreased germination compared with the

control, ($P<0.01$) (Figure 5.3). Germination results were similar when both SBS and WS were treated with the standard treatment and at 52°C for 30 minutes, indicating that treatment of SBS was no more deleterious to germination than treatment of WS. Therefore, SBS of N12 can be heat treated instead of WS to save time, electricity and water, and to obtain better control of *C. x. xyli*. Treatment of SBS and WS at 52°C for 30 minutes slightly decreased germination compared with the control, but not significantly. The germination of SBS treated at 52°C for 30 minutes was significantly higher than that of SBS treated with the standard treatment ($P=0.05$).

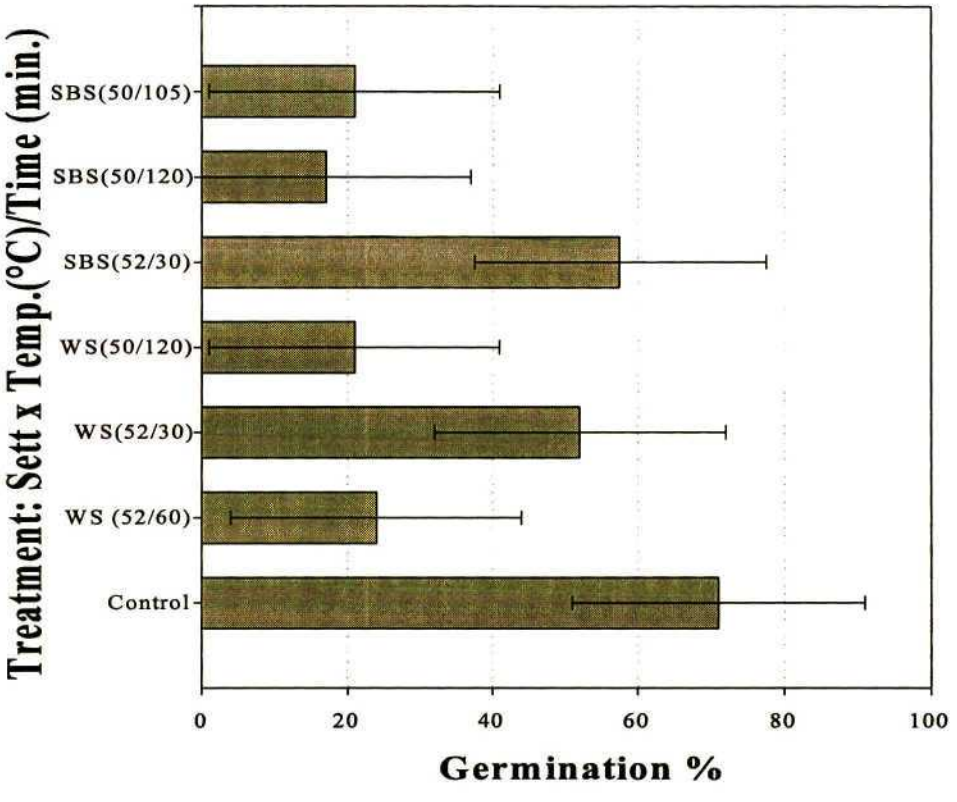

Figure 5.3 Effect of heat treatment of single-budded setts (SBS) and whole setts (WS) on germination of N12. Means whose 99% comparison intervals do not overlap are significantly different.

All treatments, except HWT at 50°C for 105 minutes, reduced stalk height at ten months, indicating that these treatments reduced plant vigour and probably would reduce yields of the plant crop. The height of the plants grown from WS treated at 50°C for 120 minutes was significantly lower than that of the control ($P=0.05$), and less than that of plants grown from

SBS treated similarly. Treatment of both SBS and WS at 52°C for 30 minutes did not reduce height to the same extent as treatment at 50°C for 120 minutes (Figure 5.4).

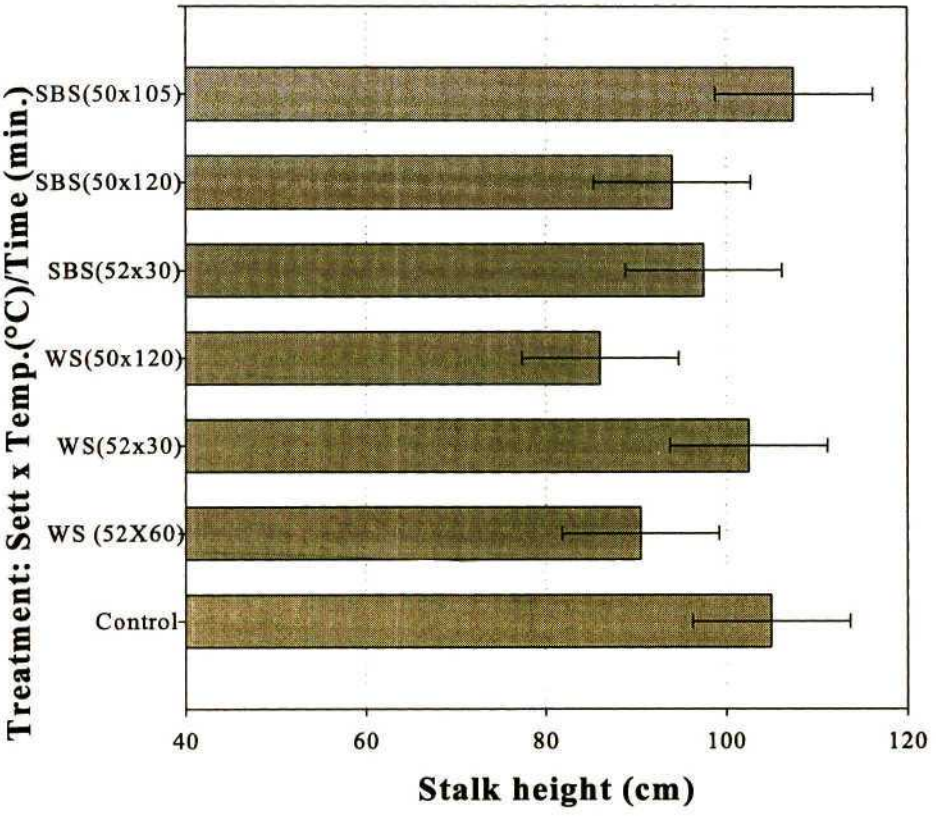

Figure 5.4 Effect of heat treatment of single-budded setts (SBS) and whole setts (WS) of the variety N12 on stalk height at 10 months. Means whose 95% comparison intervals do not overlap are significantly different.

To conclude, treatment of both SBS and WS at 52°C for 30 minutes reduced germination and height of N12, but not to the same extent as the standard treatment. In contrast, in Section 5.1, HWT of SBS and WS at 52°C for 30 minutes either improved or had no effect on germination, indicating that N14, N17 and NCo376 may be more heat tolerant than N12. However, these different responses probably not only depended on variety, but also on thickness of the stalk, age of seedcane, growing conditions and seedcane quality. Therefore, similar experiments comparing varieties of the same age and from the same plots were necessary.

5.3 Heat Treatment of Single-Budded Setts

5.3.1 Introduction

In previous experiments the standard heat treatment of SBS at 50°C for 120 minutes reduced the mean germination of varieties N12, N14 and N19 (Sections 5.1 and 5.2). In a commercial nursery this would increase production costs due to increased labour, time, nursery space, seedcane and medium. However, the mean germination obtained when SBS were treated at 52°C for 30 minutes was not as low, and this treatment appeared to control *C. x. xyli* when transplants and mature cane were tested. Since varieties differ in stalk width and in tolerance of HWT, the aim of this trial was to determine the effect of treatment of SBS at both 50°C for 120 minutes and 52°C for 30 minutes on germination of seven commercial varieties, and to test for the presence of *C. x. xyli* in mature cane grown from SBS treated at 52°C for 30 minutes.

5.3.2 Materials and methods

Twenty-two month old stalks of varieties NCo376, N12, N14, N17, N19, N24 and N25 were obtained from a *C. x. xyli*-infected variety collection at SASEX. Single-budded setts were treated at either 50°C for 120 minutes or 52°C for 30 minutes. Each treatment was represented by a total of 60 SBS, divided into three replicates of 20 SBS each. After heat treatment the SBS were soaked in 800 ppm guazatine, planted in trays containing composted bagasse and germination was recorded after 28 days. The untreated transplants were tested for the presence of *C. x. xyli* three months after planting into trays and transplants grown from SBS treated at 52°C for 30 minutes were planted in the field. Ten months after planting, xylem sap was extracted from one stalk of each plant to test for the presence of *C. x. xyli* using IFM. Data for the germination for all varieties were pooled and evaluated using two-way analysis of variance (ANOVA). Mean separation was accomplished using least significant differences.

5.3.3 Results and discussion

Germination of untreated SBS of all varieties was high (70-92%) because conditions were favourable for germination and apical dominance in the stalk had been reduced because the cane was flowering. However, since in this experiment the buds were swollen and therefore soft and vulnerable to heat damage, both heat treatments adversely affected the mean germination compared with the control ($P=0.01$; Table 5.8). The mean germination of the six varieties was significantly lower when SBS were treated at 50°C for 120 minutes than when treated at 52°C for 30 minutes ($P<0.01$) (Figure 5.5).

Table 5.8 Two-way ANOVA of the mean germination of varieties N11, N12, N14, N17, N19, N24, N25 & NCo376 after heat treatment of single-budded setts.

Factor	df	Variance-ratio	P-value
Heat treatment	2	114.654	0.0000
Variety	6	11.140	0.0000
Heat treatment x variety	12	2.052	0.0430

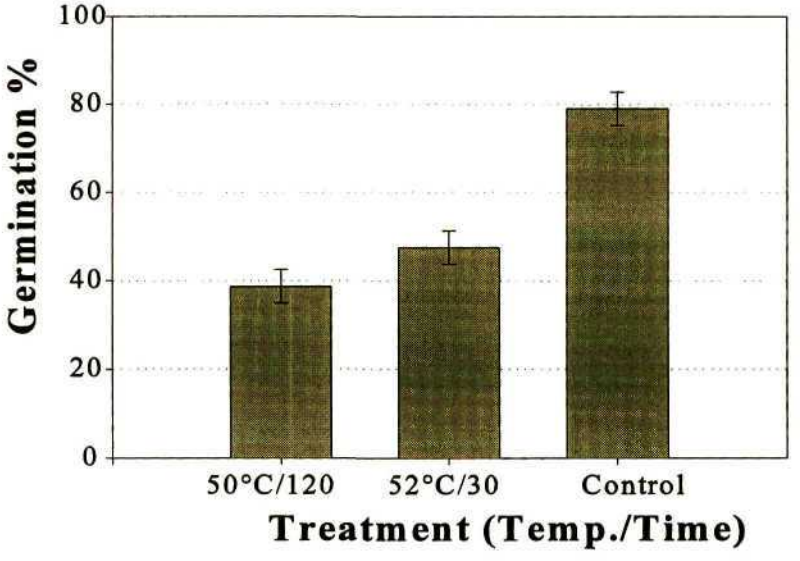

Figure 5.5 Effect of heat treatment of single-budded setts on the mean germination of varieties N11, N12, N14, N17, N19, N24, N25 and NCo376. Means whose 99% comparison intervals do not overlap are significantly different.

The results were not consistent for all varieties as indicated by the significant interaction between variety and HWT (Table 5.8). There were significant differences between treatments with varieties N12, N14, N17, N19 and N25, but not with N24 (Table 5.9). Treatment with the standard treatment at 50°C for 120 minutes significantly reduced the germination of varieties N12, N14, N17, N19 and N25 to 15-50% ($P<0.01$). Of all varieties, germination of N12 and N14 was most adversely affected, indicating their sensitivity to the standard heat treatment (Table 5.9, Figure 5.6).

Treatment at 52°C for 30 minutes also significantly reduced the germination of N12, N14, N17, N19 and N25 compared with the control. However, this treatment significantly increased germination of N12 and increased germination of the other five varieties compared with the standard heat treatment. Of all varieties, N14 was most sensitive to this treatment (-68%; $P<0.01$). Treatment of the other varieties at 52°C for 30 minutes reduced germination by 30-48% (Table 5.9, Figure 5.6).

Table 5.9 Effect of various heat treatments of single-budded setts on germination of seven sugarcane varieties.

Treatment	Germination (%)						
	NCo376	N12	N14	N17	N19	N24	N25
50°C/120 min.	43 b	15 c	23 b	50 b	38 b	50	48 b
52°C/30 min.	53 b	38 b	25 b	58 b	47 b	52	60 b
Control	82 a	73 a	79 a	83 a	70 a	75	92 a
Mean	59	42	42	65	52	59	67
LSD (P=0.05)	19.1	13.6	11.7	12.5	21.3	NS	20.5
LSD (P=0.01)	29.0	20.7	17.8	18.9	18.9		31.2

Means in a column with a letter in common are not significantly different at the 5% level.

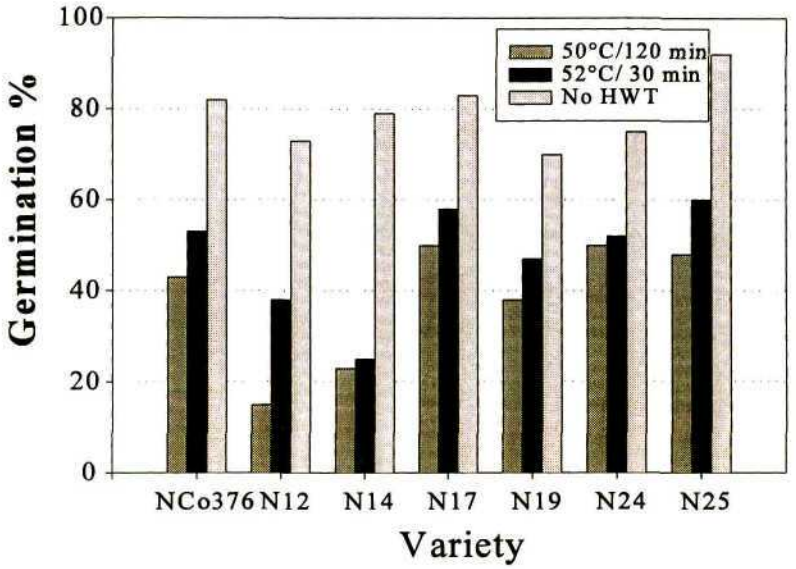

Figure 5.6 Effect of heat treatment of single-budded setts on germination of seven sugarcane varieties.

Examination of the xylem sap of untreated SBS showed that all varieties were infected with *C. x. xyli*. Treatment of N12, N17, N19, N24 and NCo376 at 52°C for 30 minutes eliminated *C. x. xyli*. However, *C. x. xyli* was present in 40% of transplants of the variety N14 treated with this treatment, confirming the high susceptibility of N14 to *C. x. xyli* (Anon., 1996a,b). These results also indicated that control of *C. x. xyli* in this variety may be difficult because it often has a thick stalk (GR Bechet, 1996, pers. comm. [1]), requiring a longer treatment period than most varieties for control of this pathogen. Therefore, control of *C. x. xyli* may not have been effective in this variety because the HWT was not long enough to kill all the bacterial cells, particularly those in the centre of the SBS. There is also a possibility that *C. x. xyli* was present in the other varieties treated at 52°C for 30 minutes, but was not detected due to low concentrations. However, since the seedcane was mature (10-months old), this is unlikely because previous research at SASEX showed that when infected cane of the varieties NCo376, N12 and N16 was planted, *C. x. xyli* was detected microscopically at eight months and the numbers of bacteria observed increased rapidly up to the age of 10-months old. Thereafter, there was no increase in concentration of bacteria (Anon., 1992).

[1] Mr GR Bechet, SASEX, Private Bag X02, Mount Edgecombe, KwaZulu-Natal.

The effect of HWT on germination depends on a number of factors, including variety, sett length, sett width, cane age and cane quality. The seedcane used in Section 5.1 was 17-months old, which is usually considered too old for use as seedcane, resulting in low germination. However, buds of this cane were resistant to treatment at both 50°C for 120 and 52°C for 30 minutes.

In this experiment the seedcane was taken from the same plot five months later when the cane was flowering. Flowering results in the release of lateral buds from correlative inhibition, which in turn results in higher germination of untreated SBS. However, HWT of the swollen buds had adverse effects on germination of NCo376, N12, N14, N17, N19 and N25 because the swollen buds were soft and vulnerable to heat damage.

Therefore, seedcane that is older than the optimum age for planting can be tolerant of HWT, but once correlative inhibition is reduced, the swollen buds are sensitive to heat treatment. Therefore, seedcane used for transplant production should not be older than 10-12 months.

In view of the marked effects of seedcane quality, stalk width and variety on germination, the results of this experiment need to be confirmed before recommendations can be made to the transplant grower. This experiment needs to be repeated, treating SBS at 52°C for 30, 40, 50 and 60 minutes and at 50°C for 120 minutes. The average stalk width of seedcane of each variety needs to be determined before planting to investigate its effect on control of *C. x. xyli*.

Recommendation of treatment at 52°C for 30 minutes to growers is highly unlikely because it did not control *C. x. xyli* in N14 in this experiment and may also be ineffective with other varieties. Since treatment at 52°C for 60 minutes controlled *C. x. xyli*, and affected germination similarly to treatment at 50°C for 120 minutes, treatment at 52°C for 40 to 60 minutes may be recommended for treatment of SBS in the future. This will result in more adequate control of *C. x. xyli* than the standard treatment and the shorter treatment time speeds up the transplant production process, decreasing labour, time and electricity costs. This in turn decreases the selling price of transplants, making their use more attractive to sugarcane growers.

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6. HEAT TREATMENT TO STIMULATE GERMINATION

Abstract

Various short hot-water treatments (SHWT) of single-budded setts (SBS) were tested. When good quality seedcane of varieties N12 and N17 was used, treatment at both 50°C and 52°C for 10 and 20 minutes had no effect on germination, but significantly improved the plant growth. However, when older seedcane from the same plot was treated, treatment for 10 minutes at 48°C and 52°C significantly increased the mean germination and increased the growth of varieties N12, N16 and NCo376. The increases in germination and growth were probably due to the change in hormonal balance within the SBS and control of systemic fungal infections.

6.1 Short Hot Water Treatment

6.1.1 Introduction

Ratoon stunting disease (RSD) is the most economically important disease of sugarcane in South Africa. An important factor in preventing *Clavibacter xyli* subsp. *xyli (C. x. xyli)*-infected material from entering commercial and nursery fields is to plant *C. x. xyli*-free seedcane or transplants. This is achieved by the use of thermotherapy. In transplant production, the direct treatment of setts at 50°C for 120 minutes is strongly recommended in areas where *C. x. xyli* is prevalent, particularly the Pongola and Umfolozi areas. In areas where *C. x. xyli* is not prevalent it may be possible to avoid thermotherapy by using seedcane grown from heat treated cane that has been consistently tested and found free of *C. x. xyli*.

When single-budded setts (SBS) are not heat treated to eliminate *C. x. xyli* they can be subjected to a short hot water treatment (SHWT) before planting to stimulate germination and early growth. Short heat treatment of setts at 52°C for 10-20 minutes before planting has significantly improved germination and growth (Benda, 1972; Anon., 1975; Comstock *et al.*, 1981; Peng, 1984; Farid, 1990). The increase in germination has been ascribed to the establishment of an appropriate hormonal balance for germination within the bud region (Benda, 1972; Peng, 1984; Farid, 1990). Although these treatments have stimulated germination when used alone or combined with fungicides, the increase in germination has not been ascribed to the control of pathogens. The aim of this experiment was to determine the effect of various SHWT on germination and growth of four commercial sugarcane varieties.

6.1.2 Materials and methods

Experiment A was carried out in November 1995 using 19-month old seedcane stalks obtained from a crop grown under hot, dry conditions, resulting in slow growth of the stalks and short internodes. Single-budded setts of varieties with poor germination, N12 and N17, were heat treated at 50°C and 52°C for 10 and 20 minutes by submerging the SBS in wire baskets into the water.

Experiment B was carried out in February 1996 using 22-month old seedcane from the same source as Experiment A. The varieties used were N16 which has rapid germination, NCo376 which has moderately rapid germination, and N12 which has unreliable germination. Single-budded setts were heat treated at temperatures of 36°, 40°, 44°, 48°, 50°, 52°, 56° and 60°C for 10 minutes before planting into trays. Each treatment in both experiments was represented by a total of 60 SBS, divided into three replicates of 20 SBS each. The germination and shoot dry mass were recorded after 28 days. For each experiment the data were pooled and evaluated using two-way analysis of variance. Mean separation was accomplished using least significant differences.

6.1.3 Results and discussion

Experiment A

Mean germination of both the control and the heat-treated SBS was high because although the seedcane was old, it was of good quality. Additionally, the warm temperatures were favourable for germination. Statistical analysis of the data indicated that SHWT had no significant effect on mean germination (Table 6.1). Treatment at 50° and 52°C for 10 minutes improved germination, and treatment 50° and 52°C for 20 minutes decreased germination, compared with the control. Therefore, germination after treatment at both 50° and 52°C was higher after treatment for 10 minutes than after treatment for 20 minutes (Figure 6.1).

Table 6.1 Two-way ANOVA of the mean germination and dry mass data of varieties N12 and N17 after short hot-water treatment (SHWT) of single-budded setts.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
SHWT	4	0.390	0.8135	17.902	0.0000
Variety	1	13.235	0.0016	87.479	0.0000
SHWT x variety	4	3.860	0.0175	7.797	0.0006

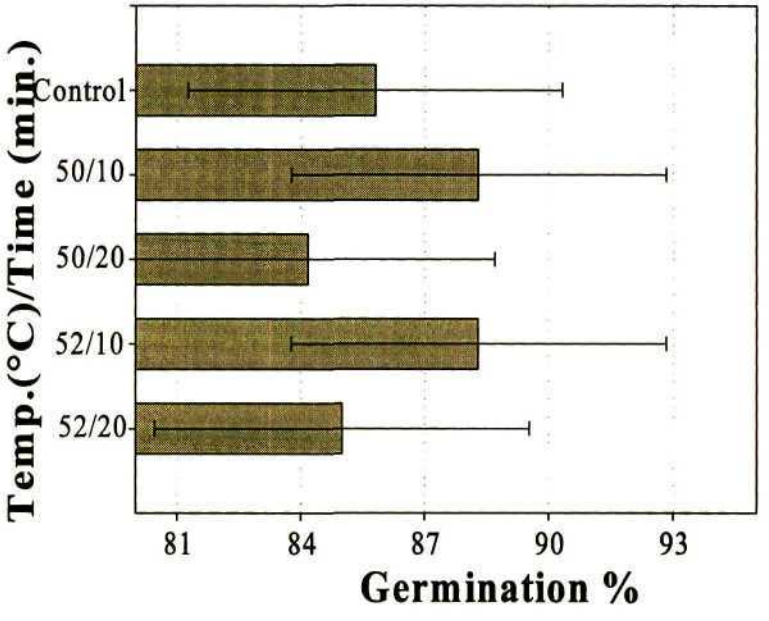

Figure 6.1 Effect of short heat treatment of single-budded setts on the mean germination of varieties N12 and N17. Means whose 95% comparison intervals do not overlap are significantly different.

The germination results were not consistent for both varieties as indicated by a significant interaction between variety and SHWT (Table 6.1). Compared with the control, treatment of N12 at both 50°C and 52°C for 10 minutes increased germination, whereas the treatment for 20 minutes decreased germination. Treatment of N17 at both 50° and 52°C for 10 minutes decreased germination, treatment at 50°C for 20 minutes had no effect on germination and treatment at 52°C for 20 minutes increased germination (Figure 6.2).

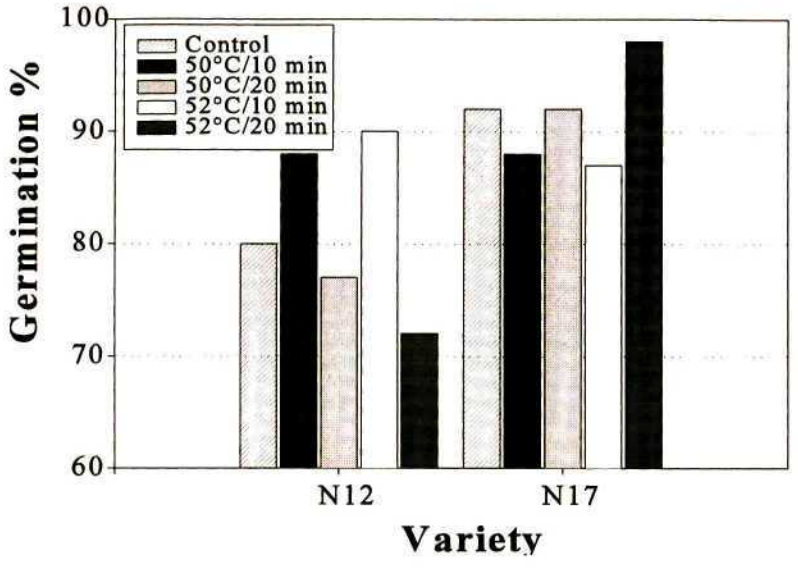

Figure 6.2 Effect of short heat treatment of single-budded setts on the germination of varieties N12 and N17.

Statistical analysis of the data indicated that SHWT had a significant effect on dry mass (Table 6.2). The mean dry mass was significantly improved by all treatments ($P < 0.01$), the SHWT at 52°C for 10 and 20 minutes being the most effective (Figure 6.3).

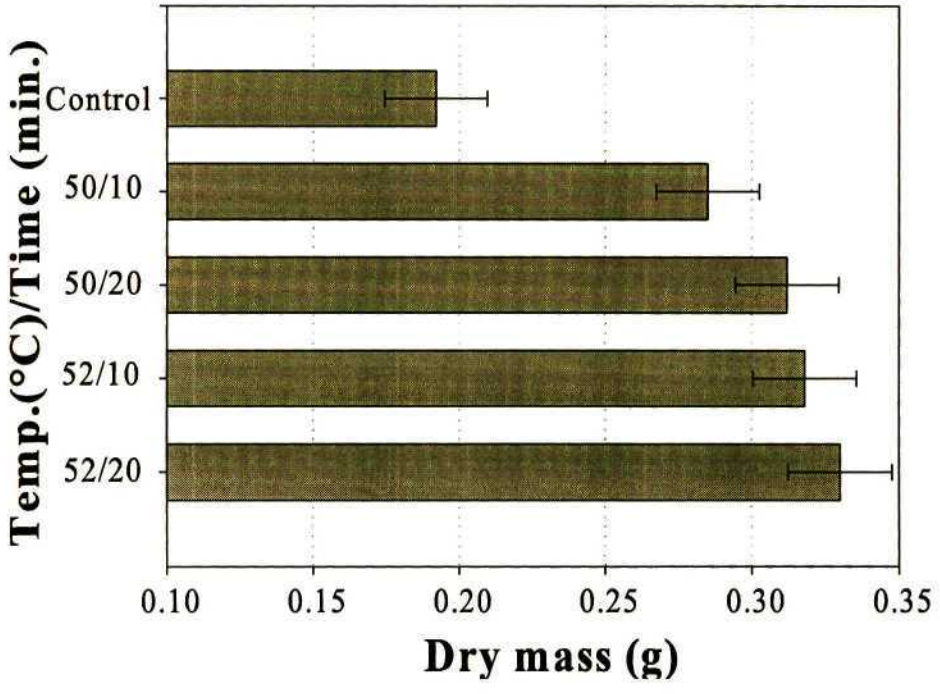

Figure 6.3 Effect of short heat treatment of single-budded setts on the mean dry mass of varieties N12 and N17. Means whose 99% comparison intervals do not overlap are significantly different.

The dry mass results were not consistent for both varieties as indicated by a significant interaction between SHWT and variety was significant (Table 6.1; $P<0.01$). Short heat treatment had a significant effect on dry mass of both varieties (Figure 6.4). Dry mass of N12 was significantly improved by treatment at both 50°C for 10 ($P<0.05$) and 20 minutes ($P<0.01$) and 52°C for 20 minutes ($P<0.01$). Dry mass of N17 was significantly improved by SHWT at 50° and 52°C for 10 and 20 minutes ($P<0.01$).

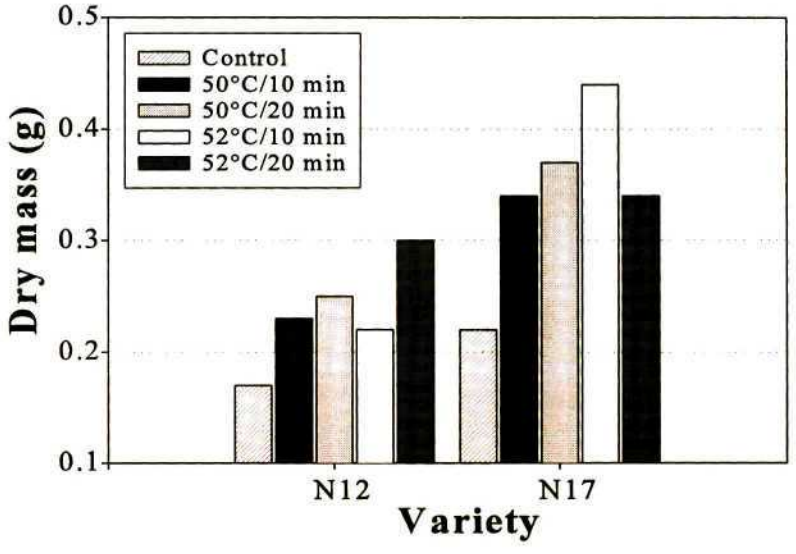

Figure 6.4 Effect of short heat treatment of single-budded setts on dry mass of varieties N12 and N17.

In summary, none of the SHWT significantly affected mean germination when cane quality was good and conditions were favourable for germination. However, all treatments significantly improved plant growth of both N12 and N17.

Experiment B

Germination of the controls was low (28%), indicating that the cane was senescent and too old for use as seedcane (Figure 6.5). Statistical analysis of the germination data indicated that SHWT had a significant effect on the mean germination of N12, N16 and NCo376. All treatments, except treatment at 56° and 60°C, improved the mean germination. Treatments

at 48° and 52°C significantly improved germination compared with the control ($P<0.01$). Treatment at 60°C had an adverse effect on germination ($P<0.01$).

Table 6.2 Two-way ANOVA of the mean germination and dry mass data of varieties N12, N16 and NCo376 after short heat treatment of single-budded setts.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
SHWT	8	11.686	0.0000	2.725	0.0134
Variety	2	13.962	0.0000	6.405	0.0032
SHWT x variety	16	1.317	0.2211	0.558	0.9011

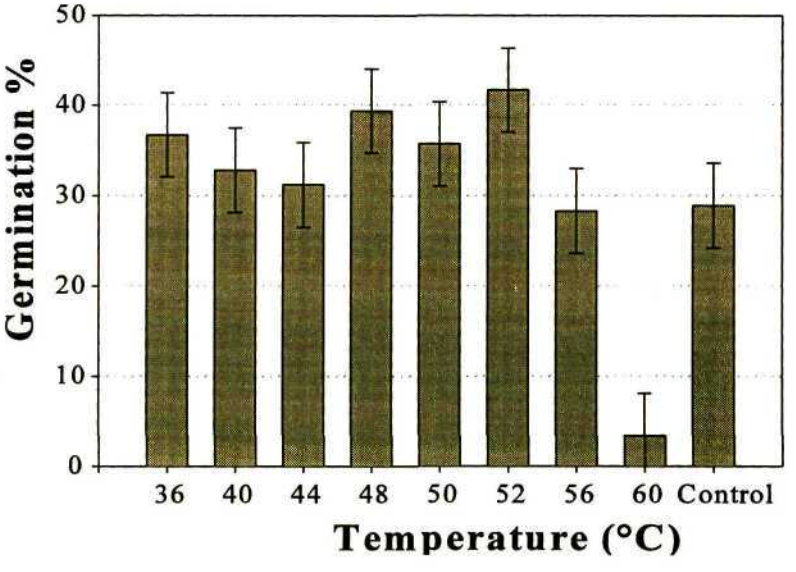

Figure 6.5 Effect of short heat treatment of single-budded setts on the mean germination of varieties N12, N16 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different.

Although the varieties differed in their ability to germinate, the germination results were consistent for all varieties as indicated by the lack of a significant interaction between SHWT and variety (Table 6.2). None of the treatments significantly improved germination of N12 or N16. However, treatment at 36°-56°C for 10 minutes improved germination of N12, and treatments at 50° and 52°C improved germination of N16. Treatment of NCo376 at 36°, 50°C, 48° and 52°C improved germination, the latter two significantly so ($P<0.05$). Treatment of all varieties at 60°C significantly reduced germination (Table 6.3).

Table 6.3 Effect of short heat treatment of single-budded setts on the germination of varieties N12, N16 and NCo376.

Treatment	Germination %		
	N12	N16	NCo376
36°C	28 a	43 ab	38 ab
40°C	33 a	37 b	28 abc
44°C	25 a	40 ab	28 abc
48°C	33 a	42 ab	43 a
50°C	28 a	45 ab	33 abc
52°C	25 a	57 a	43 a
56°C	35 a	33 b	17 cd
60°C	0 b	10 c	0 d
Control	23 a	43 ab	20 bc
Mean	26	39	28
LSD (P=0.05) LSD (P=0.01)	13.6 18.6	17.0 23.3	19.7 27.0

Means in a column with a letter in common are not significantly different at the 5% level.

Statistical analysis of the data indicated that SHWT significantly affected mean dry mass of the three varieties (Table 6.2). Treatment at 36°, 48°, 52° and 56°C for 10 minutes improved dry mass, and treatment at 60°C significantly decreased the mean dry mass (Figure 6.6).

The dry mass results were consistent for both varieties as indicated by a lack of a significant interaction between SHWT and variety (Table 6.2). There were significant differences between treatments with N12 and NCo376, but not with N16 (Table 6.4). Treatment of N12 at temperatures of 40°-52°C had no effect on dry mass, treatment at 36°C increased dry mass and treatment at 60°C adversely affected dry mass ($P<0.01$). Treatment of N16 at 48°C increased dry mass. Treatment of NCo376 at 36°, 48°, 50° and 56°C increased dry mass, treatment at 52°C for 10 minutes significantly increased dry mass ($P<0.05$) and treatment at 60°C adversely affected dry mass ($P<0.01$).

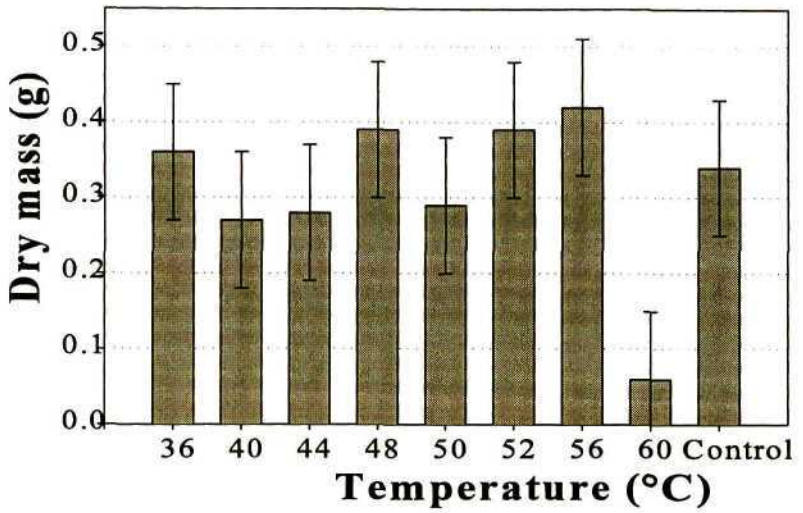

Figure 6.6 Effect of short heat treatment of single-budded setts on the mean dry mass of varieties N12, N16 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different.

Table 6.4 Effect of short heat treatment of single-budded setts on the dry mass of varieties N12, N16 and NCo376.

Treatment	Dry mass (g)		
	N12	N16	NCo376
36°C	0.34 a	0.47	0.28 ab
40°C	0.27 ab	0.31	0.22 c
44°C	0.24 ab	0.38	0.22 c
48°C	0.24 ab	0.58	0.30 ab
50°C	0.29 ab	0.26	0.33 ab
52°C	0.29 ab	0.46	0.41 a
56°C	0.19 b	0.35	0.40 ab
60°C	0 c	0.28	0 d
Control	0.31 ab	0.46	0.25 bc
Mean	0.26	0.43	0.28
LSD (P=0.05) LSD (P=0.01)	0.12 0.16	NS	0.16 0.21

Means in a column with a letter in common are not significantly different at the 5% level.

To summarise, when germination was low due to the use of poor quality seedcane in Experiment B, the mean germination of N12, N16 and NCo376 was significantly improved by heat treating the SBS at 48°C and 52°C for 10 minutes and both these treatments improved plant growth. Thus, the hormone balance of the buds may have been adjusted to an appropriate level for germination by the loss of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) during heat treatment, stimulating germination. Possible fungal infections within the SBS may have also been reduced or eliminated by the SHWT, improving germination.

When good quality seedcane was used in Experiment A, treatment at 52°C had no effect on germination. This is due to the fact that the hormonal balance was already appropriate for germination, allowing the untreated buds to germinate rapidly, surviving possible infection by systemic fungi. The significant increase in dry mass in Experiment A by treatments at 50° and 52°C for 10 and 20 minutes may have been a result of the reduction of IAA and IBA concentrations during SHWT to a level such that the higher concentration of GA_3 in the bud resulted in increased cell division and growth of the shoots.

Heat treatment at 52°C for 10 minutes can easily be implemented in the nursery to stimulate germination and growth of transplants, particularly when conditions are unfavourable for germination. Further experiments using SHWT at 52°C for 10 minutes are described in Chapters Seven and Eight, where chemicals and fungicides were added to the hot water.

6.1.4 Literature cited

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7. PLANT GROWTH REGULATORS

Abstract

Single-budded setts (SBS) were treated with ethephon and gibberellic acid (GA_3) to determine their effect on germination and growth of four commercial sugarcane varieties. In the first part of the preliminary experiment, GA_3 treatment decreased germination and had no effect on growth of N16, and had no effect on germination but increased the growth of N22. Treatment with ethephon at 7.2, 14.4 and 120 ppm significantly increased the mean germination of both varieties. In the second part of the preliminary experiment, ethephon at 14.4 ppm significantly increased the mean germination and increased the growth of varieties NCo376, N12 and N16. In a subsequent experiment, ethephon (14.4 ppm) in hot water (52°C; 10 minutes) significantly increased germination of NCo376, N12, N16, N17, N19 and N21, and growth of NCo376, N12 and N19. However, germination after treatment with ethephon in hot water was not significantly different from that of SBS treated with hot water alone.

7.1 Preliminary Experiments

7.1.1 Introduction

High germination of cane setts and rapid growth of plants has a significant effect on the establishment of a good crop resulting in better sugar yields. The indole-3-acetic acid (IAA) produced in the plant apex is inhibitory to the germination of lateral buds, and even when the apex is removed, the ability of lateral buds to germinate largely depends on the concentration of IAA within the bud relative to the concentrations of gibberellic acid (GA_3) and cytokinins.

Many attempts have been made to improve germination by treatment of setts with water, plant growth regulators, ripeners and other chemicals. The most effective treatments have involved the use of hot water, GA_3 and ethephon to establish an appropriate hormonal balance within the bud region for germination. Treatment of setts with GA_3 has been reported to stimulate germination of lateral buds (Chang & Li, 1962; Shiah & Pao, 1963; Bendigeri *et al.*, 1986), as has treatment with ethephon, which inhibits polar transport of IAA and activates its metabolism by the stimulation of peroxidase activity (Anon., 1988; Diaz *et al.*, 1995; Millhollon & Legendre, 1995). The aim of these experiments was to determine the effect of treating single-budded setts (SBS) before planting with ethephon and GA_3 on germination and growth of four commercial sugarcane varieties.

7.1.2 Materials and methods

In Experiment A, SBS of the varieties N16 and N22 were treated for ten minutes with either GA_3 (Gib Tablets®; Pazchem) at 1, 3, 5 and 7 ppm or ethephon (Ethrel®; Rhône-Poulenc) at 7.2, 14.4, 28.8, 57.6, 120, 240, 480 and 960 ppm, both dissolved in cold tap water. In Experiment B, SBS of the varieties NCo376, N12 and N16 were treated for ten minutes with 3 ppm GA_3 and 14.4 and 120 ppm ethephon, applied in cold tap water. Controls included untreated SBS, and SBS soaked in tap water for ten minutes.

Each treatment was represented by 60 SBS, divided into three replications of 20 SBS each. After treatment the SBS were planted into composted bagasse in polystyrene trays. Germination and dry mass of the transplants were recorded after 28 days and the data were pooled and evaluated using two-way analysis of variance (ANOVA). Mean separation was accomplished using least significant differences.

7.1.3 Results and discussion

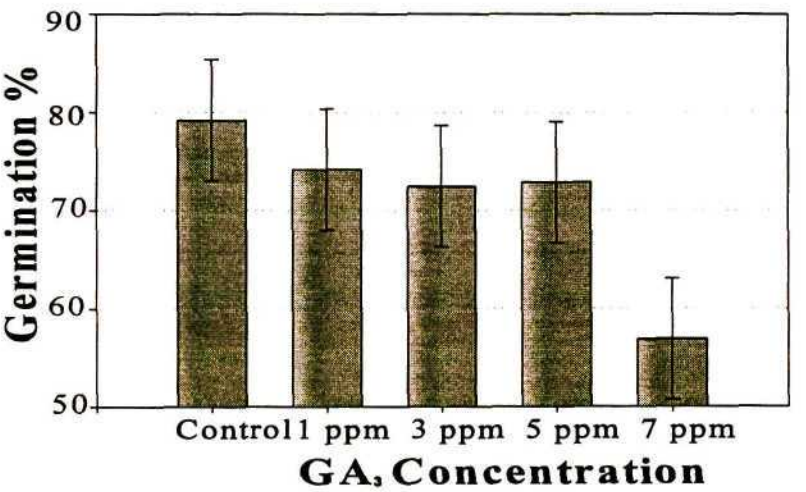
Experiment A - Gibberellic acid

Statistical analysis of the data indicated that GA_3 treatment of SBS had a significant effect on mean germination and dry mass (Table 7.1). All concentrations of GA_3 decreased the mean germination of N16 and N22, significantly so when SBS were treated with 7 ppm GA_3 (Figure 7.1a). All concentrations of GA_3 treatment increased the mean dry mass, significantly so when SBS were treated with 1 ppm ($P<0.01$) and 3 ppm GA_3 ($P<0.05$) (Figure 7.1b).

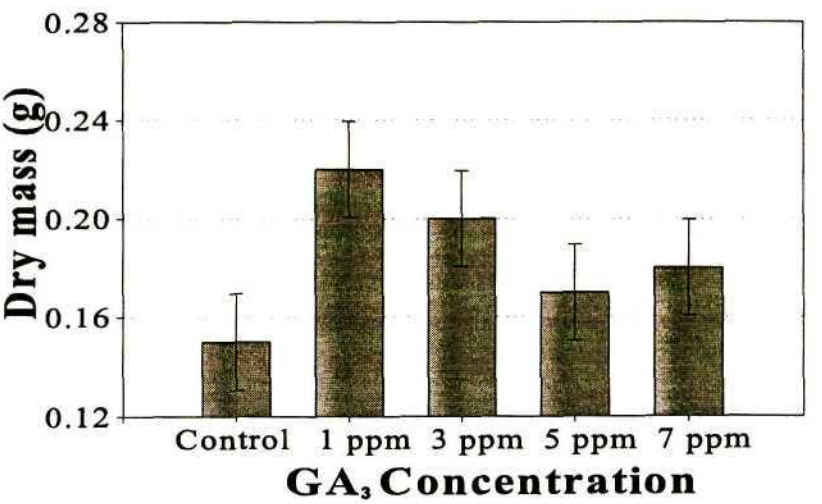
The germination and dry mass results were not consistent for both varieties as indicated by the significant interactions between GA_3 treatment and variety (Table 7.1). Compared with the control, treatment with GA_3 had no effect on germination but most concentrations (1, 3 and 5 ppm) significantly increased dry mass of N22. GA_3 treatment decreased germination and had no effect on dry mass of N16 (Table 7.2).

Table 7.1 Two-way ANOVA of mean germination and dry mass of varieties N16 and N22 after treatment of single-budded setts with gibberellic acid (GA_3).

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
GA_3	4	3.939	0.0135	3.990	0.0128
Variety	1	39.953	0.0000	35.398	0.0000
GA_3 x variety	4	3.576	0.0201	6.621	0.0010



(a)



(b)

Figure 7.1 Effect of gibberellic acid (GA_3) treatment of single-budded setts on the mean germination (a) and dry mass (b) of varieties N16 and N22. Means whose 95% comparison intervals do not overlap are significantly different.

Table 7.2 Effect of gibberellic acid (GA_3) treatment of single-budded setts on germination and dry mass of the varieties N16 and N22.

Treatment	Germination (%)		Dry mass (g)	
	N16	N22	N16	N22
Control	78 a	80	0.16	0.14 d
1 ppm GA_3	63 a	85	0.15	0.28 a
3 ppm GA_3	55 ab	90	0.13	0.27 ab
5 ppm GA_3	63 a	83	0.13	0.21 bc
7 ppm GA_3	35 b	79	0.16	0.19 cd
LSD (P=0.05) LSD (P=0.01)	25.1 35.7	NS	NS	0.06 0.08

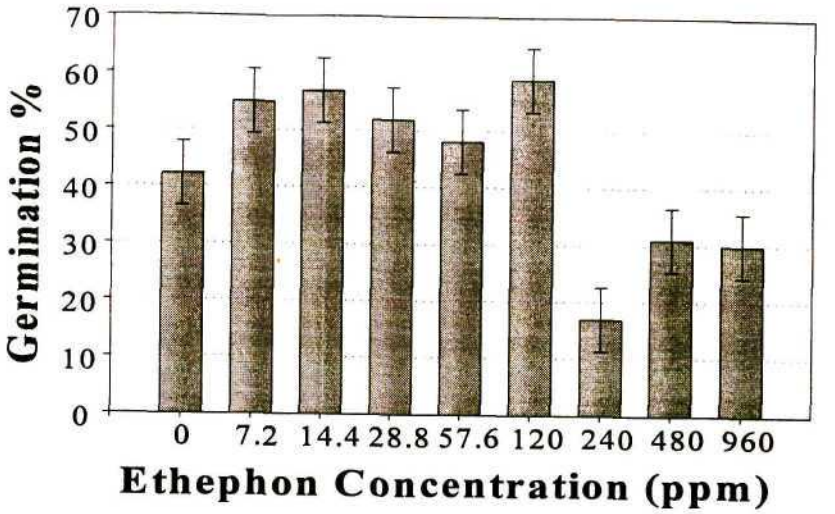
Means in a column with a letter in common are not significantly different at the 5% level.

Ethephon

Statistical analysis of the data indicated that ethephon treatment had a significant effect on mean germination and dry mass (Table 7.3). The mean germination was significantly increased by treatment of SBS with 7.2, 14.4 and 120 ppm ethephon. Treatment at concentrations higher than 120 ppm significantly decreased mean germination (Figure 7.2a). Treatment with ethephon at 7.2-240 and 960 ppm increased the mean dry mass, but not significantly. Treatment with 240 ppm ethephon decreased dry mass, and treatment with 480 ppm ethephon significantly decreased dry mass (Figure 7.2b).

Table 7.3 Two-way ANOVA of the mean germination percentages of varieties N16 and N22 after treatment of single-budded setts with ethephon.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Ethephon treatment (ET)	8	13.335	0.0000	4.432	0.0004
Variety	1	26.790	0.0000	0.125	0.7287
ET x variety	8	2.825	0.0108	1.613	0.1429



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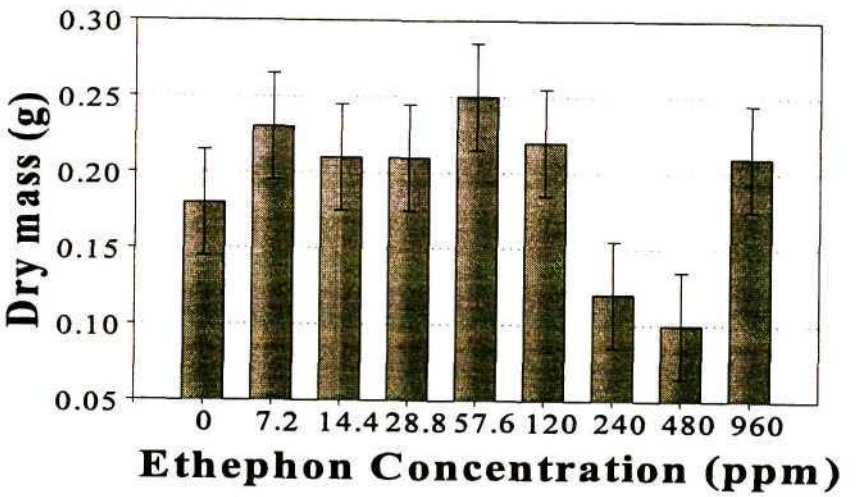


(b)

Figure 7.2 Effect of treatment of single-budded setts with ethephon on the mean germination (a) and dry mass (b) of varieties N16 and N22. Means whose 95% comparison intervals do not overlap are significantly different.

The germination results were not consistent for both varieties as indicated by a significant interaction between ethephon treatment and variety (Table 7.3). Treatment of N22 with 7.2 ppm ethephon increased germination, and treatment with 14.4 ppm and 120 ppm ethephon significantly increased germination by 46 and 37%, respectively ($P<0.05$; Table 7.4). Treatment of N16 with concentrations of ethephon of 7.2-120 ppm increased germination, and treatment at 28.8 ppm significantly increased germination by 58% ($P<0.05$). Treatment of both varieties with concentrations of ethephon greater than 120 ppm decreased germination, significantly so with N22, indicating phytotoxicity of ethephon at the higher concentrations.

Table 7.4 Effect of ethephon treatment of single-budded setts on the germination and dry mass of varieties N16 and N22.

Ethephon	Germination (%)		Dry mass (g)	
	N16	N22	N16	N22
Control	43 bc	41 bc	0.19 abc	0.17 bcd
7.2 ppm	59 ab	51 ab	0.25 a	0.21 abc
14.4 ppm	54 abc	60 a	0.21 ab	0.20 abc
28.8 ppm	68 a	36 c	0.21 ab	0.21 abc
57.6 ppm	59 ab	38 bc	0.23 a	0.26 ab
120 ppm	63 ab	56 a	0.20 abc	0.24 ab
240 ppm	23 d	13 d	0.15 bc	0.10 cd
480 ppm	39 bc	14 d	0.12 c	0.08 d
960 ppm	39 cd	21 d	0.15 bc	0.29 a
LSD (P=0.05)	19.9	14.3	0.09	0.11
LSD (P=0.01)	27.0	19.3	0.12	0.15

Means in a column with a letter in common are not significantly different at the 5% level.

The dry mass results were however consistent for varieties as indicated by the lack of a significant interaction between ethephon treatment and variety (Table 7.3). Treatment of N16 with concentrations of 7.2-120 ppm slightly increased dry mass, and concentrations higher than 120 ppm decreased dry mass (Table 7.4). Similar results occurred after treatment of N22, except that treatment with 960 ppm ethephon significantly increased dry mass ($P<0.05$) although this treatment significantly decreased mean germination.

In summary, treatment of SBS with ethephon at lower concentrations (7.2, 14.4 and 120 ppm) significantly increased the mean germination, and increased plant growth of both varieties. Treatment of SBS with concentrations greater than 120 ppm reduced germination and growth.

Experiment B

Statistical analysis of the data indicated that chemical treatment had a significant effect on germination (Table 7.5). Treatment of SBS with water and 120 ppm ethephon slightly increased the mean germination, and treatment with 14.4 ppm ethephon significantly increased germination compared with the untreated control (+30%, P=0.01) (Figure 7.3).

Table 7.5 Two-way ANOVA of the mean germination and dry mass data of varieties NCo376, N12 and N16 after treatment of single-budded setts with ethephon and gibberellic acid.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Chemical treatment (CT)	4	4.975	0.0035	2.608	0.0561
Variety	2	59.503	0.0000	18.717	0.0000
CT x variety	8	4.138	0.0022	2.437	0.0377

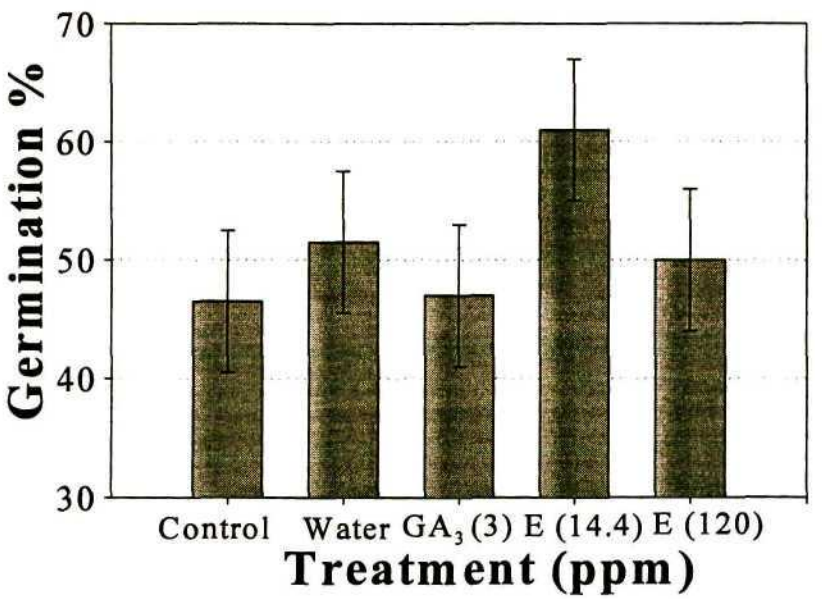


Figure 7.3 Effect of treatment of single-budded setts with gibberellic acid (GA_3) and ethephon (E) on the mean germination of NCo376, N12 and N16. Means whose 99% comparison intervals do not overlap are significantly different.

The germination results were not consistent for both varieties as indicated by the significant interaction between chemical treatment and variety (Table 7.5). The treatments had a significant effect on the germination of N16 and NCo376 (Table 7.6). Treatment with ethephon (14.4 ppm) significantly increased germination of NCo376 and N16, and treatment with 3 ppm GA_3 significantly increased germination of NCo376 ($P<0.05$) but decreased germination of N16. Similar results for N16 were obtained in Experiment A.

Table 7.6 Effect of treatment of single-budded setts with ethephon and gibberellic acid (GA_3) on germination of the varieties NCo376, N12 and N16.

Treatment	Germination %		
	NCo376	N12	N16
Control	63 b	48	28 bc
14.4 ppm ethephon	78 a	48	58 a
120 ppm ethephon	60 b	45	47 ab
3 ppm GA_3	77 a	42	23 c
Cold water	65 b	48	42 abc
LSD (P=0.05) LSD (P=0.01)	11.51 16.37	NS	19.3 27.7

Means in a column with a letter in common are not significantly different at the 5% level.

Statistical analysis of the data indicated that chemical treatment did not have a significant effect on dry mass (Table 7.5). Compared with the control, mean dry mass was increased when SBS were treated with 14.4 ppm ethephon (Figure 7.4). Ethephon at 120 ppm decreased dry mass, and treatment with 3 ppm GA_3 and the cold water treatment slightly increased dry mass.

The results were not consistent for varieties as indicated by the significant interaction between treatment and variety (Table 7.5). The treatments had a significant effect on dry mass of NCo376, but not N12 and N16. Treatment of NCo376 with 3 ppm GA_3, 14.4 ppm ethephon and cold water significantly increased dry mass ($P=0.01$). Compared with the cold water control, treatment with 3 ppm GA_3 had no effect on dry mass, and treatment with 14.4 ppm ethephon significantly improved dry mass ($P=0.01$) (Table 7.7).

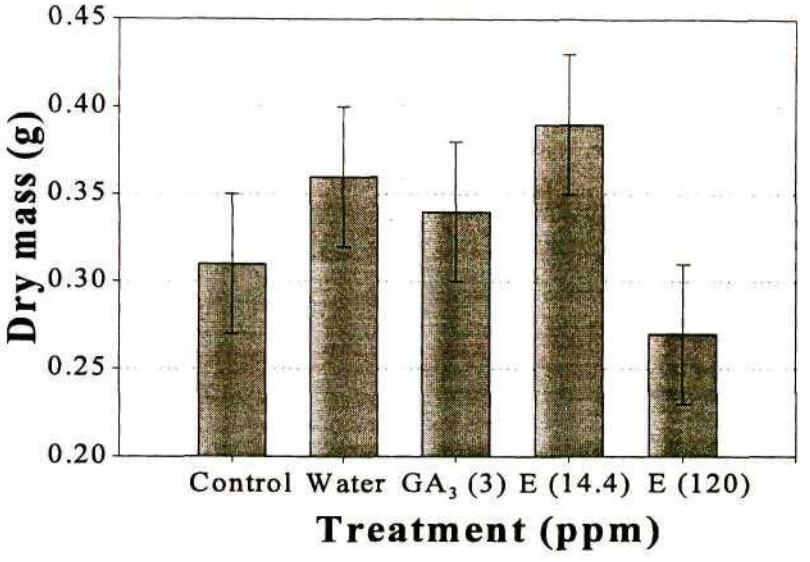


Figure 7.4 Effect of single-budded sett treatment with gibberellic acid (GA_3) and ethephon (E) on the mean dry mass of varieties NCo376, N12 and N16. Means whose 99% comparison intervals do not overlap are significantly different.

Table 7.7 Effect of treatment of single-budded setts with gibberellic acid (GA_3) and ethephon on the dry mass (g) of the varieties NCo376, N12 and N16.

Treatment	Dry mass (g)		
	NCo376	N12	N16
Control	0.24 c	0.44	0.23
14.4 ppm ethephon	0.54 a	0.43	0.20
120 ppm ethephon	0.25 c	0.37	0.20
3 ppm GA_3	0.41 b	0.35	0.25
Cold water	0.41 b	0.44	0.23
LSD (P=0.05) LSD (P=0.01)	0.08 0.12	NS	NS

Means in a column with a letter in common are not significantly different at the 5% level.

The significant increases in germination and growth after treatment with 14.4 ppm ethephon indicated that ethephon at a suitable concentration may be involved in the release of buds from correlative inhibition. Although Diaz *et al.* (1995) reported that ethephon at concentrations equal to and greater than 120 ppm significantly increased germination of Cuban varieties, these higher concentrations decreased germination and were phytotoxic to the South African commercial varieties tested in Experiments A and B.

The use of GA_3 at 3 ppm as a sett treatment was ineffective in the promotion of lateral bud germination of N12 and N16, but significantly increased germination of NCo376. These results indicated that the response to GA_3 depended on variety and may have also depended on the physiological status of the cane, which is affected by cane age, quality and growing conditions. The reduced germination of N12 and N16 indicated that GA_3 may be phytotoxic or the levels of GA_3 required to overcome apical dominance differs between varieties, making the use of GA_3 impractical. Due to these different varietal responses, no further research was carried out using this plant growth regulator.

7.2 Ethephon

7.2.1 Introduction

Ethephon has been shown to significantly improve germination of setts in South Africa, Cuba and Louisiana (Anon., 1988; Manoharan *et al.*, 1992; Diaz *et al.*,1995; Milhollon & Legendre, 1995) . In the previous experiments (Section 7.1), treatment of SBS with ethephon at 14.4 ppm for ten minutes significantly improved germination of N22, NCo376 and N16, all of which have a relatively good germination potential. Experiments in Chapter Six indicated that short hot water treatment (SHWT) of SBS at 52°C for 10 minutes stimulated germination by possibly affecting the hormonal balance. The aim of this experiment was to determine whether the addition of ethephon to the hot water could further increase germination and plant growth of six commercial varieties compared with treatment with hot water alone.

7.2.2 Materials and methods

Single-budded setts of six varieties differing in their ability to germinate (NCo376, N12, N16, N17, N19 and N21) were soaked either in hot water (52°C) or in a hot solution (52°C) of ethephon (14.4 ppm) for ten minutes. The treatments were carried out in wire baskets that were submerged in the solutions. Each treatment was represented by a total of 60 SBS, divided into three replications of 20 SBS each. After treatment, the SBS were planted into

composted bagasse in polystyrene trays. Germination and dry mass of the transplants was recorded after 28 days and pooled and evaluated using two-way analysis of variance (ANOVA). Means separation was achieved using least significant differences.

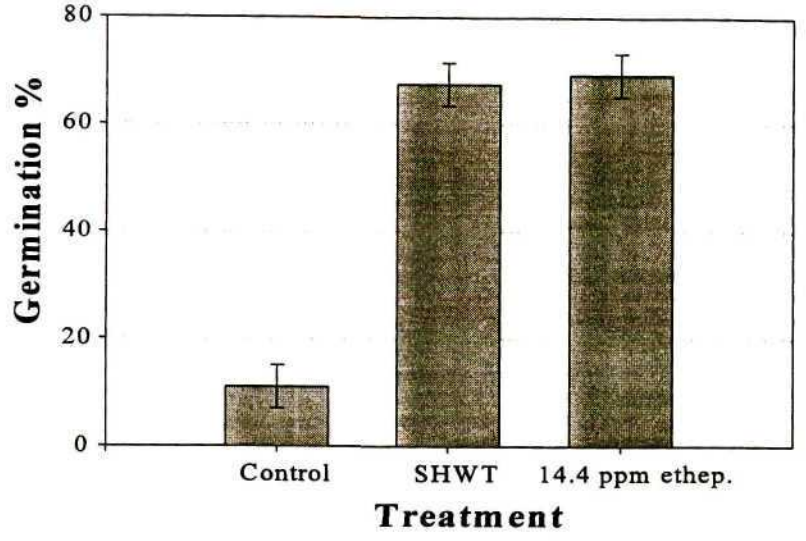
7.2.3 Results and discussion

Germination of the controls was poor because temperatures at the time of planting were low and unfavourable for germination. Furthermore, the heater fans in the germination room were not functioning due to an electrical fault, resulting in the temperature remaining at approximately 20°C, well below the optimum for germination.

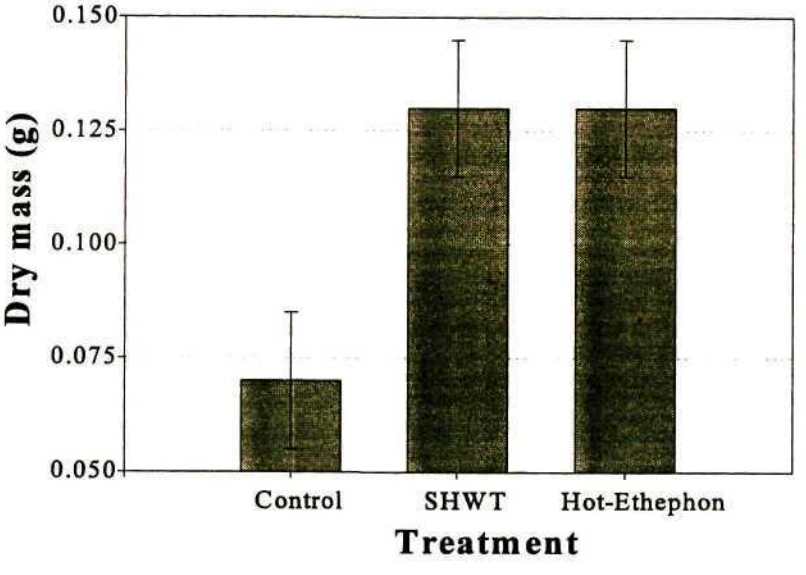
Statistical analysis of the data indicated that SBS treatment had a significant effect on germination and dry weight (Table 7.8). Treatment of SBS with the SHWT and ethephon in hot water significantly increased the mean germination by 509 and 527% respectively ($P<0.01$; Figure 7.5a) and significantly increased dry mass ($P<0.01$; Figure 7.5b). For both germination and dry mass there was no significant difference between these treatments.

Table 7.8 Two-way ANOVA of the germination and dry mass data of varieties NCo376, N12, N16, N17, N19 and N21 after treatment of single-budded setts with hot water and ethephon.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Treatment	2	249.738	0.0000	20.831	0.0000
Variety	5	8.050	0.0000	11.961	0.0000
Treatment x variety	10	2.685	0.0109	0.966	0.4854



(a)



(b)

Figure 7.5 Effect of short heat treatment (SHWT) and hot-ethephon treatment on the mean germination (a) and dry mass (b) of six sugarcane varieties. Means whose 99% comparison intervals do not overlap are significantly different.

The germination results were not consistent for all varieties as indicated by the significant interaction between treatment and variety (Table 7.8). It was interesting to note that although the varieties NCo376, N16, N19 and N21 are considered to have rapid germination, the germination of the untreated controls of these varieties was similar to that of the poor germinating varieties N12 and N17. Both the SHWT and the hot-ethephon soak significantly

improved germination of all the varieties compared with the control, and there was no significant difference between these treatments ($P<0.01$) (Table 7.9; Figure 7.6). Germination of N17, N19 and N21 was highest when SBS were treated with the SHWT, and germination of NCo376 and N16 was highest after treatment with ethephon. Therefore, both poor and good germinating varieties reacted favourably to both treatments.

Table 7.9 Effect of short heat treatment (SHWT) and hot-ethephon treatment of single-budded setts on the germination of six varieties.

Treatment	Germination (%)					
	NCo376	N12	N16	N17	N19	N21
Control	21 b	4 b	14 b	8 b	12 b	6 b
SHWT	70 a	62 a	45 a	84 a	82 a	68 a
Ethephon	79 a	62 a	60 a	78 a	78 a	59 a
Mean	57	43	40	57	57	43
LSD (P=0.05)	18.6	15.0	16.8	22.2	10.1	14.7
LSD (P=0.01)	28.2	22.2	24.2	31.9	15.0	21.3

Means in a column with a letter in common are not significantly different at the 1% level.

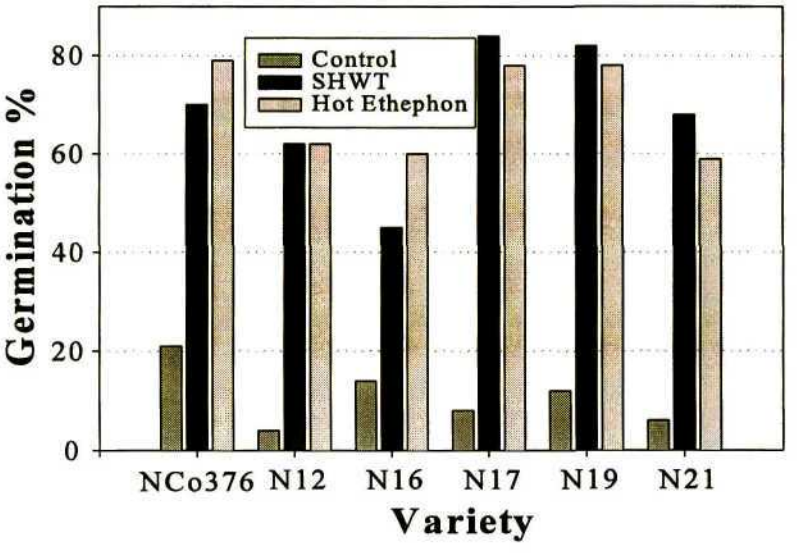


Figure 7.6 Effect of short heat treatment (SHWT) and hot-ethephon treatment of single-budded setts on the germination of six sugarcane varieties.

The dry weight results were consistent for all varieties as indicated by the lack of a significant interaction between treatment and variety (Table 7.8). There were significant differences between treatments with NCo376, N12 and N19 where dry mass was significantly increased with the hot-ethephon treatment ($P<0.01$). The SHWT also significantly increased dry mass of NCo376 ($P<0.01$), N19 ($P<0.01$) and N12 ($P<0.05$) (Table 7.10, Figure 7.7). Dry mass of N12 and NCo376 was highest when SBS were treated with ethephon, and dry mass of N19 was highest when SBS were treated with the SHWT. There was no significant difference between the SHWT and the ethephon treatment with these three varieties. Both treatments also improved dry mass of N16, N17 and N21, but not significantly.

Table 7.10 Effect of short heat treatment (SHWT) and ethephon treatment of single-budded setts on the dry mass (g) of varieties NCo376, N12, N16, N17, N19 and N21.

Treatment	Dry mass (g)					
	NCo376	N12	N16	N17	N19	N21
Control	0.08 b	0.03 b	0.07	0.06	0.06 b	0.11
SHWT	0.15 a	0.08 a	0.08	0.12	0.17 a	0.18
Ethephon	0.18 a	0.10 a	0.09	0.10	0.14 a	0.20
Mean	0.14	0.07	0.08	0.10	0.12	0.17
LSD (P=0.05) LSD (P=0.01)	0.06 0.09	0.04 0.06	NS	NS	0.17 0.02	NS

Means in a column with a letter in common are not significantly different at the 5% level.

In summary, SHWT and the hot-ethephon treatment at 14.4 ppm significantly improved the overall germination and dry mass of six sugarcane varieties ($P<0.01$) when conditions were unfavourable for germination. However, there was no added benefit of adding ethephon to the hot water because the SHWT alone significantly improved germination and growth. Additionally, in some varieties germination and growth were higher when SBS were treated with the SHWT than with the ethephon treatment. Therefore, the SHWT is highly recommended for the treatment of SBS free of *C. xyli* subsp. *xyli,* but the addition of ethephon to the hot water is not recommended for transplant production.

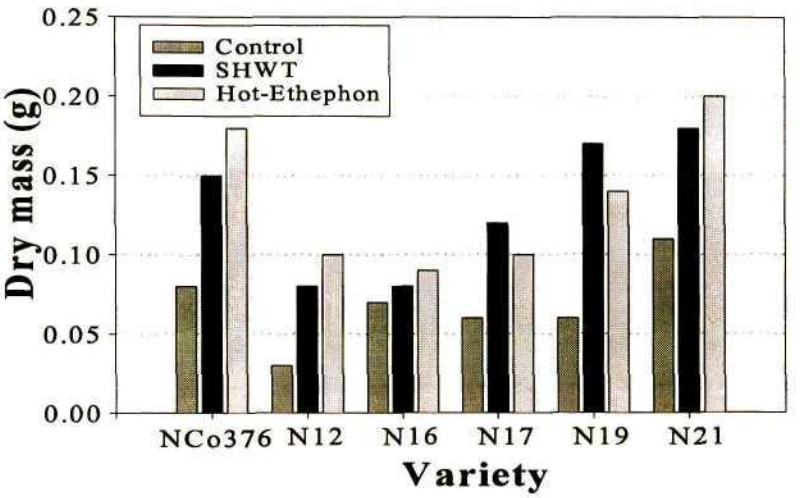


Figure 7.7 Effect of short heat treatment (SHWT) and hot-ethephon treatment of single-budded setts on dry mass of six sugarcane varieties.

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8. FUNGICIDE TREATMENT OF SINGLE-BUDDED SETTS

Abstract

Experiments were carried out to determine the effect of various fungicides on germination and plant growth. Treatment of single-budded setts (SBS) with the fungicides propamocarb-HCl, guazatine, carbendazim+ difenoconazole, carbendazim+flusilazole and propiconazole improved germination, which was highest after treatment with carbendazim+difenoconazole (Eria®; 0.5 $m\ell.\ell^{-1}$). Treatment of SBS planted in both unsterilised and sterilised media with Eria® in hot water (52°C) for 10 minutes significantly increased germination and plant growth compared with both the control and the short heat treatment (SHWT). These results indicated that the role of Eria® was not entirely fungicidal. Treatment with a propamocarb-HCl/benomyl solution had little effect on germination and growth, indicating the small role of systemic infections in germination failure. Therefore, germination and plant growth were predominantly increased by the plant growth regulatory activities of the active ingredients, carbendazim and difenoconazole. Used separately, both carbendazim and difenoconazole significantly increased germination, but only difenoconazole caused significant increases in dry mass. These results provided some evidence that germination failure is caused by the inappropriate hormonal balance within the SBS, rather than systemic infections or infection of the SBS by soilborne pathogens.

8.1 Preliminary Fungicide Experiment

8.1.1 Introduction

Due to the monoculture of sugarcane over large areas for several years, pathogens such as *Ceratocystis paradoxa* (Dade) C. Moreau, *Glomerella tucumanensis* (Speg.) V. Arx & E. Müller, *Pythium* spp., *Rhizoctonia solani* Kűhn and *Fusarium* spp. accumulate in the field. When adverse conditions prevail, germination is delayed and these pathogens can cause sett decay and germination failure. It is essential to apply fungicides as a sett treatment when temperatures are low, the seedcane is old or the setts have been heat treated (Barnes, 1974). In most transplant nurseries pathogens do not accumulate because growing media are usually discarded after use. Therefore, setts are only treated in the winter months when germination is delayed by low temperatures.

In South Africa, benomyl (Benlate®, Du Pont de Nemours; Fundazol®, Sanachem) and guazatine (Panoctine®, Rhône-Poulenc) are registered as sett treatments against pineapple disease (*C. paradoxa*) and sett decay caused by *R. solani* and *Fusarium* spp. (Krause *et al.*, 1996). However, there are problems associated with the use of these fungicides. The toxicity of a fungicide must be taken into account when choosing a fungicide for the treatment of SBS

because setts are always planted into trays by hands which are not usually protected by water resistant gloves. Pesticides can be absorbed through the skin and accumulate in the body, causing acute poison symptoms or death (Vermeulen *et al.*, 1990). Guazatine is classified as a Group II pesticide, indicating that it is poisonous and should not be handled without gloves. At the time of this study guazatine was not available or imported into South Africa as it is only registered for use on sugarcane and wheat. However, it has recently been re-introduced and is now readily available.

Benomyl is relatively harmless (Group IV). This fungicide is available as a wettable powder that is insoluble in water. Therefore, it tends to settle out of solution, resulting in poor coating of the setts by the fungicide unless constantly stirred (Mitchell-Innes & Thomson, 1973).

The aim of this experiment was to determine the value of using fungicides in the nursery, and to test which of eight fungicides were most efficacious in contributing to increased germination and plant growth. All but one of the fungicides were less toxic than guazatine and most were soluble in water. The fungicides tested and their toxicity rating were propamocarb-HCl (Previcur N®, AgrEvo; IV), benomyl (Benlate®; IV), guazatine (Panoctine®; II), carbendazim+ difenoconazole (Eria®, Novartis; III), carbendazim+flusilazole (Punch-Xtra®, Du Pont de Nemours; III), propiconazole (Tilt®, Novartis; III), captab (Captab®, Agricura; IV) and triadimefon (Bayleton®, Bayer; II).

Most of the fungicides tested are systemic and have a curative effect after penetrating plant tissue. Guazatine was the only protective fungicide tested which inhibits fungal growth or spore germination on the treated surface (Table 8.1).

Table 8.1. Fungicides tested for treatment of single-budded setts (DS = powder for dry seed treatment; SL = soluble concentrate; WP = wettable powder; EC = emulsifiable concentrate; SC = suspension concentrate; FC = fungicide; SDI = steroid demethylation inhibitor) (Worthing & Hance, 1991; Krause *et al.*, 1996).

Active ingredient	Activity of a.i.	Trade name	Fungal class controlled	Diseases controlled*	Target organisms*
propamocarb-hydrochloride SL 72.2%	carbamate FC	Previcur N*	Oomycetes	damping-off, seed decay & root rot.	*Phytophthora, Pythium, Peronospora, Pseudoperonospora*
benomyl WP 50%	benzimidazole FC	Benlate*	Asco-, Basidio-, & Deuteromycetes	black- & ring spot; blossom blight; botrytis-, brown-, bulb-, root- & sclerotinia rot; freckle; fruit spot; grey leaf spot; pineapple disease; post-harvest decay; powdery mildew; scab & sett decay.	*Actinonema, Alternaria, Botrytis, Ceratocystis, Cercospora, Colletotrichum, Diplodia, Fusarium, Fusicladium, Guignardia, Hendersonia, Monilinia, Mycosphaerella, Oidiopsis, Oidium, Penicillium, Podosphaera, Pseudocercospora, Rhizoctonia, Sclerotinia, Thielaviopsis, Venturia, Verticillium.*
guazatine SC 40%	guanidine FC	Panoctine*	Asco-, Basidio-, & Deuteromycetes	pineapple disease, sett decay & stinking smut	*Ceratocystis, Fusarium, Rhizoctonia, Tilletia.*
carbendazim SC 12.5% difenoconazole SC 6.25%	benzimidazole FC/ conazole FC, SDI	Eria*	Asco-, Basidio-, & Deuteromycetes	grey leaf spot & leaf spot	*Cercospora, Phoma.*
carbendazim SC 25% flusilazole SC 12.5%	benzimidazole FC conazole FC, SDI	Punch-Xtra*	Asco-, Basidio-, & Deuteromycetes	eye spot, grey leaf spot, rust.	*Cercospora, Puccinia, Pseudocercosporella.*
propiconazole EC 25%	conazole FC, SDI	Tilt*	Asco-, Basidio-, & Deuteromycetes	blossom blight; eye- & leaf spot; leaf rust & powdery mildew.	*Erysiphe, Monilinia, Oidium, Pseudocercospora, Puccinia, Rhyncosporium.*
captab WP 50%	phthalimide FC	Captab*	Asco-, Basidio-, Deutero-, & Oomycetes	anthracnose, black spot, dollar spot, early blight, fairy ring & scab.	*Actinonema, Fusicladium, Helminthosporium, Marasimus, Rhizoctonia, Sclerotinia, Sphaceloma, Venturia.*
triadimefon DS 25%	conazole FC, SDI	Bayleton*	Asco-, Basidio-, & Deuteromycetes	cob & tassel smut, pineapple disease, sett decay.	*Cercospora, Fusarium, Hemileia, Podosphaera, Rhizoctonia, Rhynchosporium, Sphacelotheca.*

* = registered at the given concentration of active ingredient for the control of these diseases and pathogens in South Africa (Krause *et al.*, 1996).

8.1.2 Materials and methods

Single-budded setts (SBS) of varieties N17 and NCo376 were hot water treated at 50°C for 120 minutes and then soaked for five minutes in the relevant fungicides which were mixed with tap water at the appropriate concentrations (Table 8.2). Two experiments were carried out because of the large quantities of seedcane required for each treatment. Single-budded setts were treated with captab, propamocarb-HCl, benomyl, guazatine and triadimefon in Experiment A, and with carbendazim+difenoconazole (C+D), propiconazole, and carbendazim+flusilazole (C+F) in Experiment B. The control consisted of untreated SBS. Each treatment was represented by a total of 60 SBS, divided into three replications of 20 SBS each. The SBS were planted into trays, left in a germination room (28°C) for seven days and then placed into the nursery. The germination and dry mass were recorded after 30 days and the data analysed using two-way analysis of variance (ANOVA). Separation of mean differences was accomplished using least significant differences.

Table 8.2 Rates of fungicides used for the treatment of the varieties N17 and NCo376.

Commercial name (active ingredient)	**Rate**	**Concentration of active ingredient**
Previcur N® (propamocarb-HCl)	0.5 mℓ.ℓ$^{-1}$ 1.0 mℓ.ℓ$^{-1}$ 1.5 mℓ.ℓ$^{-1}$ 2 mℓ.ℓ$^{-1}$	360 ppm 722 ppm 1083 ppm 1444 ppm
Benlate® (benomyl)	0.5 g.ℓ$^{-1}$*	250 ppm
Panoctine® (guazatine)	2 mℓ.ℓ$^{-1}$*	800 ppm
Eria® (carbendazim+difenoconazole)	0.2 mℓ.ℓ$^{-1}$ 0.5 mℓ.ℓ$^{-1}$ 1 mℓ.ℓ$^{-1}$ 1.5 mℓ.ℓ$^{-1}$	25 ppm : 12.5 ppm 62.5 ppm : 31.25 ppm 125 ppm : 62.5 ppm 187.5 ppm : 94 ppm
Punch-Xtra® (carbendazim+flusilazole)	0.5 mℓ.ℓ$^{-1}$ 1 mℓ.ℓ$^{-1}$ 1.5 mℓ.ℓ$^{-1}$ 2 mℓ.ℓ$^{-1}$	125 ppm : 62.5 ppm 250 ppm : 125 ppm 375 ppm : 187.5 ppm 500 ppm : 250 ppm
Tilt® (propiconazole)	0.02 mℓ.ℓ$^{-1}$ 0.2 mℓ.ℓ$^{-1}$ 1.0 mℓ.ℓ$^{-1}$ 2 mℓ.ℓ$^{-1}$	5 ppm 50 ppm 250 ppm 500 ppm

Commercial name (active ingredient)	Rate	Concentration of active ingredient
Captab* (captab)	0.5 g.ℓ^{-1} 1 g.ℓ^{-1} 2 g.ℓ^{-1} 3 g.ℓ^{-1}	250 ppm 500 ppm 1000 ppm 1500 ppm
Bayleton* (triadimefon)	0.2 g.ℓ^{-1} 0.5 g.ℓ^{-1} 1 g.ℓ^{-1}*	50 ppm 125 ppm 250 ppm

* = registered concentration for the control of pineapple disease and sett decay in South Africa.

8.1.3 Results and discussion

Experiment A

Statistical analysis of the data indicated that the mean germination of N17 and NCo376 was significantly affected by fungicide treatment ($P<0.05$; Table 8.3). The germination of the control was high (72.5%), suggesting that there was little SBS infection. None of the fungicide treatments were significantly different from the control, but germination was increased by treatment with guazatine (800 ppm) and propamocarb-HCl (722, 1083 and 1444 ppm), the latter indicating the possible presence of *Pythium* spp. (Figure 8.1).

Table 8.3 Two-way ANOVA of the mean germination and dry mass data of varieties N17 and NCo376 after fungicide treatment of single-budded setts.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Fungicide	13	2.318	0.0153	2.414	0.0116
Variety	1	73.9626	0.0000	2.619	0.1112
Fungicide x variety	13	3.468	0.0006	1.796	0.0664

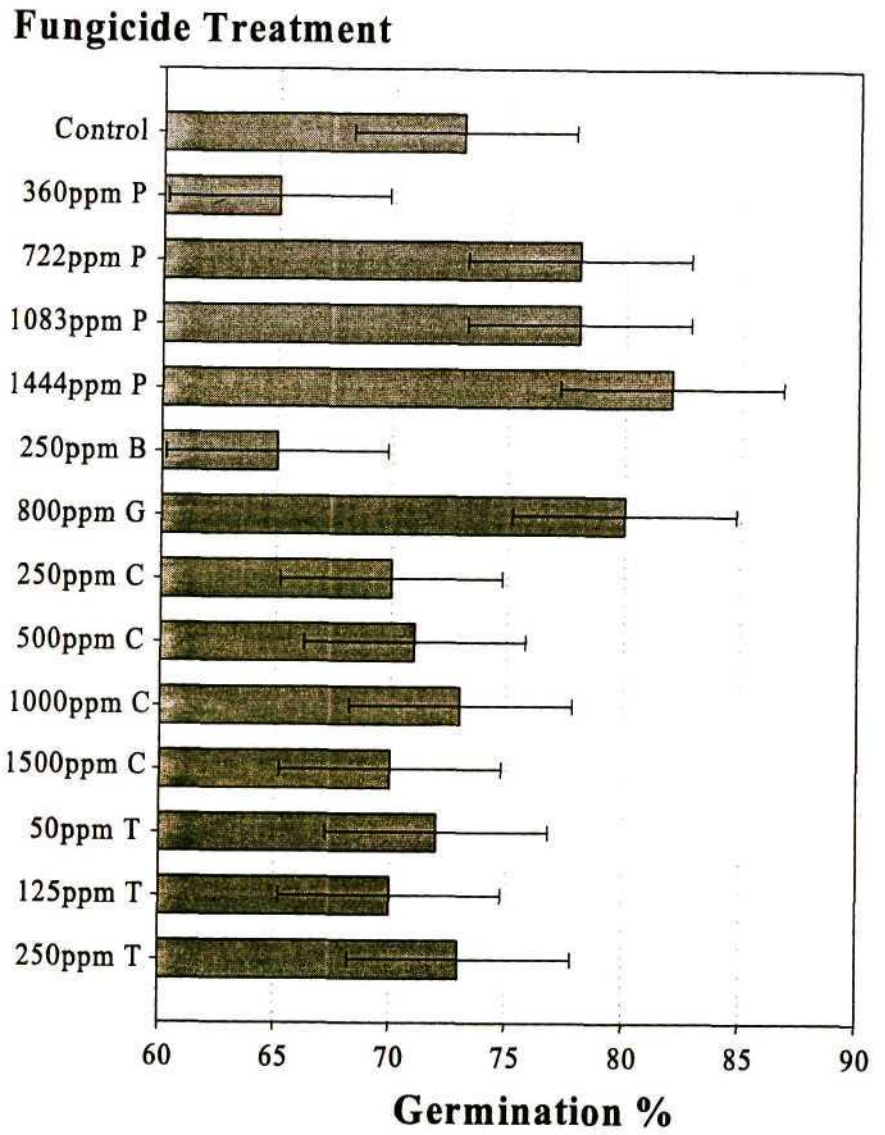


Figure 8.1 Effect of fungicide treatment on the mean germination of varieties N17 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different. (P=propamocarb; B=benomyl; G=guazatine; C= captab; T=triadimefon).

The results were not consistent for both varieties as indicated by the significant interaction between fungicide and variety (Table 8.3). Although there were significant differences between fungicide treatments for both varieties, none of the fungicides significantly increased germination compared with the control (Table 8.4).

All fungicides, except benomyl, increased germination of N17 and NCo376. Germination of N17 was highest after treatment with 722 and 1083 ppm propamocarb-HCl (+15-18%) and the germination of NCo376 was increased by 15-16% after treatment with 50 ppm triadimefon, 1444 ppm propamocarb-HCl and guazatine. The registered treatment with 800 ppm guazatine increased germination of both varieties. However, the registered treatment with benomyl had no effect on germination of N17 and decreased germination of NCo376 (Table 8.4).

Table 8.4 Effect of fungicide treatment of single-budded setts on the germination and dry mass of varieties N17 and NCo376.

Treatment		Germination %		Dry mass (g)	
Fungicide	**Concentration**	**N17**	**NCo376**	**N17**	**NCo376**
triadimefon	**50 ppm**	67 f	77 a	0.18	0.17
	125 ppm	77 def	63 abcd	0.15	0.22
	250 ppm	83 abcd	62 abcd	0.24	0.33
captab	**250 ppm**	83 abcde	57 bcd	0.23	0.19
	500 ppm	78 cdef	65 abc	0.16	0.18
	1000 ppm	85 abcd	62 abcd	0.29	0.14
	1500 ppm	68 ef	72 ab	0.24	0.15
propamocarb-HCl	**360 ppm**	82 abcde	48 d	0.22	0.14
	722 ppm	90 abc	67 abc	0.25	0.25
	1083 ppm	92 abc	63 abcd	0.32	0.23
	1444 ppm	87 abcd	78 a	0.20	0.20
benomyl	**250 ppm**	77 def	53 cd	0.19	0.20
guazatine	**800 ppm**	83 abcd	77 a	0.16	0.17
control	-	78 bcdef	67 abc	0.26	0.19
LSD (P=0.05)		14.58	15.74	NS	NS
LSD (P=0.01)		19.76	21.25		

Means in a column with a letter in common are not significantly different at the 5% level.

The wettable powders captab, benomyl and triadimefon had no effect on germination compared with the control, indicating that the powders may have adhered poorly to the SBS after the five

minute treatments due to settling out of solution. Germination of SBS after treatment with the soluble concentrates guazatine and propamocarb-HCl (722, 1083 and 1444 ppm) was significantly greater than after treatment with benomyl. These results indicated that guazatine and propamocarb-HCl may have adhered to the SBS surface more efficiently than the wettable powders, providing better disease control.

Statistical analysis of the data indicated that fungicide treatment had a significant effect on dry mass ($P < 0.05$; Table 8.3). However, none of the treatments significantly increased the mean dry mass compared with the control. The dry mass was increased by treatment with propamocarb-HCl (722 and 1083 ppm), and decreased by treatment with both benomyl (250 ppm) and guazatine (800 ppm) compared with the control (Figure 8.2).

The dry mass results were consistent for both varieties as indicated by the lack of a significant interaction between variety and fungicide treatment (Table 8.3). The fungicide treatments had no significant effects on the dry mass of N17 and NCo376 (Table 8.4). Most fungicide treatments, except captab (1000 ppm) and propamocarb-HCl (1083 ppm), decreased dry mass of N17. Dry mass of NCo376 was increased by treatment with triadimefon (250 ppm) and propamocarb-HCl (722 and 1083 ppm).

Results from this experiment indicated that the most promising fungicide was propamocarb-HCl at 722, 1083 and 1444 ppm. Since this fungicide only controls Oomycetes, its success indicated that *Pythium* or *Phytophthora* may have been present in the growing medium, causing sett mortality. Germination was also increased by treatment with guazatine indicating the control of pathogenic fungi belonging to the Asco-, Basidio- or Deuteromycetes.

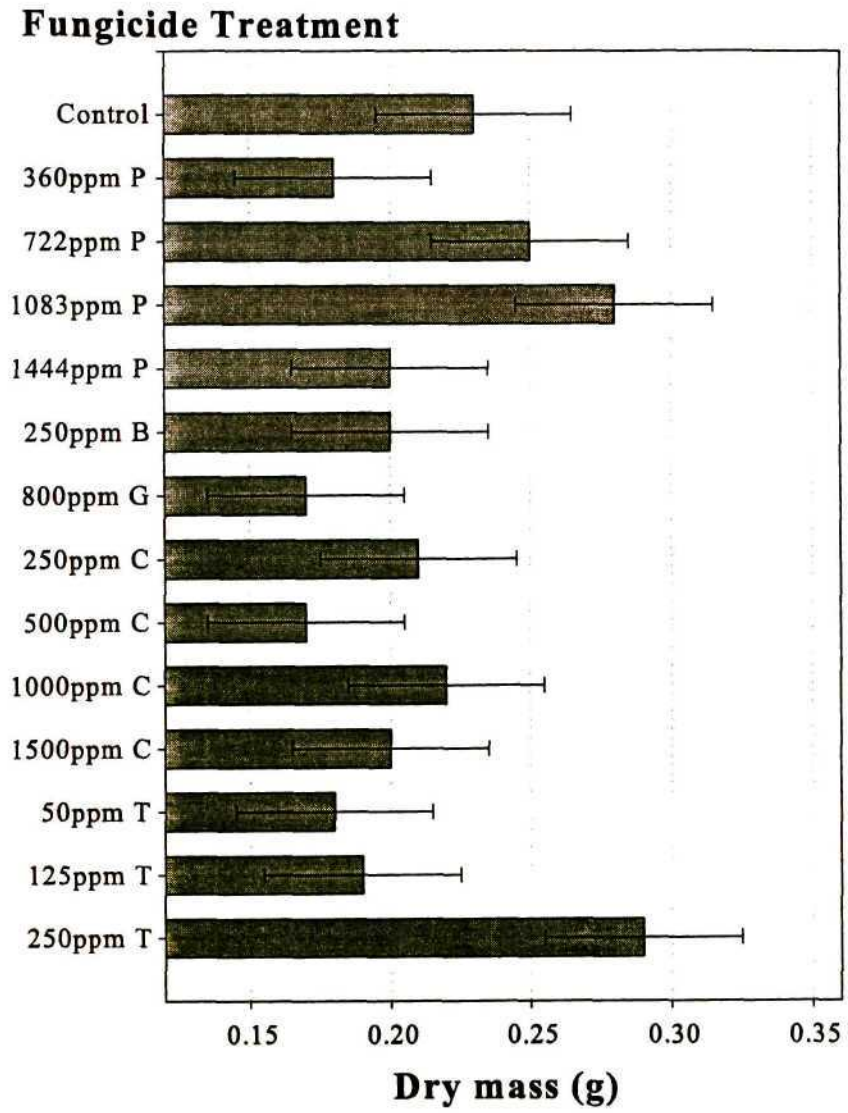


Figure 8.2 Effect of fungicide treatment of single-budded setts on the mean dry mass of varieties N17 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different. (P=propamocarb; B=benomyl; G= guazatine; C=captab; T=triadimefon).

Experiment B

The mean germination of the untreated controls of the two varieties was high (78%), indicating that there was little SBS infection. Statistical analysis of the data indicated that fungicide treatment did not have a significant effect on germination (P=0.05; Table 8.5). Most concentrations of C+F and propiconazole decreased germination (Figure 8.3).

Table 8.5 Two-way ANOVA of the mean germination and dry mass data of varieties N17 and NCo376 after fungicide treatment of single-budded setts.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Fungicide	12	1.937	0.0543	4.816	0.0000
Variety	1	47.9626	0.0000	4.512	0.0384
Fungicide x variety	12	2.628	0.0092	0.596	0.8351

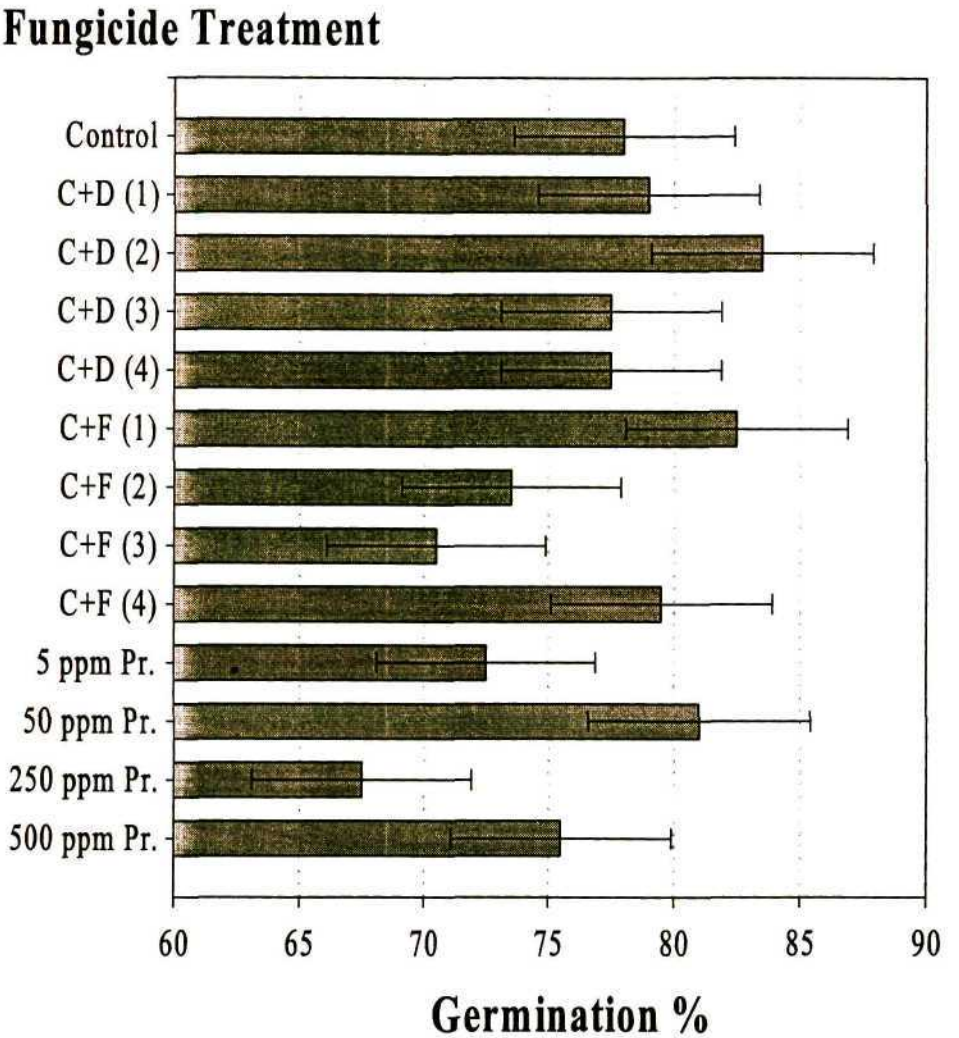


Figure 8.3 Effect of fungicide treatment of single-budded setts on the mean germination of varieties N17 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different. (C+D=carbendazim+ difenoconazole; C+F=carbendazim+flusilazole; Pr.=propiconazole).

The germination results were consistent for both varieties as indicated by the significant interaction between fungicide treatment and variety (Table 8.5). Treatment with C+D, propiconazole and C+F at most concentrations improved germination of N17, the treatments with 50 ppm propiconazole, C+F(1) and C+F(2) significantly so (+15-18%, $P<0.05$) (Table 8.6). Treatment with C+D at three concentrations slightly increased germination, and both propiconazole and C+F decreased germination of NCo376. These results indicated that there were differing varietal responses to treatment with both C+F and propiconazole, but not to C+D(2).

Table 8.6 Effect of fungicide treatment on the germination and dry mass of varieties N17 and NCo376.

Treatment		Germination (%)		Dry mass (g)	
Conc. of active ingredient	Abbrev.	N17	NCo376	N17	NCo376
25 ppm C + 12.5 ppm D	C+D(1)	78 bc	80 abc	0.34 a	0.24
62.5 ppm C + 31.25 ppm D	C+D(2)	85 abc	82 a	0.24 abc	0.25
125 ppm C + 62.5 ppm D	C+D(3)	75 c	80 abc	0.25 ab	0.20
187.5 ppm C + 94 ppm D	C+D(4)	82 abc	73 abcd	0.26 ab	0.18
5 ppm propiconazole	-	82 abc	63 bcd	0.14 bcd	0.13
50 ppm propiconazole	-	90 a	72 abcd	0.12 cd	0.13
250 ppm propiconazole	-	78 bc	57 d	0.12 cd	0.12
500 ppm propiconazole	-	85 abc	66 abcd	0.09 d	0.11
125 ppm C + 62.5 ppm F	C+F(1)	92 a	73 abcd	0.15 bcd	0.14
250 ppm C + 125 ppm F	C+F(2)	92 a	55 d	0.19 bcd	0.17
375 ppm C + 187.5 ppm F	C+F(3)	78 bc	63 cd	0.20 bcd	0.14
500 ppm C + 250 ppm F	C+F(4)	87 ab	72 abcd	0.19 bcd	0.19
Control	-	78 bc	78 abc	0.17 bcd	0.12
LSD (P=0.05) LSD (P=0.01)		10.88 14.72	16.28 22.13	0.12 0.16	NS

Means in a column with a letter in common are not significantly different at the 5% level

Eria® contains difenoconazole, which is a triazole. Triazoles are sterol biosynthesis inhibitors that are highly systemic and have activity against all fungal groups except Oomycetes. Difenoconazole provides a preventative and curative activity, increasing germination by the prevention of both spore germination on the SBS surface and subsequent fungal penetration, and the elimination of existing systemic infections. Eria® is registered in South Africa for the control of the Deuteromycetes causing grey leaf spot of maize (*Cercospora zeae-maydis)*, and leaf spot (*Cercospora arachidicola* and *C. personata*) and web spot (*Phoma arachidicola*) of groundnuts.

The increase in germination of both varieties after treatment with Eria® may not have been purely fungicidal and could have been due to plant growth regulator properties of the triazole difenoconazole. Triazoles block the gibberellin biosynthesis pathway of plants, promoting growth of lateral buds (Werbrouck & Debergh, 1996; Werbrouck *et al.*, 1996).

Germination was probably also improved by carbendazim, the second active ingredient in Eria® which together with butyl isocyanate, is a breakdown product of benomyl, and is registered to control pathogens in the Ascomycetes, Basidiomycetes and Deuteromycetes. Bechet (1977) reported that both 375 ppm carbendazim (Bavistin®, BASF) and 500 ppm benomyl gave good control of pineapple disease when infected SBS were treated with the fungicides and planted into the field.

Carbendazim also has auxin-like properties which have been used for adventitious rooting and callus formation in stem cuttings of *Taxus baccata* L. and other plant species (Nandi *et al.*, 1996). Mitchell-Innes & Thomson (1974) reported that 300 ppm carbendazim and benomyl were both effective in promoting root and shoot growth of sugarcane. Therefore, the increase in germination caused by treatment of SBS with Eria® was probably caused by the fungicidal and plant growth regulator properties of both carbendazim and difenoconazole.

Statistical analysis of the data indicated that fungicide treatment had a significant effect on dry mass ($P<0.01$; Table 8.5). The mean dry mass of N17 and NCo376 was significantly increased by treatment with C+D(1), C+D(2) and C+D(3). This could have been due to the

activity of both carbendazim and difenoconazole against Ascomycetes, Basidiomycetes and Deuteromycetes, the plant growth regulator properties of both active ingredients, or both. Plant growth was not affected by C+F, indicating that carbendazim was not responsible for the increased dry mass caused by C+D treatment. Additionally, these results indicated that the triazole active ingredient flusilazole in C+F was not as effective as difenoconazole. Dry mass was slightly decreased by the propiconazole treatments, indicating that it was slightly phytotoxic, particularly at the higher concentrations (Figure 8.4).

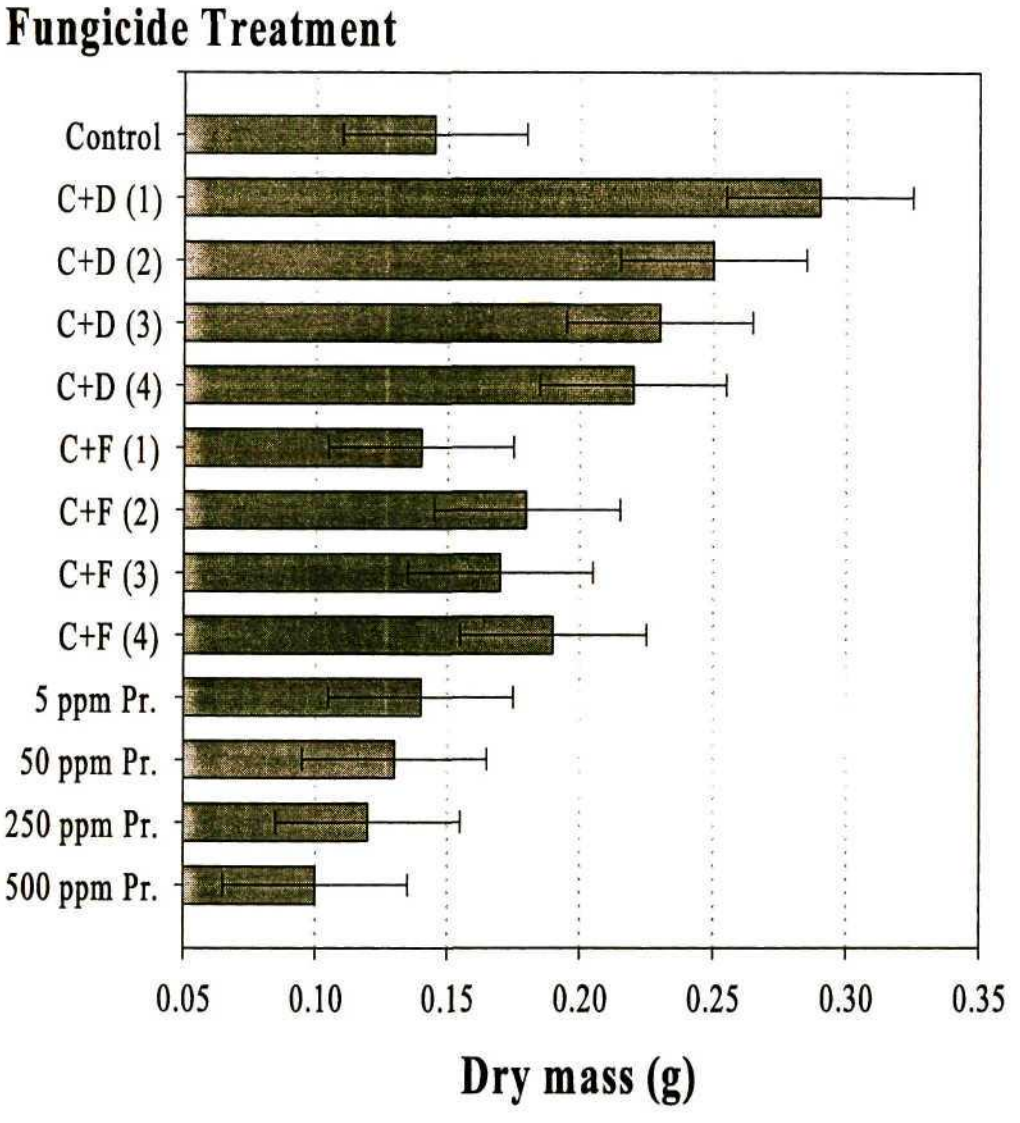


Figure 8.4 Effect of fungicide treatment of single-budded setts on the mean dry mass of varieties N17 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different. (C+D=carbendazim+ difenoconazole; C+F=carbendazim+flusilazole; Pr.=propiconazole).

The results were consistent for both varieties as indicated by the lack of a significant interaction between fungicide treatment and variety (Table 8.5). Fungicide treatment significantly affected dry mass of N17 but not NCo376 (Table 8.6). All treatments with C+D increased dry mass of N17 (+41-100%) and NCo376 (+50-108%). Treatment of SBS with all propiconazole concentrations decreased dry mass of N17 and had little effect on dry mass of NCo376. Treatment of SBS with most concentrations of C+F slightly increased dry mass of both varieties.

In summary, the fungicides propamocarb-HCl, guazatine, C+D, C+F and propiconazole improved germination. Treatments with propamocarb-HCl (722, 1083 and 1444 ppm), C+D(1) and C+D(2) were the most successful in improving both germination and growth. In commercial nurseries, SBS are currently only treated with fungicides before planting, and frequent irrigation rapidly leaches fungicides from the SBS and medium. Therefore, the persistent systemic fungicides, Eria® and Previcur®, are ideal for treatment of SBS. Both the non-persistent fungicides and protectants were not as effective. Germination of the untreated controls in this experiment was high because temperatures were favourable for germination. As a result, fungicide treatment caused only small increases in germination. However, even when conditions were favourable for germination, the application of C+D significantly increased plant growth.

8.2 Treatment with Hot- and Cold-Fungicide Treatments

8.2.1 Introduction

Results from the first fungicide experiments (Section 8.1) indicated that when germination was high under favourable germination conditions, treatment with certain fungicides further improved germination and significantly improved plant growth. Since SBS are only treated with a fungicide immediately before planting in the trays and frequent irrigation causes rapid loss of fungicides from the SBS and medium, persistent systemic fungicides such as Eria® and Previcur® would be most effective in improving plant growth.

The aim of this experiment was to compare the most effective fungicides from Experiments A and B with the registered fungicides, guazatine and benomyl. The fungicides were tested on six commercial sugarcane varieties. In order to determine the effect of cold- and hot-fungicide treatments on germination and growth, two experiments were carried out. In the first experiment the fungicides were applied as cold treatments, and in the second experiment, the fungicides were applied in hot water at 52°C for 10. The effect of the hot-fungicide treatments on root length and ratings of root disease was also determined. Additionally, fungi were isolated from the SBS and roots of the controls, and from the growing medium, to identify fungi that may adversely affect germination and growth of transplants.

8.2.2 Materials and methods

The fungicides were mixed in tap water at the appropriate concentrations (Table 8.7). For the hot-fungicide treatments, tap water was heated to 52°C and transferred to buckets, where the fungicides were added and mixed in thoroughly. Stalks of NCo376, N12, N14, N16, N17 and N22 were obtained from a variety collection at SASEX and cut into SBS. The SBS of N16, N17 and N22 were treated in cold-fungicide solutions for five minutes. The SBS of NCo376, N12 and N14 were treated for 10 minutes either in hot water (SHWT) or with the fungicides in hot water. The temperature of the hot water was initially 52°C, but when SBS were submerged in the water, the temperature dropped to between 48-50°C.

Each cold treatment was represented by a total of 80 SBS, divided into four replicates of 20 SBS each; and each hot fungicide treatment was represented by a total of 60 SBS, divided into three replications of 20 SBS each. The SBS were planted into trays, left in a germination room for seven days and then placed on benches outdoors. The germination and dry mass of the transplants were recorded after 30 days and data for all varieties in each experiment were pooled and evaluated using two-way analysis of variance (ANOVA). Mean results were separated using least significant differences.

Table 8.7 Concentrations of the fungicides tested for treatment of single-budded setts.

Commercial name	Rate	Concentration of active ingredient
Panoctine®	2.0 $m\ell.\ell^{-1}$*	800 ppm guazatine
Benlate®	0.5 $g.\ell^{-1}$*	250 ppm benomyl
Previcur®	1.0 $m\ell.\ell^{-1}$	722 ppm propamocarb-HCl
Tilt®	0.2 $m\ell.\ell^{-1}$	50 ppm propiconazole
Punch-Xtra®	0.5 $m\ell.\ell^{-1}$	125 ppm C + 62.5 ppm F [C+F(1)]
Eria®	0.5 $m\ell.\ell^{-1}$	62.5 ppm C + 31.25 ppm D [C+D(2)]

* = registered at the given concentration for control of sett decay of sugarcane in South Africa (Krause *et al.*, 1996).

After the shoots were harvested for dry mass recordings, transplants of NCo376, N12 and N14 were pulled out of the trays and any medium was washed off the roots. The mean length of the sett roots on each SBS was determined and the extent of root infection was given a numerical rating according to the following scale:

1 normal, healthy appearance
2 occasional lesions or slight discolouration
3 ≤ 25% of sett roots with lesions or discolouration
4 26-50% of sett roots with lesions or discolouration
5 51-80% of sett roots with lesions or discolouration
6 80-100% of sett roots with lesions or discolouration
7 most of the sett roots were absent
8 all sett roots were absent

Selective media were used to isolate *Pythium* spp., *Ceratocystis paradoxa* and *Fusarium* spp. from the untreated SBS (control). *Pythium* spp. were isolated from the sett roots by rinsing the roots in tap water and then sterile water before blot drying on sterile paper towel and plating onto P_5ARP (Appendix 1). *Pythium* spp. were isolated from the composted bagasse using the method of Stanghellini and Kronland (1985). A petri dish was half-filled with composted bagasse, which was moistened with distilled water. Cubes of potato (10 mm x 10 mm x 2 mm) were washed under running tap water for five minutes, washed with distilled water and placed onto the composted bagasse. Thin blocks of water agar (5 mm x 5 mm x 2 mm) were placed on top of the potato. After 24 hours, the agar pieces were removed and plated on P_5ARP.

A selective medium described by Rashid & Trujillo (1974) was used to isolate *C. paradoxa* from SBS and composted bagasse. Inner tissues (1 cm^3) of the SBS were surface sterilised in 0.1% sodium hypochlorite for five minutes and rinsed three times in sterile distilled water. The outer tissues were cut away aseptically, and the 0.5 cm^2 inner piece was sterilised in sodium hypochlorite for one minute and then plated onto the *C. paradoxa*-selective medium.

C. paradoxa and *Fusarium* spp. were isolated from the composted bagasse using the method of Srinivasan (1969). Boiled pieces of sugarcane stems and roots were buried in the composted bagasse. After three days the stem pieces were sterilised and plated onto the *C. paradoxa*-selective medium, and the roots were sterilised and plated onto PDA containing 100 ppm streptomycin sulphate for the isolation of *Fusarium* spp.

Fusarium spp. were isolated from the sett roots by washing roots in deionised water. The roots were then cut into 5 mm sections, surface sterilised for one minute in 0.1% sodium hypochlorite, washed twice in sterile distilled water and then dried on sterile tissue paper. The roots were then plated onto PDA containing 100 ppm streptomycin sulphate.

8.2.3 Results and discussion

Cold-fungicide treatments

Statistical analysis of the germination data indicated that fungicide treatment had no significant effect on germination (Table 8.8). Compared with the control, germination was highest after treatment with (C+D(2)) (+6%) and propiconazole (+5%) (Figure 8.5) and guazatine decreased germination (-5%).

Table 8.8 Two-way ANOVA of the mean germination and dry mass of varieties N16, N17 and N22 after cold-fungicide treatment of single-budded setts.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Fungicide treatment	6	0.917	0.4891	1.702	0.1353
Variety	2	5.020	0.0095	79.9	0.0000
Fungicide x variety	12	0.676	0.7675	1.928	0.0475

Although the germination of N16 and N22 is considered to be rapid and germination of N17 is considered to be slow, the results were consistent for all varieties as indicated by the lack of a significant interaction between fungicide treatment and variety (Table 8.8). There were no significant differences between treatments of all varieties (Table 8.9). Germination of the untreated controls of N16 and N17 was high (83 and 86%) indicating that there was little inhibition of germination by fungal infection. Germination of the untreated control of N22 was lower (69%), indicating that this variety has a low inherent germination ability, was possibly more susceptible to fungal infection, or the SBS contained systemic infections before planting. Most fungicide treatments increased germination of N22, indicating some pathogen control. Guazatine had little effect on germination of this variety, indicating that the infection was systemic, and could not be controlled by the protective action of guazatine. Germination of N22 was highest when SBS were treated with 250 ppm benomyl, C+D(2) and C+F(1), indicating the control of fungi belonging in the Asco-, Basidio- and Deuteromycetes.

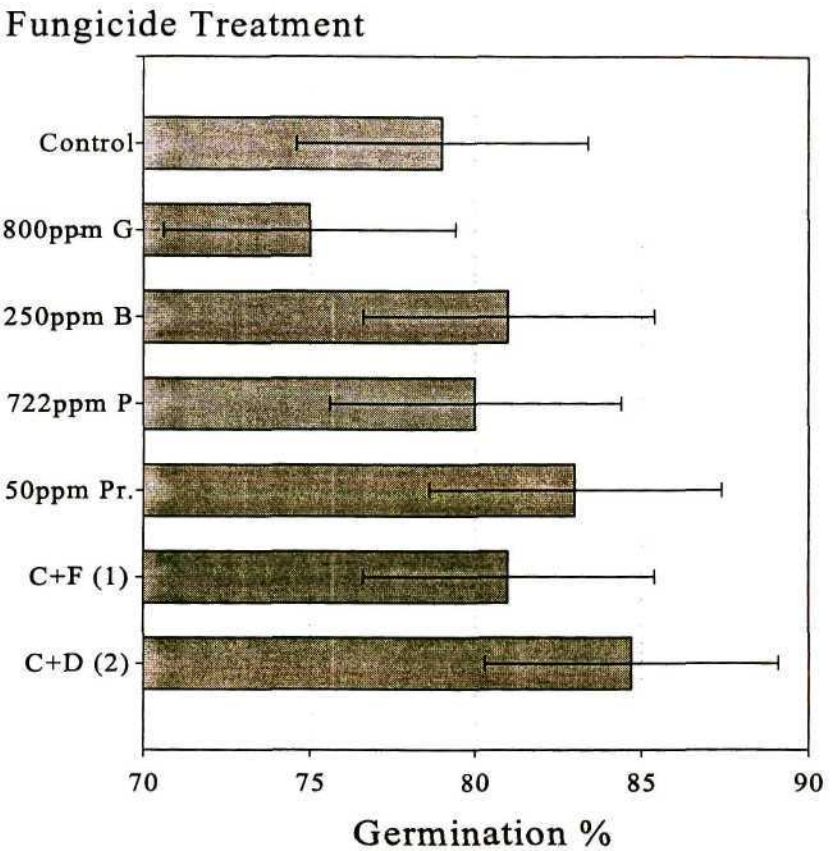


Figure 8.5 Effect of cold-fungicide treatment of SBS on the mean germination of varieties N16, N17 and N22. Means whose 95% comparison intervals do not overlap are significantly different. (G=guazatine; B=benomyl; P=propamocarb; Pr.= propiconazole; C+F=carbendazim+flusilazole; C+D=carbendazim+ difenoconazole).

Table 8.13 Effect of cold-fungicide treatments of single-budded setts on the germination and dry mass of varieties N16, N17 and N22.

Treatment	Germination (%)			Dry mass (g)		
	N16	N17	N22	N16	N17	N22
Control	83	86	69	0.24 a	0.39	0.21
800 ppm guazatine	78	79	70	0.06 c	0.33	0.26
250 ppm benomyl	78	83	84	0.19 ab	0.35	0.29
722 ppm propamocarb HCl	76	85	79	0.20 ab	0.36	0.25
50 ppm propiconazole	83	92	76	0.13 bc	0.39	0.25
C+F(1)	76	86	81	0.17 ab	0.38	0.28
C+D(2)	81	92	81	0.16 ab	0.38	0.26
LSD	NS	NS	NS	0.09	NS	NS

Means in a column with a letter in common are not significantly different at the 5% level.

Statistical analysis of the data indicated that fungicide treatment had little effect on mean dry mass (Table 8.8). Treatment with benomyl, C+F, propiconazole, C+D and propamocarb-HCl had no effect on mean dry mass, whereas treatment with guazatine considerably decreased dry mass indicating its phytotoxicity (Figure 8.9).

The dry mass results were not consistent for all varieties as indicated by the significant interaction between fungicide treatment and variety (Table 8.8). There were significant differences between treatments with N16, but not with N17 and N22 (Table 8.9). Treatment of N16 with guazatine ($P<0.01$) and propiconazole ($P<0.05$) significantly decreased dry mass, but these treatments had little effect on the dry mass of N17 and N22. Dry mass of N16 was also decreased by treatment with propamocarb-HCl, benomyl, C+F(1) and C+D(2).

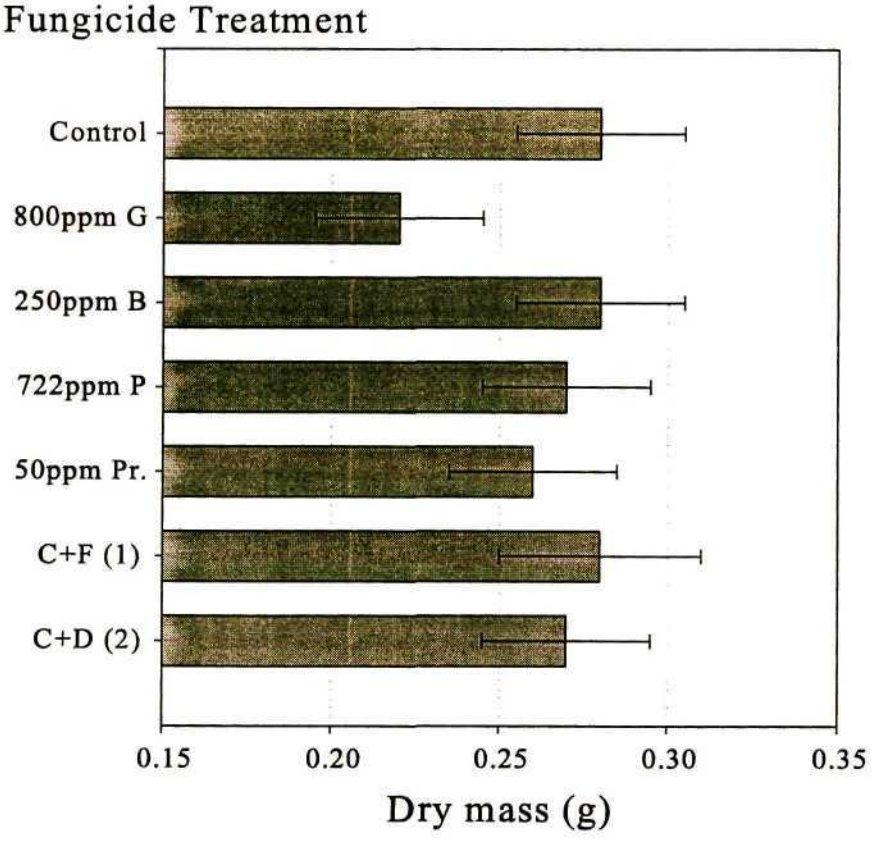


Figure 8.6 Effect of cold-fungicide treatment of single-budded setts on the mean dry mass of varieties N16, N17 and N22. Means whose 95% comparison intervals do not overlap are significantly different. (G=guazatine; B=benomyl; P=propamocarb; Pr.=propiconazole; C+F=carbendazim+ flusilazole; C+D=carbendazim+difenoconazole).

Hot-fungicide treatments

Germination and dry mass

Statistical analysis of the data indicated that fungicide treatment had a significant effect on mean germination and dry mass of varieties NCo376, N12 and N16, indicating that the fungicides were more effective when applied in hot water than in cold water (Table 8.10). The mean germination was significantly increased by treatment with C+D(2) (+44%), SHWT (+29%), C+F(1) (+20%) (P=0.01), 250 ppm benomyl (+16%) and 800 ppm guazatine (+16%) (P=0.05) compared with the control (Figure 8.7a). Dry mass was significantly increased by treatment with C+D(2) (+92%, P<0.01) and SHWT (+46%, P<0.05), and increased by C+F(1) (+38%) compared with the control (Figure 8.7b).

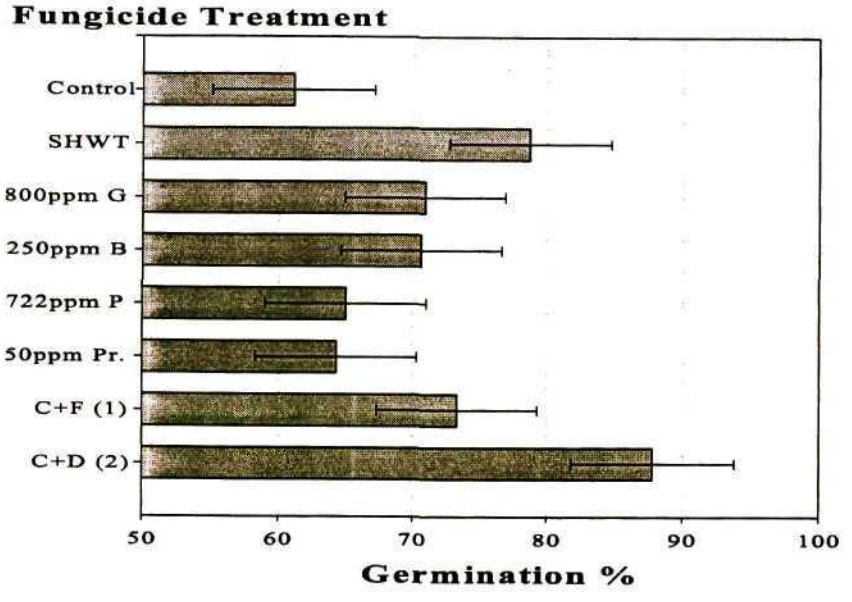
Table 8.10 Two-way ANOVA of the mean germination of varieties NCo376, N12 and N14 after hot-fungicide treatment of single-budded setts.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Fungicide treatment	7	7.469	0.0000	4.656	0.0005
Variety	2	6.006	0.0047	2.666	0.0798
Fungicide x variety	14	1.401	0.1896	0.645	0.8140

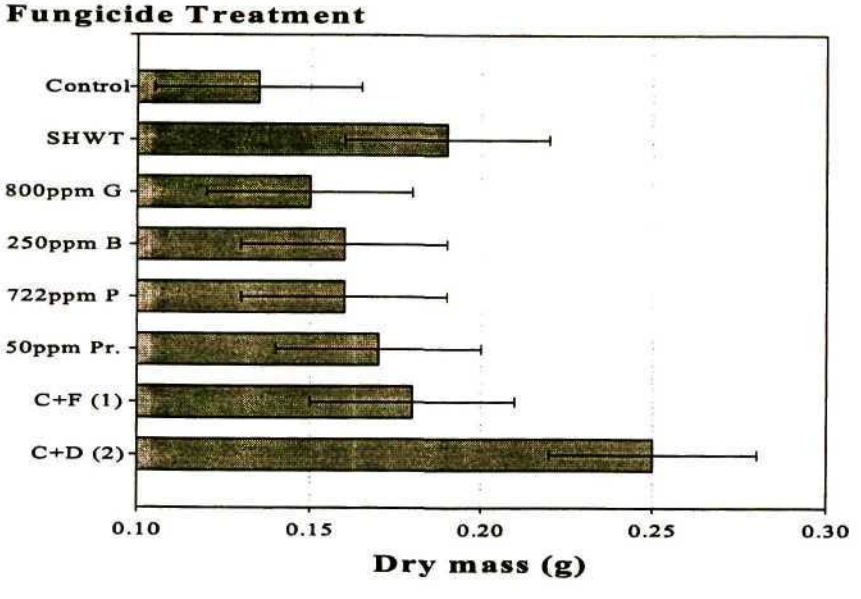
Compared with the SHWT, treatment with guazatine, benomyl and C+F(1) decreased germination and dry mass, and treatment with propamocarb-HCl and propiconazole significantly decreased germination and decreased dry mass (P=0.01) (Figure 8.7). These results indicated that these fungicides used in hot water were not as effective as heat treatment alone. The SHWT may not only stimulate germination by adjusting the hormonal balance, but may also eliminate systemic pathogenic fungi within the SBS.

Treatment of SBS with Eria® (0.5 $m\ell.\ell^{-1}$) in hot water significantly increased germination (+12%, P=0.01) and dry mass (+32%, P=0.05) compared with the SHWT, indicating that the ability of Eria® to control pathogenic fungi and to stimulate germination was enhanced when applied in hot water. These results indicated that the fungicide treatments of SBS in cold

water had no effect on plant growth. However, when fungicides were added to hot water (52°C), plant growth was greatest when SBS were treated for 10 minutes with both C+D(2) (Eria® at 0.5 mℓ.ℓ$^{-1}$; 52°C) and hot water (52°C).



(a)



(b)

Figure 8.7 Effect of hot-fungicide treatment of single-budded setts on the mean germination (a) and dry mass (b) of varieties NCo376, N12 and N14. Means whose 99% comparison intervals do not overlap are significantly different. (SHWT=short hot-water treatment; G=guazatine; B=benomyl; P=propamocarb; Pr.=propiconazole; C+F=carbendazim+flusilazole; C+D= carbendazim+ difenoconazole).

The germination results were consistent for all varieties as indicated by the lack of a significant interaction between fungicide treatment and variety (Table 8.10). There were significant differences between treatments of varieties NCo376, N12 and N14 (Table 8.11). Compared with the control, the SHWT at 52°C for 10 minutes improved germination of NCo376 and N12, and significantly improved germination of N14 (+55%, $P<0.01$). Treatment with the Eria in hot water significantly improved germination of NCo376 (+40%, $P<0.05$), N12 (+34%, $P<0.01$) and N14 (+60%, $P<0.01$). Treatment of SBS with the C+F(1) in hot water improved germination of NCo376 and N12, and significantly improved germination of N14 (+33%, $P<0.05$). Addition of 50 ppm propiconazole to the hot water decreased germination of NCo376 and N12, and significantly improved germination of N14 (+42%, $P<0.01$). Germination of N14 was significantly improved after treatment with 250 ppm benomyl and 800 ppm guazatine ($P<0.01$), and improved with 722 ppm propamocarb-HCl, all of which had little effect on germination of NCo376 and N12.

Table 8.11 Effect of hot-fungicide treatments of single-budded setts on the germination and dry mass of varieties NCo376, N12 and N14.

Treatment	Germination (%)			Dry mass (g)		
	NCo376	N12	N14	NCo376	N12	N14
Control	63 bc	65 b	55 c	0.13	0.13 c	0.14 d
SHWT	78 ab	73 ab	84 ab	0.19	0.17 bc	0.21 ab
800 ppm guazatine	62 bc	68 b	83 ab	0.16	0.13 c	0.17 cd
250 ppm benomyl	62 bc	68 b	82 ab	0.12	0.19 bc	0.17 cd
722 ppm propamocarb-HCl	63 bc	62 b	70 bc	0.15	0.17 bc	0.16 d
50 ppm propiconazole	53 c	61 b	78 ab	0.13	0.17 bc	0.20 bc
C+F(1)	72 abc	75 ab	73 ab	0.15	0.23 ab	0.18 bcd
C+D(2)	88 a	87 a	88 a	0.22	0.28 a	0.25 a
LSD (P=0.05)	18.5	15.2	15.8	NS	0.09	0.04
LSD (P=0.01)	25.5	21.1	21.8		0.13	0.06

Means in a column with a letter in common are not significantly different at the 5% level.

The dry mass results were consistent for both varieties as indicated by the lack of a significant interaction between variety and fungicide treatment (Table 8.10). There were significant differences between treatments with N12 and N14, but not with NCo376 (Table 8.11). Treatment of SBS with C+D(2) improved dry mass of NCo376 (+69%), and significantly improved dry mass of N12 (+115%) and N14 (+79%) ($P<0.01$). Treatment with C+F(1) had little effect on dry mass of NCo376, improved dry mass of N14 (+29%), and significantly improved dry mass of N12 (+77%) ($P<0.05$). Dry mass of N14 was also significantly improved after treatment with both 50 ppm propiconazole (+42%) and the SHWT (+50%). The standard treatments with guazatine and benomyl had no significant effect on germination of all varieties. Treatment with benomyl increased dry mass of N12 (+46%) and N14 (+21%), and guazatine increased dry mass of NCo376 (+23%) and N14 (+21%).

To summarise, analysis of the germination data of these experiments indicated that when fungicides were applied in cold water, the best treatment was C+D(2), and when the fungicides were applied in hot water, germination was highest after treatment with C+D(2) and the SHWT at 52°C for 10 minutes. Addition of the other fungicides to the hot water was not as effective as the SHWT alone.

Disease ratings and root length

Fungicide treatment had a significant effect on mean disease rating of NCo376, N12 and N14. All fungicide treatments and the SHWT significantly decreased disease rating ($P<0.05$). The disease rating was lowest after treatment with benomyl, propamocarb-HCl and propiconazole and highest after treatment with guazatine (Figure 8.8). The mediocre disease control provided by C+F(1) and C+D(2) is contrasted by their ability to increase germination and dry mass, which shows their hormonal activities may be more important than disease control.

Table 8.12 Two-way ANOVA of the mean disease ratings and root lengths of varieties NCo376, N12 and N14 after hot-fungicide treatment of single-budded setts.

Factor	df	Disease rating		Root length (mm)	
		Variance-ratio	P-value	Variance-ratio	P-value
Fungicide treatment	7	3.439	0.0046	0.838	0.5613
Variety	2	11.585	0.0001	1.818	0.1736
Fungicide x variety	14	1.806	0.0653	0.652	0.8070

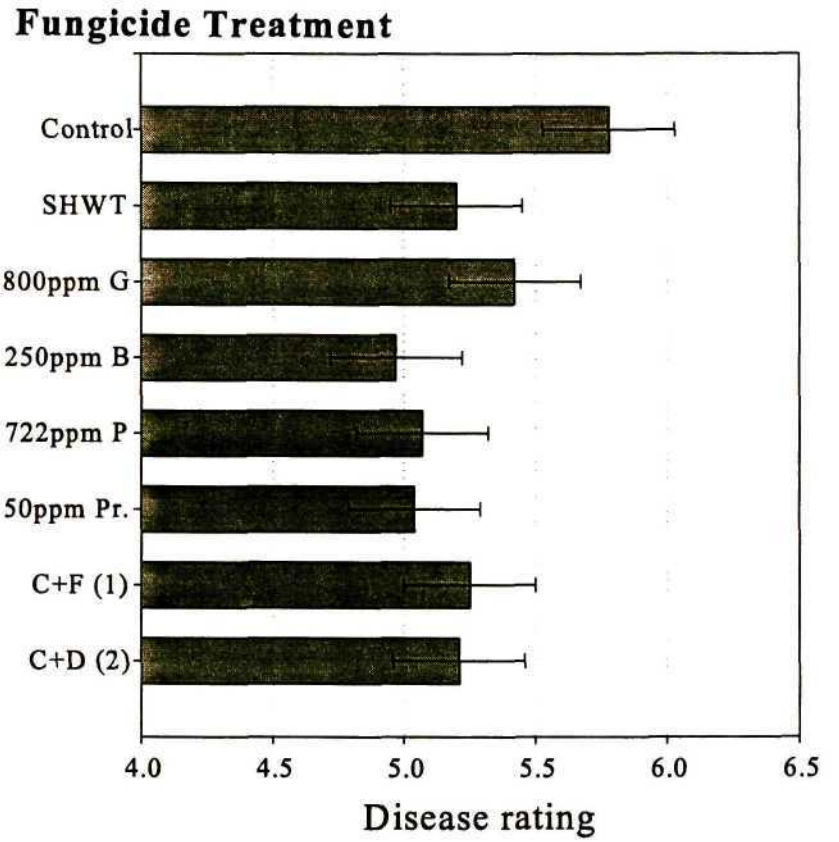


Figure 8.8 Effect of hot-fungicide treatment on the mean disease rating of varieties NCo376, N12 and N14. Means whose 99% comparison intervals do not overlap are significantly different. (SHWT=short hot-water treatment; G=guazatine; B=benomyl; P=propamocarb; Pr.=propiconazole; C+F= carbendazim+flusilazole; C+D=carbendazim+difenoconazole).

The disease rating results were consistent for all varieties as indicated by the lack of a significant interaction between variety and fungicide (Table 8.12). There were significant differences between treatments with N12, but not with NCo376 and N14 (Table 8.13). Treatment of NCo376, N12 and N14 with all fungicides and the SHWT decreased the disease

rating, suggesting fungal control by these treatments. The lowest disease ratings resulted from treatment of NCo376 with the SHWT, N14 with 800 ppm guazatine and 50 ppm propiconazole and N12 with 722 ppm propamocarb-HCl, 250 ppm benomyl and C+D(2).

Table 8.13 Effect of hot-fungicide treatments of single-budded setts on the disease ratings of varieties NCo376, N12 and N14.

Treatment	Disease rating			Root length (mm)		
	NCo376	N12	N14	NCo376	N12	N14
Control	5.5	5.7 a	6.1	71	55	57
SHWT	4.5	5.5 ab	5.5	61	62	61
800 ppm guazatine	5.3	5.6 ab	5.3	52	48	63
250 ppm benomyl	5.0	4.2 c	5.7	54	64	74
722 ppm propamocarb-HCl	5.1	4.8 bc	5.3	54	65	71
50 ppm propiconazole	4.9	4.9 abc	5.4	62	49	67
C+F(1)	5.0	5.1 ab	5.6	52	60	64
C+D(2)	5.0	4.9 bc	5.8	67	70	69
LSD (P=0.05) LSD (P=0.01)	NS	0.85 1.17	NS	NS	NS	NS

Means in a column with a letter in common are not significantly different at the 5% level.

Statistical analysis of the mean root length data of varieties NCo376, N12 and N14 indicated that fungicide treatment had no effect on root length. The mean root length was greatest after treatment with C+D(2) and shortest after treatment with 800 ppm guazatine (Figure 8.9).

The root length results were consistent for all varieties as indicated by the lack of a significant interaction between fungicide treatment and variety (Table 8.12). Compared with the control, all fungicide treatments decreased root length of NCo376 and increased root length of N14 (Table 8.13). Treatment of N12 with all fungicides except 800 ppm guazatine and 50 ppm propiconazole increased root length.

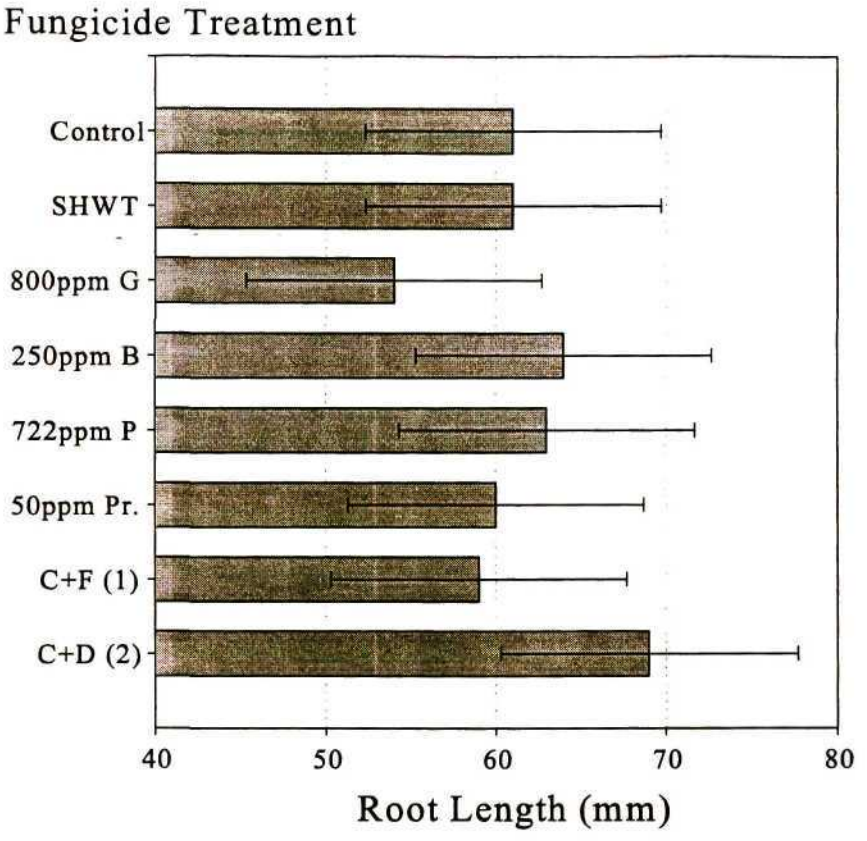


Figure 8.9 Effect of hot-fungicide treatment of single-budded setts on the mean root length of varieties NCo376, N12 and N14. Means whose 99% comparison intervals do not overlap are significantly different. (SHWT=short hot-water treatment; G= guazatine; B=benomyl; P=propamocarb; Pr.=propiconazole; C+F= carbendazim +flusilazole; C+D=carbendazim+difenoconazole).

Fungus isolations

Pythium spp., *C. paradoxa* and *Trichoderma* spp. were isolated from the untreated SBS; *Pythium* spp. and *Trichoderma* spp. were isolated from the roots; and *Pythium* spp. and *Fusarium* spp. were isolated from the composted bagasse (Table 8.13). These fungi may be pathogenic to transplants, adversely affecting germination and growth, particularly when germination is delayed by low temperatures or the use of old or heat-treated seedcane.

Table 8.14 Fungi isolated from sugarcane roots, setts and composted bagasse

Isolation method	Fungi isolated
Pythium baiting with potato	4 *Pythium* spp.
PDA+streptomycin	7 *Pythium* spp, 2 *Fusarium* spp., 2 unknown
PDA	1 *Pythium* spp, 6 *Trichoderma*
C. paradoxa-selective medium	1 *C. paradoxa*
P_5ARP	1 *Fusarium* spp., 1 unknown

Summary

The results of this section are summarized in Table 8.14, which shows the effect of each fungicide treatment on germination and dry mass expressed as a percentage of the control. As in Section 8.1, the best germination and growth was associated with C+D(2) (Eria® at 0.5 $m\ell.\ell^{-1}$) which, when compared with the control, improved the germination when used as a cold treatment and significantly improved germination when used as a hot treatment ($P<0.01$). Compared with the SHWT, treatment with C+D(2) in hot water significantly increased germination ($P<0.05$), and this treatment significantly increased dry mass compared with the control and the SHWT ($P<0.01$).

Table 8.15 Summary of the effects of hot- and cold-fungicide treatments on the mean germination and dry mass of varieties NCo376, N12, N14, N16, N17 and N22 compared with the untreated control.

Fungicide	Germination			Dry mass		
	Cold	Hot	Mean	Cold	Hot	Mean
SHWT	-	+ 30%	-	-	+ 36%	-
guazatine	- 5%	+ 16%*	+ 4%	- 21%	+ 15%	- 14%
benomyl	+ 3%	+ 16%*	+ 8%	0%	+ 23%	+ 5%
propamocarb	+ 1%	+ 7%*	+ 3%	- 4%	+ 23%	+ 5%
propiconazole	+ 5%	+ 5%	+ 6%	- 7%	+ 31%	0%
C+F(1)	+ 3%	+ 20%**	+ 10%*	- 4%	+ 38%*	+ 10%
C+D(2)	+ 6%	+ 44%**	+ 23%**	- 4%	+ 92%**	+ 24%**

* = significantly different from the untreated control at the 5% level
** = significantly different from the untreated control at the 1% level

Good germination and plant growth were also associated with treatment with C+F(1) (Punch-Xtra® at 0.5 $m\ell.\ell^{-1}$), but it was not as effective as the SHWT alone. This treatment increased germination when used as a cold treatment, and significantly improved germination ($P<0.01$) and dry mass ($P<0.05$) and when used as a hot treatment.

The mean germination was slightly improved by treatment of the SBS with benomyl, propiconazole, propamocarb-HCl and guazatine applied in cold and hot water. These results indicate the presence of low levels of pathogenic fungi such as *Pythium, C. paradoxa, Trichoderma* and *Fusarium*. The significant increase in mean germination after treatment with C+D(2) ($P<0.01$) and C+F(1) ($P<0.05$) indicates that these fungicides probably had fungicidal and plant growth regulatory effects on germination and growth. The registered fungicides, benomyl and guazatine were far more effective when used in hot water than when used in cold water, but they were not as effective as the SHWT alone.

In summary, treatment with the fungicides propamocarb-HCl, guazatine, C+D, C+F and propiconazole improved germination when used in cold water. Additionally, the SHWT and C+D(2) in hot water significantly improved germination and growth by controlling systemic fungal infections, and probably by changing the hormonal balance within the bud to one appropriate for germination. The inherent ability of a variety to germinate had no effect on a variety's response to heat treatment and fungicide treatment.

8.3 Heat Treatment with Eria®

8.3.1 Introduction

Results from Sections 8.1 and 8.2 indicated that Eria® (0.5 mℓ.ℓ$^{-1}$; C+D (2)) was the most successful fungicide in improving germination and growth when used in cold water. In addition, treatment of SBS with the Eria® in hot water (52°C) for ten minutes significantly improved germination and dry mass compared with the control ($P<0.01$), and significantly improved germination compared with the SHWT ($P<0.01$). These results indicated that the activity of Eria® were enhanced when added to hot water. Single-budded setts are often treated at 50°C for 120 minutes or may be treated at 52°C for 30 minutes to control *Clavibacter xyli* subsp. *xyli*. The aim of this experiment was to determine the effect on germination and growth of adding Eria® to the hot water when SBS were treated at 52°C for both 10 and 30 minutes, and at 50°C for 120 minutes.

8.3.2 Materials and methods

Single-budded setts prepared from 10-month old stalks of the varieties N12 and N17 were subjected to the following treatments in a 40 *l* heat treatment tank before planting into trays:

a) Control
b) 52°C for 10 minutes
c) 52°C for 30 minutes
d) 50°C for 120 minutes
e) 52°C for 10 minutes + Eria® (0.5 mℓ.ℓ$^{-1}$)
f) 52°C for 30 minutes + Eria® (0.5 mℓ.ℓ$^{-1}$)
g) 50°C for 120 minutes + Eria® (0.5 mℓ.ℓ$^{-1}$)

The fungicide was mixed thoroughly with the hot water before SBS in wire baskets were lowered into the water for the required treatment period before planting into trays. Each treatment was represented by a total of 60 SBS, divided into three replications of 20 SBS each. Trays were placed in the germination room. To allow fungal infection of the SBS to occur, the temperature was kept at 20°C for five days and then raised to 32°C for four days before trays were placed on benches in the nursery. Germination and dry mass of the transplants were recorded after 35 days.

8.3.3 Results and discussion

Statistical analysis of the data indicated that treatment did not have a significant effect on mean germination of N12 and N17 (Table 8.16). All treatments increased germination compared with the control. Compared with the control, the mean germination was increased by treatments in hot water at 52°C (+19-21%) and treatment with Eria® in hot water (52°C) for ten minutes (+30%). Treatment at 50°C for 120 minutes improved germination (+9%), as did treatment with Eria® at 50°C for 120 minutes (+13%) (Figure 8.10).

Table 8.16 Two-way ANOVA of the mean germination of varieties N12 and N17 after treatment of single-budded setts.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
Treatment	6	2.209	0.0720	13.348	0.0000
Variety	1	27.344	0.0000	49.060	0.0000
Treatment x variety	6	1.408	0.2466	10.069	0.0000

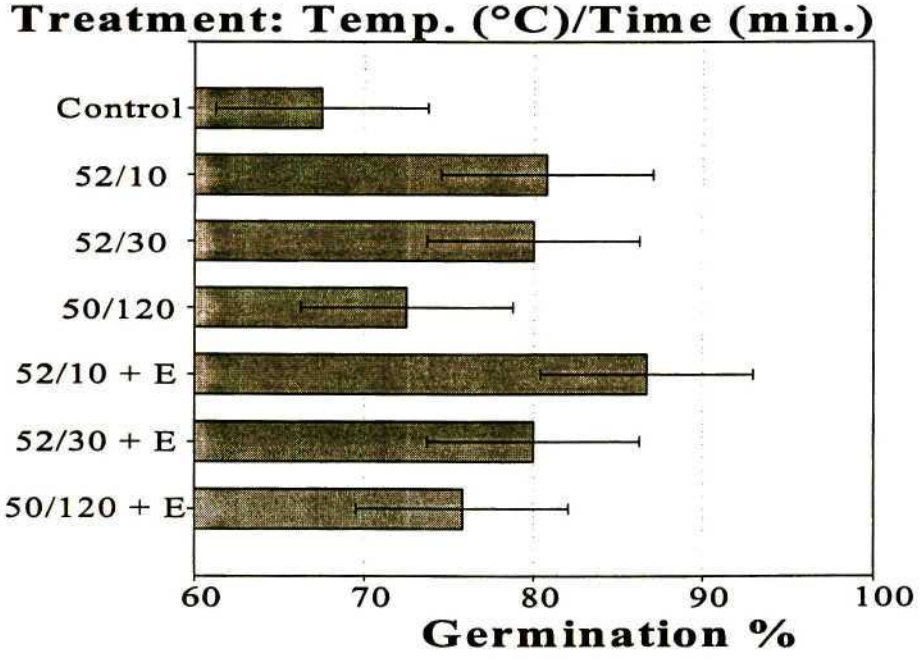


Figure 8.10 Effect of hot-water treatment of single-budded setts with and without Eria® (E) on the mean germination of varieties N12 and N17. Means whose 95% comparison intervals do not overlap are significantly different.

The results were consistent for both varieties as indicated by a lack of significant interaction between treatment and variety (Table 8.16). The germination of the untreated SBS of N12 was low, but there was a substantial increase in germination caused by all treatments (+18-49%; Table 8.17). These results suggested that the heat treatments with and without Eria® possibly controlled systemic infections in the SBS and altered the hormonal balance to one favourable for germination. Treatment at 50°C for 120 minutes improved germination suggesting that the seedcane was mature and more tolerant of treatment than the immature or senescent seedcane used in previous heat treatment experiments. Addition of Eria® to the treatments at 52°C for 10 and 30 minutes was more beneficial than the heat treatments alone. However, addition of Eria® to water at 50°C for treatment for 120 minutes decreased germination compared with heat treatment alone (-11%).

Germination of untreated SBS of N17 was relatively high (80%) and all treatments except 50°C for 120 minutes increased germination by 9-12% (Table 8.16). Contrary to the results obtained with N12, addition of Eria® to the tank for treatment at 50°C for 120 minutes improved germination (+21%) but had little effect after treatments at 52°C.

Table 8.17 Effect of hot water treatment of single-budded setts with and without Eria® on the germination and dry mass of varieties N12 and N17.

Treatment	Germination (%)		Dry mass (g)	
	N12	N17	N12	N17
Control	55	80	0.13 bc	0.12 d
52°C/10 min	68	93	0.22 a	0.21 c
52°C/30 min	68	92	0.15 b	0.27 ab
50°C/120 min	73	72	0.15 b	0.23 bc
52°C/10 min + Eria®	82	92	0.23 a	0.28 ab
52°C/30 min + Eria®	73	87	0.24 a	0.26 abc
50°C/120 min + Eria®	65	87	0.10 c	0.30 a
LSD (P=0.05) LSD (P=0.01)	NS	NS	0.049 0.068	0.054 0.075

Means in a column with a letter in common are not significantly different at the 5% level.

Statistical analysis of the data indicated that the mean dry mass of the varieties N12 and N17 was significantly affected by treatment (Table 8.16). All treatments significantly improved dry mass compared with the control. Addition of Eria® to the hot water significantly increased dry mass compared with treatments at 52°C for 10 and 30 minutes alone ($P<0.05$), but had little effect when added to the treatment at 50°C for 120 minutes (Figure 8.11).

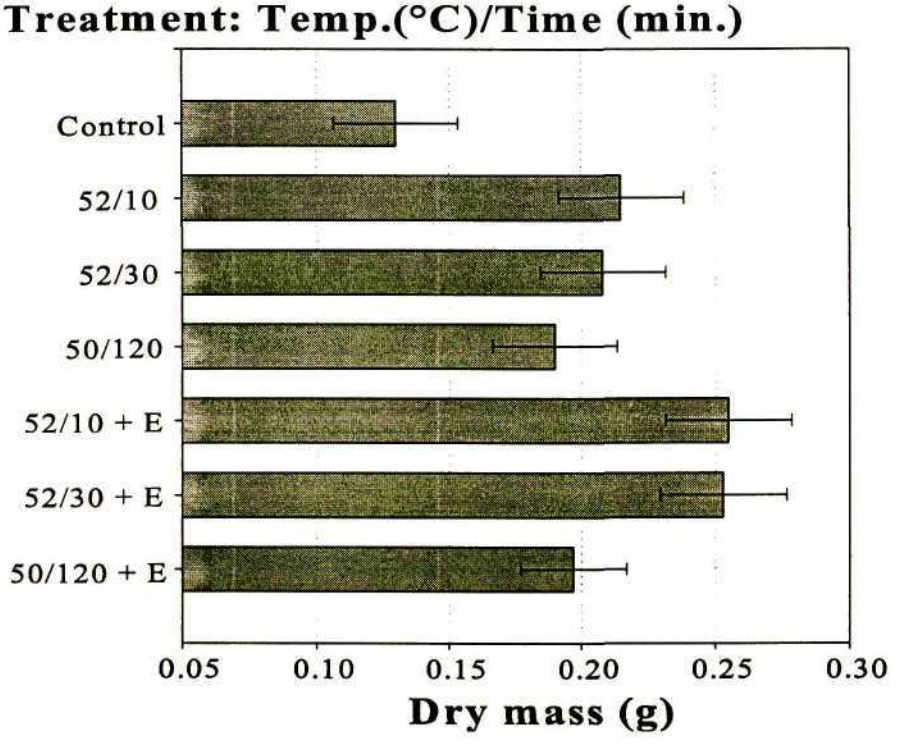


Figure 8.11 Effect of hot water treatment of single-budded setts, with and without Eria® (E) on the mean dry mass of varieties N12 and N17. Means whose 95% comparison intervals do not overlap are significantly different.

The results were not consistent for both varieties as indicated by a significant interaction between treatment and variety (Table 8.16). Compared with the control, dry mass of N12 was significantly increased by treatments at 52°C for both 10 minutes (+69%) with and without Eria® (+85 & 77%) and 30 minutes with Eria® (+85%) ($P<0.01$) (Table 8.17). Compared with the control, treatment of N12 at 50°C for 120 minutes slightly increased dry mass, and addition of Eria® slightly decreased dry mass. However, compared with the heat treatment alone, the addition of Eria® significantly decreased dry mass ($P=0.05$). Dry mass of N12 treated at 52°C for 30 minutes with Eria® was significantly higher than treatment at 52°C for

30 minutes (+60%, $P<0.01$), and addition of Eria® to the treatment at 52°C for 10 minutes had no effect on dry mass. All treatments significantly increased dry mass of N17 (+75-150%, $P<0.01$). Addition of Eria® to the tank significantly increased dry mass compared with treatments at 52°C for 10 minutes (+33%, $P=0.05$) and 50°C for 120 minutes (+30%, $P=0.05$).

To summarise, addition of Eria® to water at 50°C for 120 minutes had no effect on germination and plant growth compared with treatment at 50°C for 120 minutes alone. When SBS were treated with Eria® at 52°C for 10 and 30 minutes, germination and dry mass were improved compared with treatment in hot water alone ($P=0.05$). The increases in germination and growth after treatment with Eria® in hot water was probably due to the plant growth regulatory effects of carbendazim and difenoconazole and their control of subsequent infections by soil pathogens. These results indicated that Eria® can be safely added to the tank when SBS are treated at 52°C for 10 and 30 minutes and has little effect on germination and plant growth when SBS are treated at 50°C for 120 minutes.

8.4 Fungicide Drench

8.4.1 Introduction

Treatment of SBS with a systemic fungicide before planting inhibits the development of existing infections and prevents further infection by soil pathogens. The activity of some systemic fungicides is short-lived, particularly when the fungicide is highly soluble, allowing pathogens present in the medium to infect the SBS when the fungicide has washed off. It is common practice in the vegetable and flower seedling industries to drench the medium in the trays with a mixture of propamocarb-HCl (1083 ppm; Previcur®) and benomyl (250 ppm; Benlate®) to control *Pythium, Rhizoctonia, Phytophthora* and *Rhizoctonia*, the causal organisms of damping off, seed decay and root rots of many crops. Propamocarb-HCl and benomyl are both systemic fungicides which, when used as soil treatments, are absorbed by roots to give prolonged disease control. Benlate® is a wettable powder active against a wide range of Asco-, Deutero- and some Basidiomycetes. Previcur® is a soluble concentrate active against Oomycetes.

Since members of the above Classes infect sugarcane, the aim of these experiments was to determine the effect of drenching the trays with propamocarb-HCl and benomyl on the germination and growth of sugarcane transplants grown from untreated, Eria®- and Panoctine®-treated SBS. In addition, propamocarb-HCl and benomyl were used together and separately to determine which was the most effective active ingredient in order to determine which soil pathogens may inhibit germination and plant growth.

8.4.2 Materials and methods

Experiment A

Single-budded setts of varieties N14 and NCo376 were treated as follows:

SBS treatment
a. no SBS treatment
b. hot-guazatine treatment (52°C, 800 ppm, 10 minutes)

Fungicide drench
a. no fungicide drench
b. propamocarb-HCl (1083 ppm) and benomyl (250 ppm) (P/B) drench

Experiment B

Single-budded setts of varieties N11, N12, NCo376, N16 and N17 were treated as follows:

a. control (no treatment)
b. SHWT (52°C, 10 minutes)
c. hot-Eria® treatment (52°C, 0.5 mℓ.ℓ$^{-1}$, 10 minutes)
d. hot-Eria® treatment + P/B drench

Experiment C

Single-budded setts of NCo376 were treated as follows:

SBS treatment
a. no SBS treatment
b. hot-Eria® treatment (52°C, 0.5 mℓ.ℓ$^{-1}$, 10 minutes)

Fungicide drench
a. no fungicide drench
b. propamocarb-HCl (1083 ppm) drench
c. benomyl (250 ppm) drench
b. propamocarb-HCl (1083 ppm) and benomyl (250 ppm) drench

The propamocarb-HCl and benomyl were mixed into a paste, to which 10 *l* of cold water was added and mixed thoroughly. After SBS treatment the SBS were planted into composted bagasse in 24-celled (Experiment A) or 98-celled trays (Experiments B & C) which were drenched with either the fungicide solution or water at a rate of 20 mℓ per cell. Each treatment in Experiment A was represented by a total of 72 SBS which were divided into three replications of 24 SBS each, and each treatment in Experiments B and C was represented by a total of 60 SBS which were divided into three replications of 20 SBS each. The trays were left in a germination room and transferred outdoors once most of the SBS had germinated. Germination and plant dry mass were recorded after 28 days and the data for each experiment were pooled and evaluated using two- or three-way analysis of variance (ANOVA). Mean separation was accomplished using least significant difference.

8.4.3 Results and discussion

Experiment A

Statistical analysis of the germination data of varieties NCo376 and N14 indicated that germination was significantly affected by SBS treatment but not affected by the fungicide drench. The mean germination was significantly increased by the guazatine in hot water (+45%; $P<0.01$) (Figure 8.12a) and was slightly improved after the fungicide drench (+10%) (Figure 8.12b).

Table 8.18 Three-way ANOVA of the mean germination and dry mass data of varieties NCo376 and N14 after single-budded sett (SBS) treatment with guazatine, with and without a fungicide drench with propamocarb-HCl/benomyl.

Factor	df	Germination %		Dry mass (g)	
		Variance-ratio	P-value	Variance-ratio	P-value
SBS treatment (T)	1	47.908	0.0000	21.5	0.0003
Fungicide drench (FD)	1	3.457	0.0815	7.267	0.0159
Variety (V)	1	8.093	0.0117	128.198	0.0000
T x FD	1	1.630	0.2199	3.360	0.0855
T x V	1	24.065	0.0002	0.291	0.6029
FD x V	1	0.843	0.3817	2.616	0.1253

The results after drenching the trays were consistent for both guazatine-treated and untreated SBS as indicated by the lack of a significant interaction between SBS treatment and fungicide drench (Table 8.18). Drenching the medium with P/B slightly improved germination of both untreated and guazatine-treated SBS (Figure 8.13a). Germination was highest when guazatine-treated SBS were subsequently drenched with P/B. This treatment significantly increased germination compared with the control (no SBS treatment or fungicide drench) (+59%, $P<0.01$). The hot water probably adjusted the hormonal balance to one favourable for germination and killed systemic pathogens, and guazatine controlled surface infections and soil

pathogens infecting the SBS after planting. The benomyl and propamocarb-HCl remaining in the medium would have subsequently been absorbed by developing roots, affording control of late infections.

Fungicide drenching of untreated SBS with poor root growth only increased germination slightly because there was little absorption of P/B, making the application of the fungicide drench to untreated SBS ineffective.

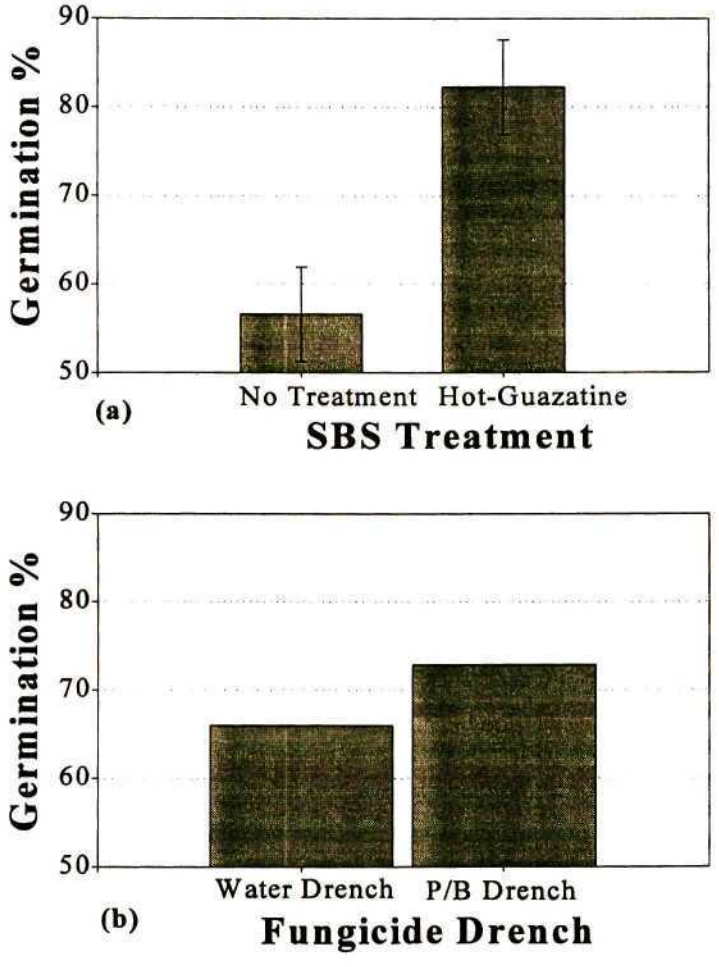


Figure 8.12 Effect of single-budded sett treatment with guazatine (a), and drenching of medium with propamocarb-HCl/benomyl (P/B) (b) on the mean germination of varieties N14 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different.

The reaction of both varieties to the drench was consistent, as indicated by the lack of a significant interaction between fungicide drench and variety (Table 8.18). Drenching improved germination of NCo376 and N14, but not significantly (Figure 8.13b).

However, the reaction of both varieties to the SBS treatment was not consistent, as indicated by the significant interaction between variety and SBS treatment (Table 8.18). The guazatine treatment significantly increased germination of both N14 (+104%, $P<0.01$) and NCo376 (+11%, $P<0.05$) compared with the untreated control (Figure 8.13c).

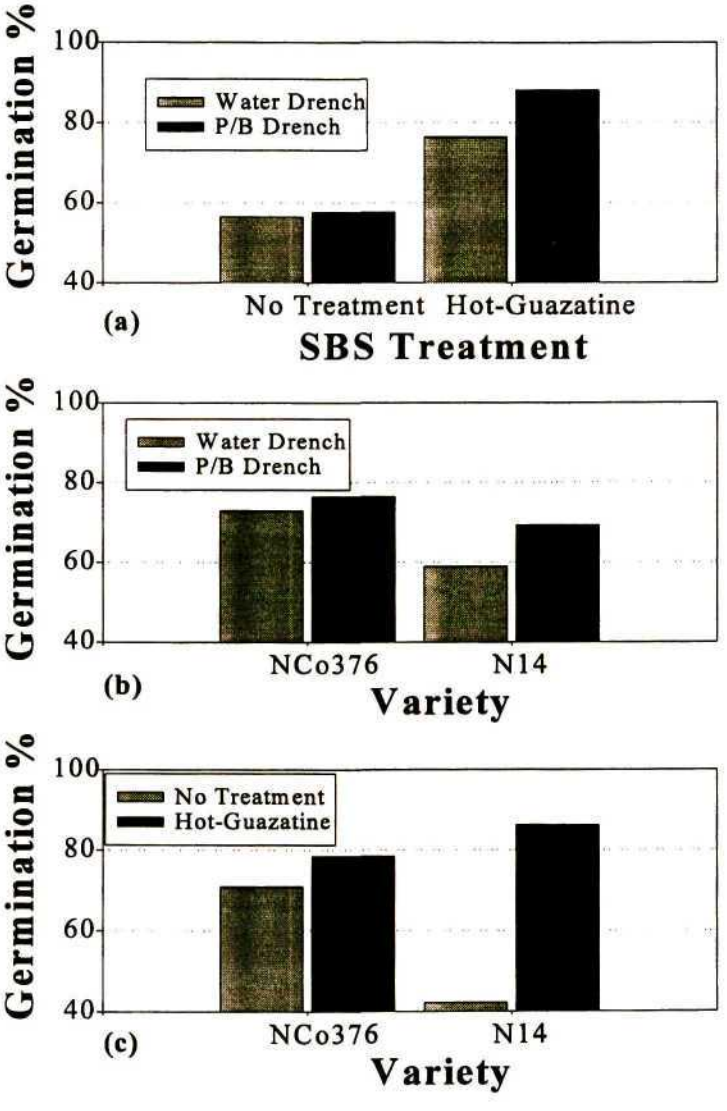


Figure 8.13 Interaction between (SBS) treatment with guazatine and drenching of medium with propamocarb-HCl/benomyl (P/B) (a) and their effect on the mean germination of varieties NCo376 and N14 (b & c).

Statistical analysis of the dry mass results indicated that both SBS treatment and fungicide drench had a significant effect on mean dry mass of NCo376 and N14 (Table 8.18). The dry mass was significantly increased by the hot-guazatine treatment (+29%, $P<0.01$) and the propamocarb-HCl/benomyl drench (+16%, $P<0.01$) (Figure 8.14).

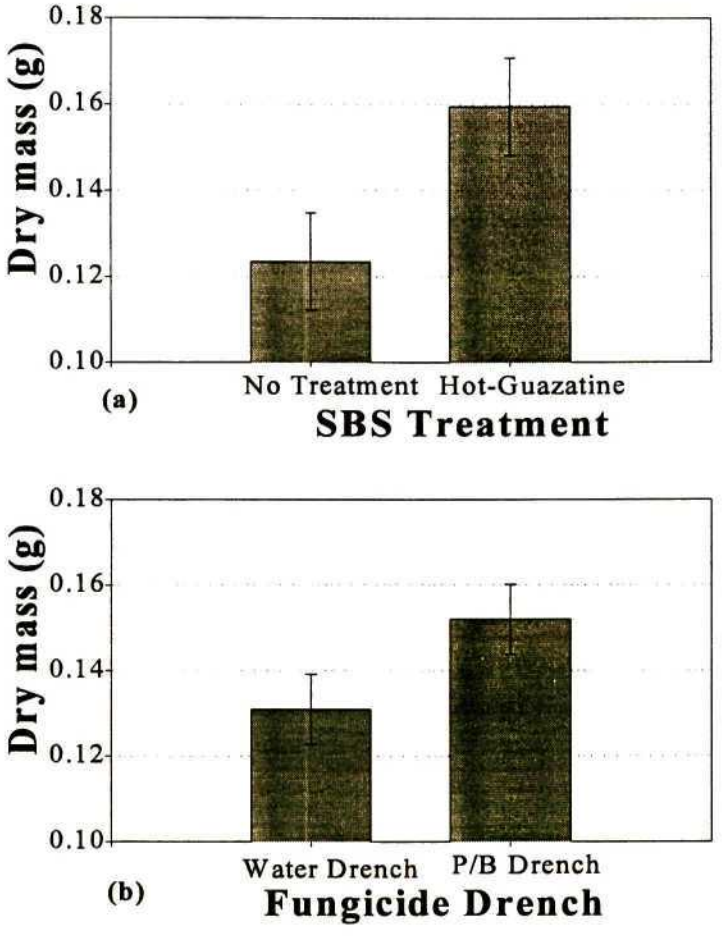


Figure 8.14 Effect of single-budded sett (SBS) treatment with guazatine (a), and drenching of medium with propamocarb-HCl/benomyl (P/B) (b) on the mean dry mass of varieties N14 and NCo376.

The dry mass results were consistent for both varieties and treatments as indicated by the lack of significant interactions between variety and SBS treatment, variety and fungicide drench, and SBS treatment and fungicide drench (Table 8.18). Drenching of trays with P/B improved dry mass of both untreated and guazatine-treated SBS. Dry mass was greatest when guazatine-treated SBS were subsequently drenched with P/B (Figure 8.15a). Compared with the control (no SBS treatment or fungicide drench) this treatment significantly increased the

mean dry mass (+48%; $P<0.01$). However, the use of guazatine or the drench alone only slightly increased dry mass indicating that they had significant complementary effects when combined.

Drenching of the trays with P/B improved dry mass of both varieties, significantly so for NCo376 ($P<0.05$) (Figure 8.15b). The guazatine treatment significantly increased dry mass of both N14 ($P<0.05$) and NCo376 ($P<0.01$) (Figure 8.15c).

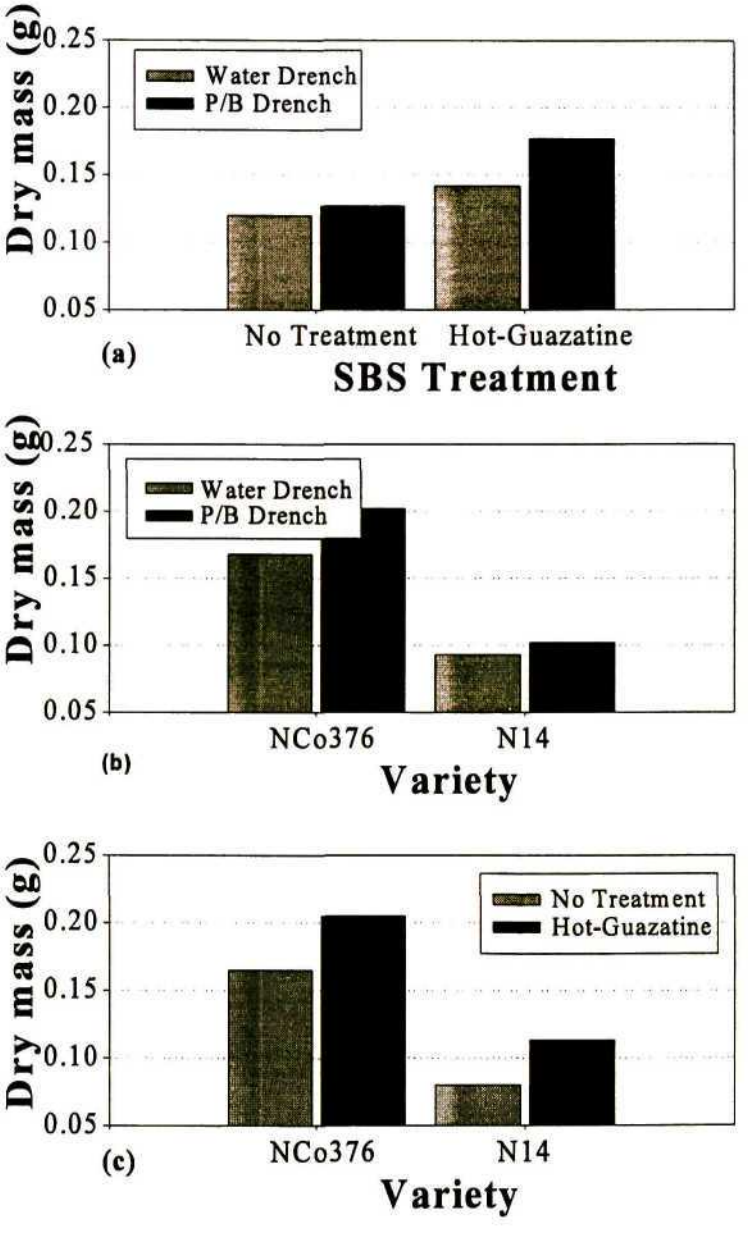


Figure 8.15 Interaction between single-budded sett (SBS) treatment with guazatine and drenching of medium with propamocarb-HCl/benomyl and their effect on the mean dry mass of varieties N14 and NCo376.

To summarise, compared with the control, treatment of SBS with guazatine in hot water significantly increased the mean germination and growth of both varieties. When pre-germination infections were not controlled by treatment with guazatine, application of P/B had little effect on germination and plant growth. However, after treatment with guazatine, germination was increased by treatment with a mixture of the fungicides Previcur® and Benlate®. Therefore, the significant increases in germination and plant growth were probably due to the control of both pre-and post-germination infections.

Experiment B

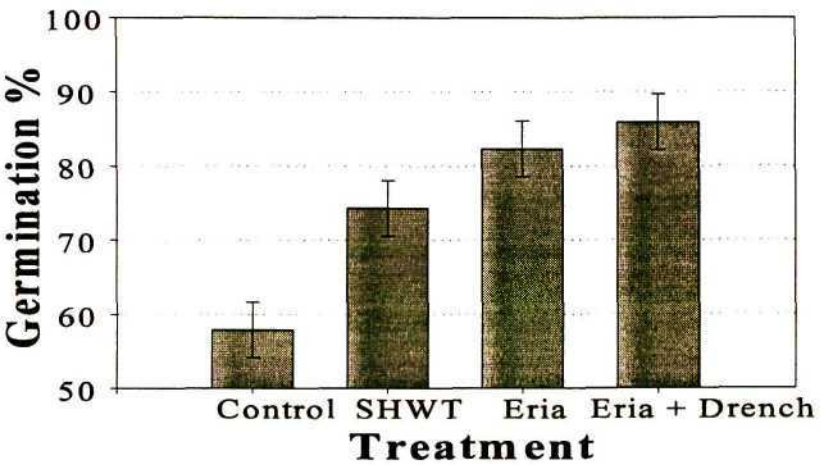
The mean germination of the untreated SBS of the five varieties was poor (58%), indicating that fungal pathogens possibly inhibited germination. Statistical analysis of the data indicated that treatments had a significant effect on germination and dry mass (Table 8.19). Compared with the control, the mean germination and dry mass were significantly increased by the heat treatment (+28%, P=0.01; +43%, P=0.05). This treatment adjusted the hormone balance and probably controlled systemic fungi, increasing the speed of germination and allowing shoots to develop faster than those grown from untreated SBS, resulting in increased plant growth.

Addition of Eria® to the hot water significantly increased both germination and growth compared with the heat treatment alone (P=0.05), and compared with the control (+41%, P=0.01; +114%, P=0.01). These increases were probably due to the persistent fungicidal and plant growth regulatory activity of carbendazim and difenoconazole.

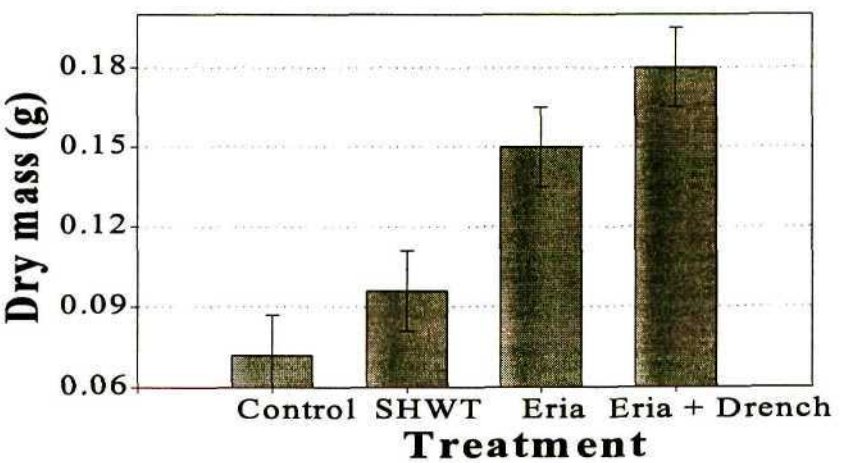
Drenching trays containing Eria®-treated SBS with a propamocarb-HCl/benomyl further improved germination and significantly improved dry mass compared with the Eria® treatment, and significantly improved germination and plant growth compared with the heat treatment (P=0.01) and the untreated control (+48%, P=0.01; +157%, P=0.01) (Figure 8.16). Since Eria® is a long-lasting systemic fungicide and both propamocarb-HCl and benomyl were slowly absorbed by the developing roots, these treatments probably controlled diseases for a long period after planting.

Table 8.19 Two-way ANOVA of the mean germination and dry mass data of five sugarcane varieties after single-budded sett treatment and drenching of the medium with propamocarb-HCl/benomyl.

Factor	df	Germination		Dry mass	
		F-variance	P-value	F-variance	P-value
Treatment	3	22.313	0.0000	52.370	0.0000
Variety	4	3.6840	0.0120	26.110	0.0000
Treatment x variety	12	2.783	0.0075	2.092	0.0410



(a)



(b)

Figure 8.16 Effect of single-budded sett treatments on the mean germination (a) and dry mass (b) of varieties NCo376, N11, N12, N16 and N17. Means whose 99% comparison intervals do not overlap are significantly different.

The germination and dry mass results were not consistent for all varieties as indicated by a significant interaction between variety and treatment (Table 8.19). There were significant differences between treatments with NCo376, N11, N12 and N17, but not with N16 (Table 8.20). Germination of SBS treated with the SHWT ranged from 55-92%. The SHWT significantly increased germination of NCo376 and N17 ($P<0.01$), increased germination of N12 and N16 and had no effect on germination of N11.

Germination of SBS treated with Eria® ranged from 65-93%, and germination after treatment with Eria® and the fungicide drench ranged from 77-93%. Compared with the control, treatment with Eria® or the drenching of Eria®-treated SBS with the fungicide solution significantly increased germination of N11 (+43-50%, $P<0.05$), NCo376 (+60%, $P<0.01$), N12 (+47-51%, $P<0.01$) and N17 (+92-93%, $P<0.01$). Compared with the SHWT, treatment of SBS with Eria®, and the drenching of Eria®-treated SBS with the P/B solution significantly increased germination of N11, and increased germination of N12.

Table 8.20 Effect of fungicide treatments on germination of five varieties after treatment of single-budded setts and drenching with propamocarb-HCl/benomyl.

Treatment	Germination %				
	NCo376	N11	N12	N16	N17
Control	58 b	58 b	53 b	65	55 b
Short hot water treatment	85 a	55 b	67 ab	73	92 a
Eria®	83 a	93 a	78 a	65	92 a
Eria® + drench	87 a	93 a	80 a	77	93 a
LSD (P=0.05)	18.8	24.8	16.3	NS	18.8
LSD (P=0.01)	27.4	37.6	23.7		27.4

Means in a column with a letter in common are not significantly different at the 5%

There were highly significant effects of treatment on dry mass with all varieties (Table 8.19). Compared with the control, the SHWT increased dry mass of N12, N16 and N17, and the hot-Eria® treatment significantly increased dry mass of NCo376 (+110%, $P<0.01$), N12 (+250%, $P<0.05$), N16 (+90%, $P<0.01$) and N17 (+120%, $P<0.01$) (Table 8.21).

Drenching Eria®-treated SBS significantly increased dry mass of all the varieties (+90-400%, $P<0.01$). Compared with the SHWT, the hot-Eria® treatment significantly increased dry mass of NCo376 ($P<0.01$) and N11 ($P<0.05$) and increased dry mass of N12, N16 and N17.

Table 8.21 Effect of fungicides on the dry mass (g) of five varieties after treatment of single-budded setts and drenching with propamocarb-HCl/benomyl.

Treatment	Dry mass (g)				
	NCo376	N11	N12	N16	N17
Control	0.10 b	0.06 bc	0.04 b	0.10 b	0.05 b
Short hot water treatment	0.12 b	0.05 c	0.08 ab	0.14 ab	0.09 ab
Eria®	0.21 a	0.10 ab	0.14 a	0.19 a	0.11 a
Eria® + drench	0.25 a	0.14 a	0.20 a	0.19 a	0.12 a
LSD (P=0.05)	0.05	0.04	0.07	0.05	0.04
LSD (P=0.01)	0.07	0.06	0.11	0.08	0.06

Means in a column with a letter in common are not significantly different at the 5% level

Since the efficacy of Eria® was enhanced when augmented with hot water and the P/B drench, this treatment would increase the number of transplants produced in a transplant nursery. This in turn would lower the production costs and the selling price of transplants, and make the transplant option more viable for farmers intending on establishing their own seedcane nurseries. Therefore, the drenching of Eria®-treated SBS in trays with P/B is strongly recommended for commercial transplant nurseries.

The use of Eria® instead of Panoctine® also reduces the risk of pesticide poisoning of labourers because Eria® has a lower dermal toxicity than Panoctine®, and need only be treated with caution and not as a poison. However, when using Eria®, Panoctine® or Benlate®, gloves should always be worn. The use of Eria® is cheaper than Panoctine® as it is required in smaller quantities. Using prices quoted in 1997, to make up a 100 l fungicide solution it cost R 4.40 for Benlate®, R 7.28 for Eria® and R 22.60 for Panoctine®. Therefore, in addition to improving germination, Eria® is less hazardous and cheaper that the currently registered fungicides Panoctine® and Benlate®.

Experiment C

Statistical analysis of the data indicated that SBS treatment had a significant effect on germination and dry mass, whereas the fungicide drench did not (Table 8.22). Treatment with Eria® significantly increased germination ($P=0.05$; Figure 8.17a) and dry mass ($P=0.01$; Figure 8.18a). Drenching trays with propamocarb-HCl and/or benomyl had no effect on germination (Figure 8.17b) and significantly increased dry mass ($P<0.05$) when used separately (Figure 8.18b).

Table 8.22 Two-way ANOVA of the mean germination and dry mass of NCo376 after Eria®-treatment of single-budded setts (SBS) and drenching of medium with propamocarb-HCl and benomyl.

Factor	df	Germination		Dry mass	
		Variance-ratio	P-value	Variance-ratio	P-value
SBS treatment	1	4.405	0.0422	99.995	0.0000
Fungicide drench	3	0.278	0.8409	2.548	0.0694
SBS treatment x drench	3	10.562	0.2137	0.202	0.8943

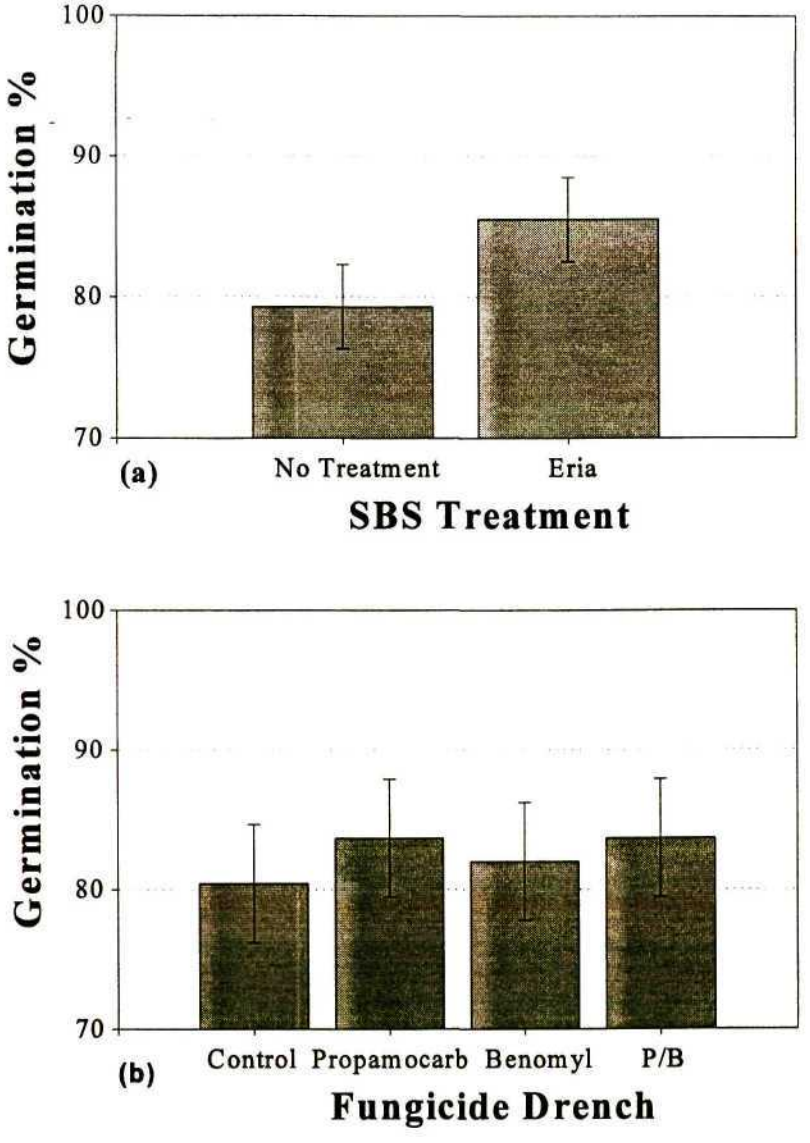
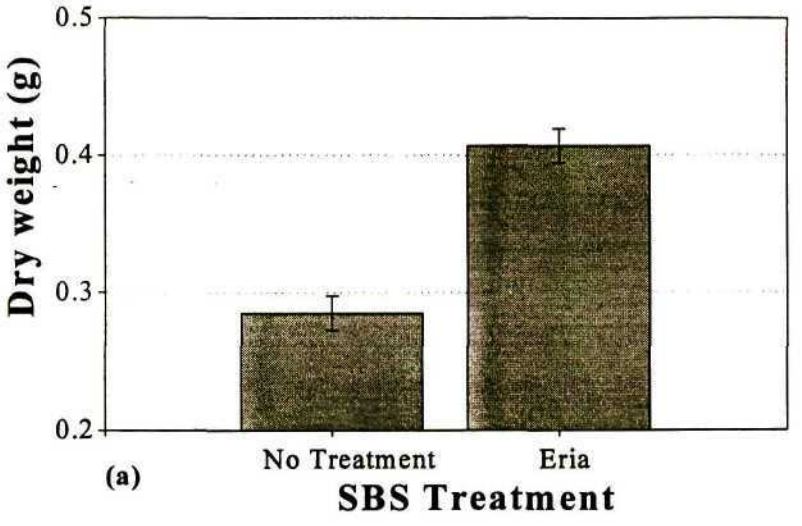


Figure 8.17 Effect of single-budded sett (SBS) treatment with Eria® (a), and drenching of trays with propamocarb-HCl, benomyl and a mixture of both (P/B) (b) on the mean germination of NCo376. Means whose 95% comparison intervals do not overlap are significantly different.



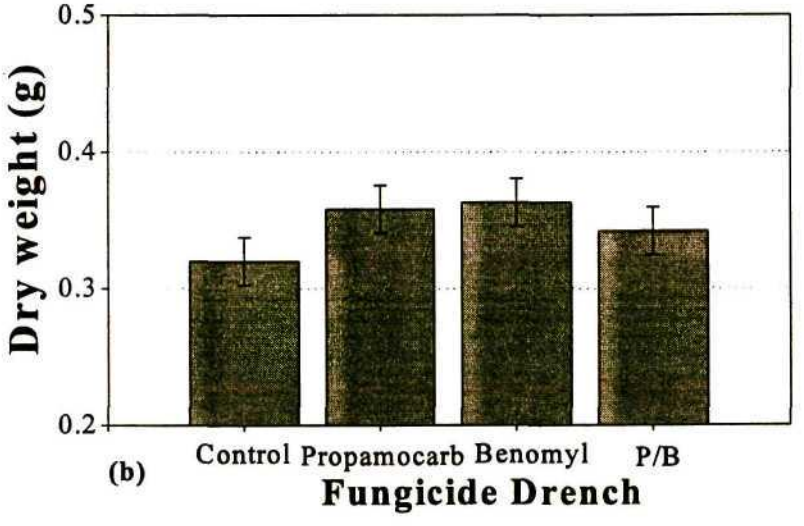


Figure 8.18 Effect of single-budded sett (SBS) treatment with Eria® (a), and drenching of trays with propamocarb-HCl, benomyl and a mixture of both (P/B) (b) on the mean dry mass of NCo376. Means whose 95% comparison intervals do not overlap are significantly different.

The germination and dry mass results were consistent for Eria®-treated and untreated SBS as indicated by the lack of a significant interaction between SBS treatment and fungicide drench (Table 8.22). Compared with the untreated control, the drenches had no effect on germination of untreated SBS and germination was increased when Eria®-treated SBS were drenched with propamocarb-HCl and benomyl (Figure 8.19). Dry mass of both untreated SBS and Eria®-treated SBS was increased by drenching the medium with propamocarb-HCl, benomyl and a mixture of propamocarb-HCl/benomyl (Figure 8.20). The combination of propamocarb-HCl and benomyl was less effective than when the fungicides were used separately.

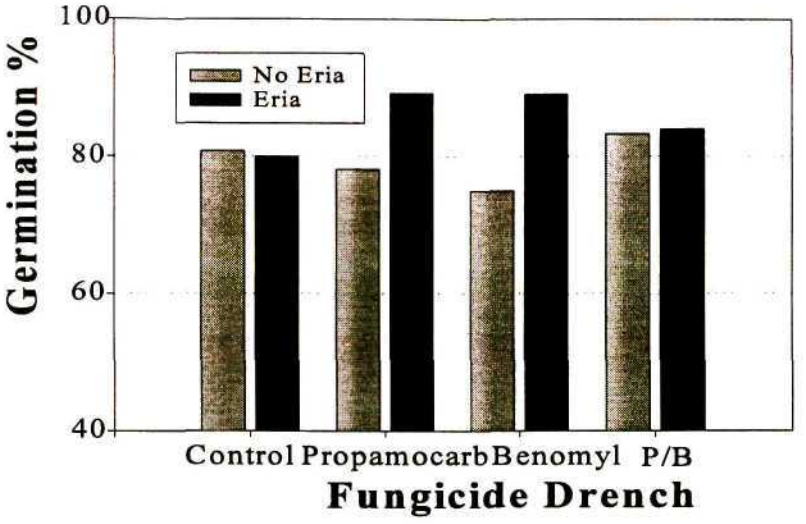


Figure 8.19 Effect of single-budded sett (SBS) treatment with Eria® and drenching of trays with propamocarb-HCl and/or benomyl on the germination of NCo376.

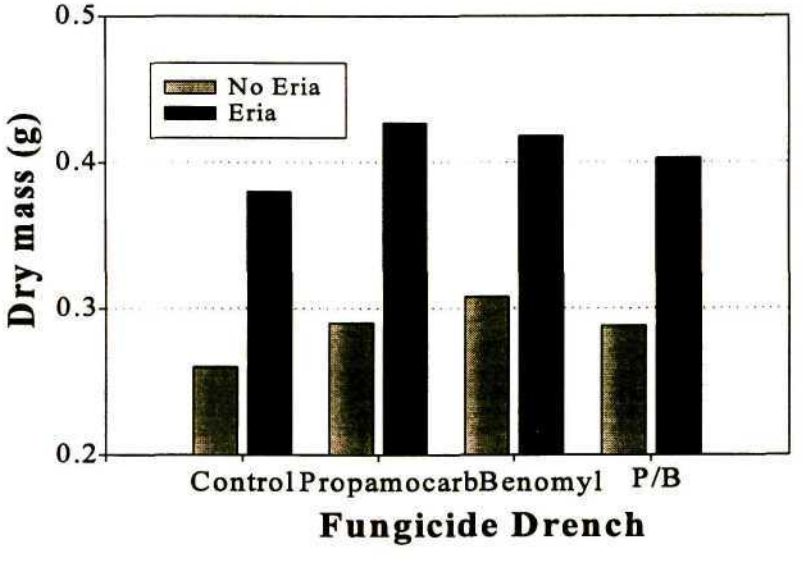


Figure 8.20 Effect of single-budded sett treatment with Eria® and fungicide drenching of trays with propamocarb-HCl and/or benomyl on dry mass of NCo376.

The efficacy of both propamocarb-HCl and benomyl indicated that reduced germination and growth were partly due to infections by fungi belonging to all fungal groups. However, use of propamocarb-HCl and benomyl only increased the mean germination by 5 and 3%, and dry mass by 13%, and Eria® significantly increased germination by 9% and dry mass by 46%. Therefore, treatment with Eria® was more effective than the fungicide drenches, suggesting that the increase in germination and growth after Eria®-treatment was caused by the plant growth regulatory activity of both difenoconazole and carbendazim.

8.5 Mode of Action of Eria®

8.5.1 Introduction

In the previous experiments treatment with Eria® significantly increased germination and plant growth when used as a hot treatment and was more effective than the other fungicides tested in Section 8.2 when used as a cold-treatment. The high efficacy of Eria® suggests that fungi belonging to the Ascomycetes, Basidiomycetes or Deuteromycetes were involved in germination failure and poor growth. These fungi might include *Fusarium* spp. and *C. paradoxa* that were isolated from untreated SBS tissues, roots and growing media (Section 8.2). The systemic fungicides Eria® and Punch-Xtra® were more effective than the protectant fungicide guazatine, indicating that systemic infections may have been the major cause of germination failure. Further increases in germination after drenching of trays containing the fungicide-treated SBS with the P/B solution indicated control of soil-pathogens such as *Pythium* spp. and *Fusarium* spp. The results indicated that the promotive effect of Eria® treatment on germination and growth could not be ascribed only to protection against fungi, but may have also been due to the plant growth regulatory effects of both difenoconazole and carbendazim.

The aim of this experiment was to determine the extent to which the large increases in germination and growth after treatment with Eria® were due to fungicidal activity and to plant growth regulator properties. In these experiments SBS were treated with the systemic fungicides propamocarb-HCl and benomyl before Eria® treatment and after planting. Together with these treatments, further increases in germination or growth afforded by Eria® treatment could be ascribed to the plant growth regulatory activity of Eria® or the control of fungi not controlled by Benlate® or Previcur®. Single-budded setts were also treated with carbendazim (62.5 ppm; Bavistin®, BASF), difenoconazole (31.25 ppm; Score®, Novartis) and carbendazim+difenoconazole (62.5 ppm, 31.25 ppm; Eria®, Novartis) to determine which active ingredient of Eria® was implicated in the stimulation of germination and growth. In addition, SBS were planted either in steam-sterilised or unsterilised composted bagasse to determine whether germination failure was principally due to systemic fungi within the SBS or pathogens in the growing medium.

8.5.2 Materials and methods

Experiment A

Single-budded setts of variety NCo376 were treated as follows:

a. Control
b. SHWT (52°C for 10 minutes)
c. Eria® (0.5 $m\ell.\ell^{-1}$, 52°C, 10 minutes)
d. Eria® (0.5 $m\ell.\ell^{-1}$; 52°C, 10 minutes) + propamocarb-HCl (1083 ppm) and benomyl (250 ppm) drench.

The SBS were planted into trays containing either steam-sterilised or unsterilised composted bagasse. Each treatment was represented by a total of 60 SBS, divided into three replications of 20 SBS each. Trays were left in a germination room and transferred to the nursery once most SBS had germinated.

Experiment B

Single-budded setts of varieties NCo376 and N12 were dipped in either water or a propamocarb-HCl (1083 ppm) and benomyl (250 ppm) solution before a five minute treatment in the following fungicides:

a. Control
b. Eria® at 0.5 $m\ell.\ell^{-1}$ (62.5 ppm carbendazim and 31.25 ppm difenoconazole)
c. Bavistin® at 125 $\mu\ell.\ell^{-1}$ (62.5 ppm carbendazim)
d. Score® at 125 $\mu\ell.\ell^{-1}$ (31.25 ppm difenoconazole)

The SBS were then planted into trays and those previously dipped in the P/B solution were drenched with the same solution. Each treatment was represented by a total of 60 SBS divided into three replications of 20 SBS each. The trays were initially placed at 20°C and 55-95% relative humidity for five days to delay germination, then four days at 32°C (75-95% relative humidity) and then on benches outdoors. The germination and dry mass of the transplants from both experiments were recorded after 35 days and the data pooled and evaluated using either two- or three-way analysis of variance (ANOVA). The means were separated using least significant differences.

8.5.3 Results and discussion

Experiment A

Statistical analysis of the data indicated that SBS treatment and steam sterilisation of the growing medium had no significant effect on the mean germination of NCo376 (Table 8.23, Figure 8.21). These results indicated that systemic infections and soil pathogens did not affect germination.

Table 8.23 Two-way ANOVA of the mean germination and dry mass of NCo376 after treatment of single-budded setts (SBS).

Factor	df	Germination		Dry mass	
		Variance-ratio	P-value	Variance-ratio	P-value
SBS treatment	3	0.326	0.8065	13.697	0.0001
Steam sterilisation	1	0.018	0.8972	0.0000	1.0000
SBS treatment x steam sterilisation	3	2.439	0.1021	1.333	0.2985

The germination results were consistent for SBS planted in both steam-sterilised and unsterilised composted bagasse as indicated by the lack of a significant interaction between SBS treatment and steam sterilisation (Table 8.23). Germination of the controls was high (73 and 89%) and treatment of SBS with both the SHWT and Eria® before planting into either medium had no significant effect on germination (Figure 8.22).

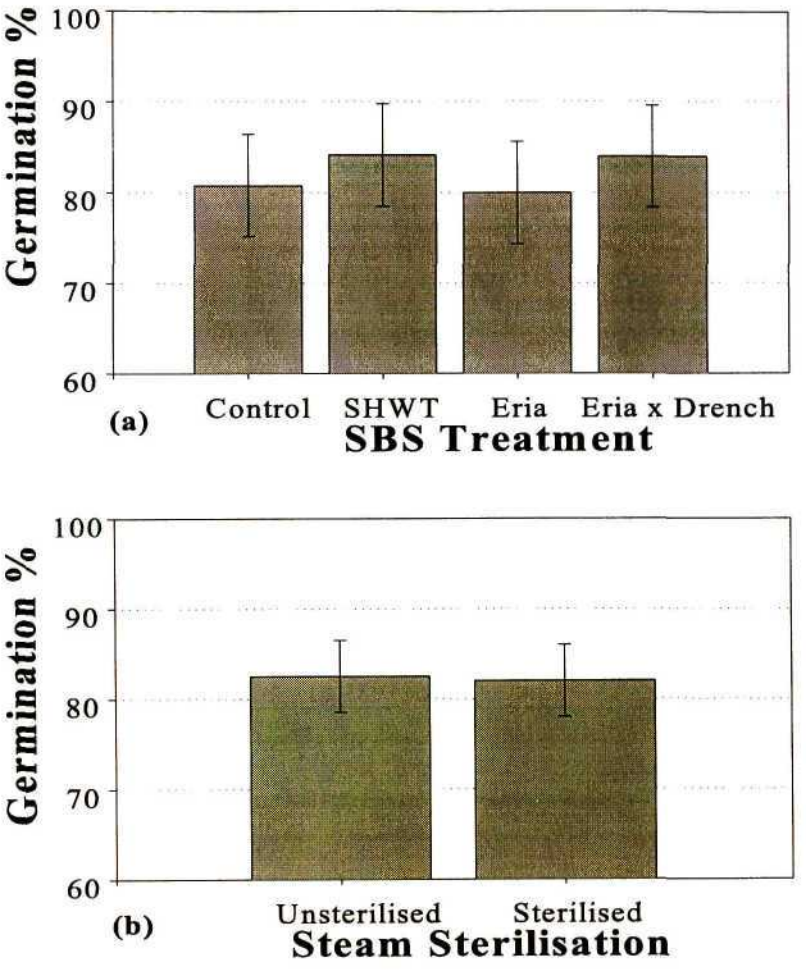


Figure 8.21 Effect of single-budded sett (SBS) treatment (a) and steam sterilisation of medium (b) on germination of NCo376. Means whose 95% comparison intervals do not overlap are significantly different.

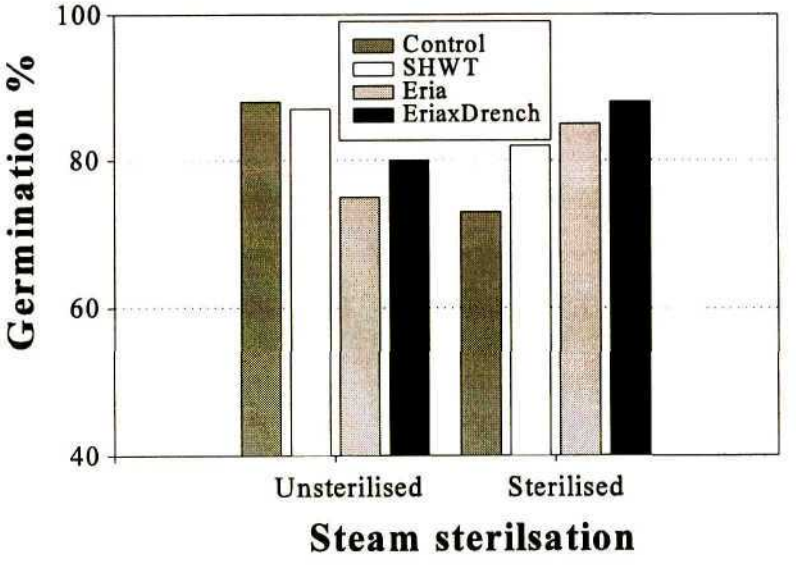


Figure 8.22 Effect of fungicide treatment and steam-sterilisation of medium on germination of NCo376.

Statistical analysis of the data indicated that the mean dry mass of NCo376 was significantly affected by SBS treatment but not by steam sterilisation of growing medium (Table 8.23). Compared with the control, mean dry mass was significantly increased by the SHWT (+23%, $P<0.05$), Eria® (+46%, $P<0.01$) and drenching Eria®-treated SBS with the P/B solution (+54%, $P<0.01$). Dry mass of transplants of the latter two treatments was significantly higher than that of the transplants treated with hot water only (+19-25%, $P<0.05$) (Figure 8.23 a). Sterilisation of the growing medium had no effect on dry mass, indicating that the unsterilised medium did not contain pathogens inhibiting germination (Figure 8.23b).

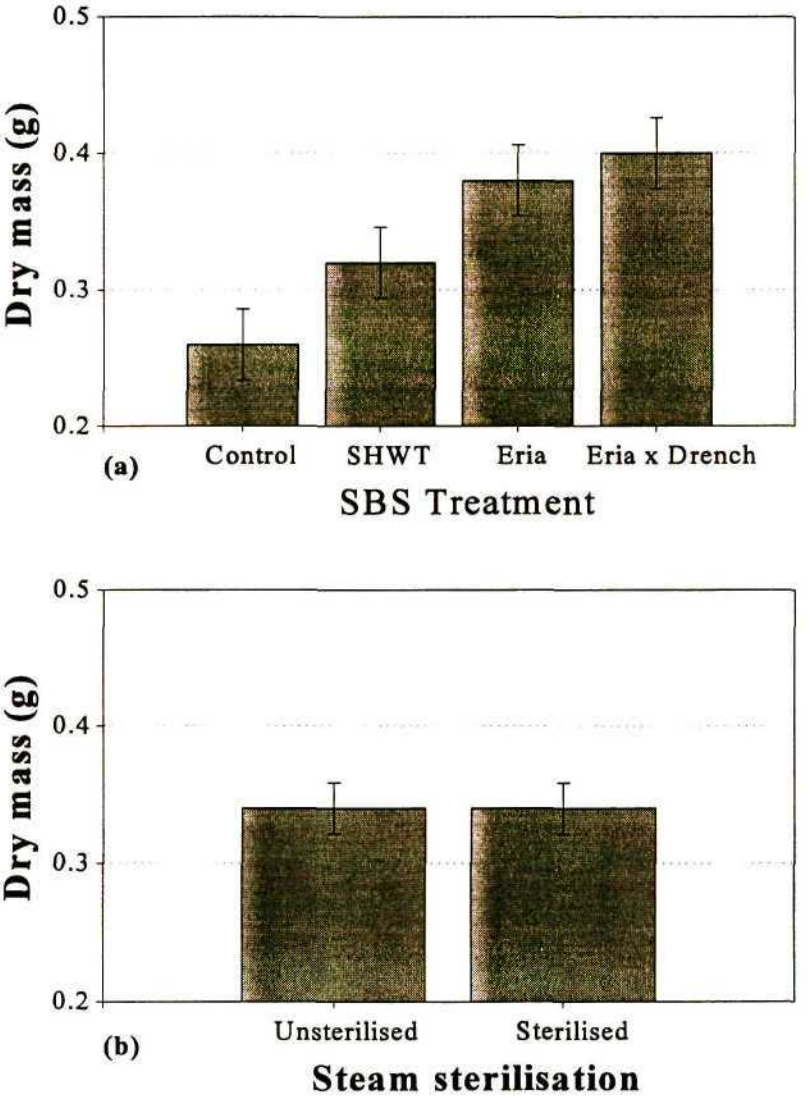


Figure 8.23 Effect of single-budded sett (SBS) treatment (a), and steam sterilisation of medium (b) on the mean dry mass of NCo376. Means whose 95% comparison intervals do not overlap are significantly different.

The dry mass results were consistent for SBS planted in both steam-sterilised and unsterilised composted bagasse as indicated by the lack of a significant interaction between SBS treatment and steam sterilisation (Table 8.23). Dry mass of the controls was similar in both unsterilised and sterilised medium. Compared with the control, the SHWT increased dry mass of transplants in both unsterilised (+15%) and sterilised (+32%) media. The Eria® treatment in hot water significantly increased dry mass of the transplants in both the unsterilised (+33%, $P<0.01$) and sterilised (+60%, $P<0.05$) media. The increase in dry mass of transplants in sterilised media after Eria® treatment indicated that systemic pathogens within the SBS were eliminated or the hormonal balance was affected. Drenching the Eria®-treated SBS with P/B significantly increased dry mass of transplants in both unsterilised (+56%, $P<0.01$) and sterilised (+52%, $P<0.05$) media compared with the controls, but not compared with Eria® treatment of SBS without a drench, indicating that soil pathogens did not affect plant growth (Figure 8.24).

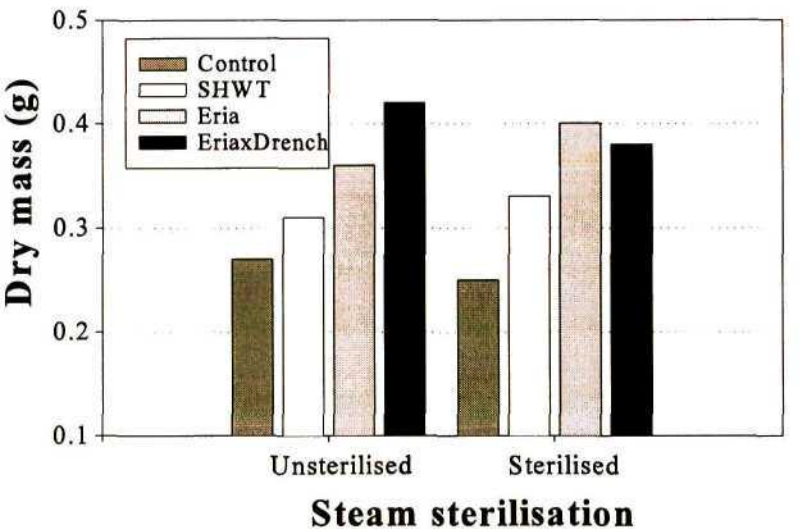


Figure 8.24 Effect of single-budded sett treatment and steam sterilisation of growing media on dry mass of NCo376.

Experiment B

Statistical analysis of the data indicated that the mean germination of NCo376 and N12 was significantly affected by SBS treatment but not by P/B treatment (Table 8.24). Compared with the control, mean germination was significantly increased by 24-25% when SBS were treated

with carbendazim, difenoconazole and carbendazim+difenoconazole ($P < 0.01$) (Figure 8.25a). Therefore, both active ingredients controlled the same fungi or had similar plant growth regulator properties. When trays were removed from the germination room, definite increases in germination after treatment with carbendazim and difenoconazole were observed compared with the control. This observation suggests that the increased germination recorded 28 days after planting was not due to control of late infections).

Treatment of SBS and drenching of trays with P/B had no effect on germination (Table 8.24). Propamocarb-HCl is a persistent fungicide that usually remains active against *Pythium* spp. for 30-45 days (Laing, 1996, unpublished[1]). However, benomyl only remains active against a broad range of pathogens for 8-10 days. Since both active ingredients had no effect on germination when applied as an SBS treatment and as a drench, *Pythium* spp. and other pathogenic fungi may have not been present in the medium or in the SBS. If this is the case, unless carbendazim and difenoconazole control fungi which are not controlled by propamocarb-HCl and benomyl, the increase in germination of SBS given this treatment was due to the plant growth regulatory properties of both carbendazim and difenoconazole.

Table 8.24 Three-way ANOVA of the mean germination and dry mass of varieties NCo376 and N12 after single-budded sett (SBS) treatment and propamocarb-HCl/benomyl (P/B) treatment.

Factor	df	Germination		Dry mass	
		Variance-ratio	P-value	Variance-ratio	P-value
SBS treatment	3	11.105	0.0000	4.307	0.0116
P/B treatment	1	0.006	0.9375	0.238	0.6342
Variety	1	64.103	0.0000	17.190	0.0002
SBS treatment x P/B treatment	3	0.892	0.4558	0.565	0.6420
P/B treatment x variety	1	4.046	0.0151	1.458	0.2447
SBS treatment x variety	3	0.463	0.5083	0.729	0.4087

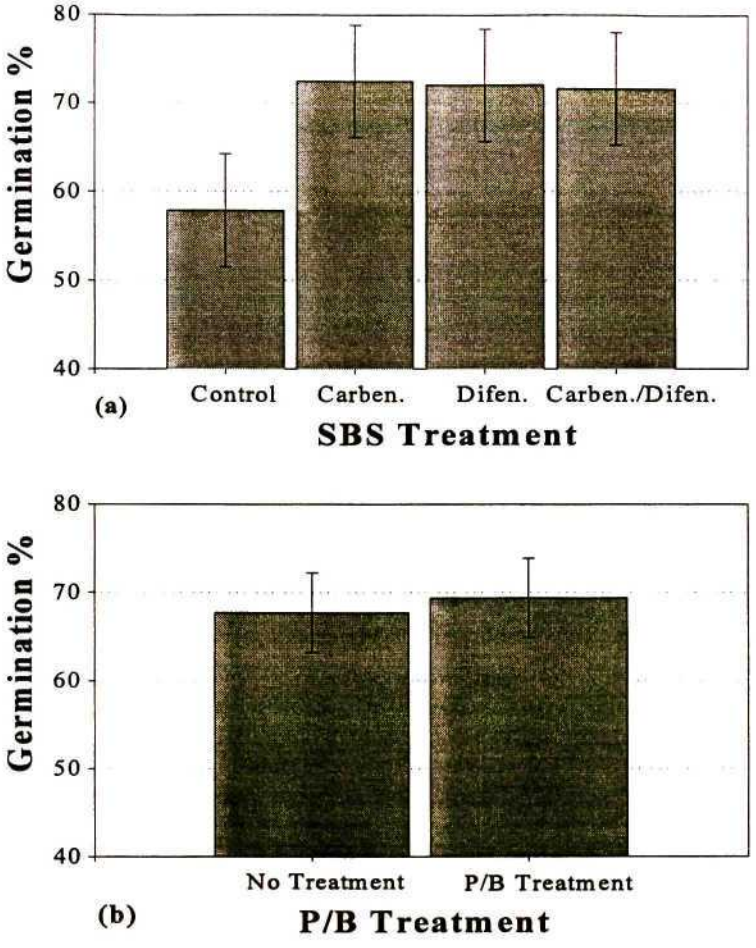


Figure 8.25 Effect of single-budded sett (SBS) treatment (a), and propamocarb-HCl/ benomyl (P/B) treatment (b), on the mean germination of the varieties NCo376 and N12. Means whose 99% comparison intervals do not overlap are significantly different.

The germination results were consistent for both varieties and treatments as indicated by the lack of significant interactions between SBS treatment and P/B treatment, SBS treatment and variety, and P/B treatment and variety (Table 8.24). Combined with the P/B treatment, germination was improved by treatment with carbendazim (+24%), difenoconazole (+28%) and carbendazim+difenoconazole (+17%), but not significantly. Therefore, although systemic infections and soil pathogens should have been eliminated by treatment with P/B, germination was further increased by treatment with both carbendazim and difenoconazole suggesting that these active ingredients had plant growth regulatory activity (Figure 8.26a). Germination of SBS not treated with P/B was increased by treatment with difenoconazole (+20%) and significantly increased by treatment with carbendazim (+26%, $P<0.05$) and carbendazim+difenoconazole (+31%, $P<0.01$).

Germination of N12 was not significantly affected by SBS treatment but was increased by treatment with carbendazim (+35%), difenoconazole (+26%) and carbendazim+ difenoconazole (+15%). Germination of NCo376 was increased by treatment with carbendazim (17%) and significantly increased by treatment with difenoconazole (+23%, $P<0.05$) and carbendazim+difenoconazole (31%, $P<0.01$) (Figure 8.26b). Treatment with P/B did not affect germination of both varieties (Figure 8.26c).

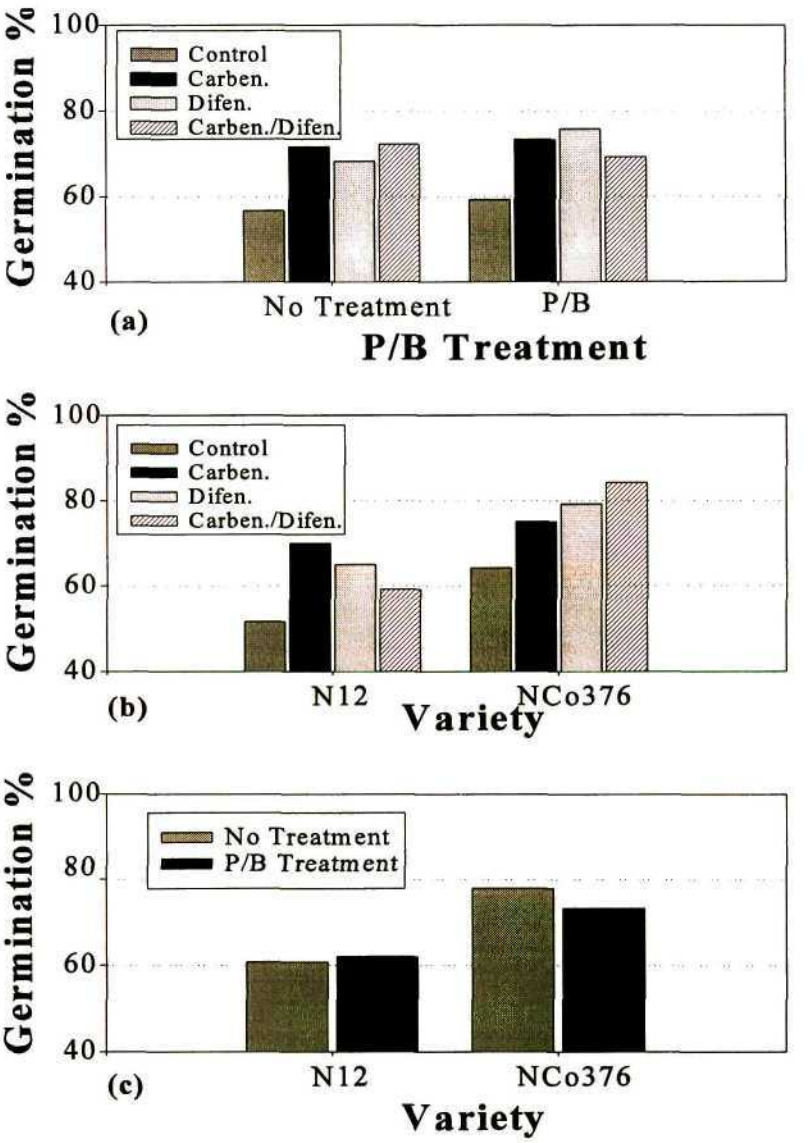


Figure 8.26 Effect of single-budded sett (SBS) treatment and propamocarb-HCl/benomyl (P/B) treatment on germination of the varieties NCo376 and N12. The interactions between SBS treatment and P/B treatment (a), SBS treatment and variety (b), and P/B treatment and variety (c) were non-significant.

Statistical analysis of the data indicated that the mean dry mass of NCo376 and N12 was significantly affected by SBS treatment, but not by the P/B treatment (Table 8.24). Compared with the control, the mean dry mass was significantly increased when SBS were treated with carbendazim+difenoconazole (+83%, P=0.01) and difenoconazole (+72%, P=0.01), and increased after treatment with carbendazim (+28%) (Figure 8.27a). These results indicated that the growth stimulation after treatment of SBS with carbendazim+difenoconazole was mainly due to the activity of difenoconazole. The P/B treatment had no effect on dry mass (Figure 8.27b).

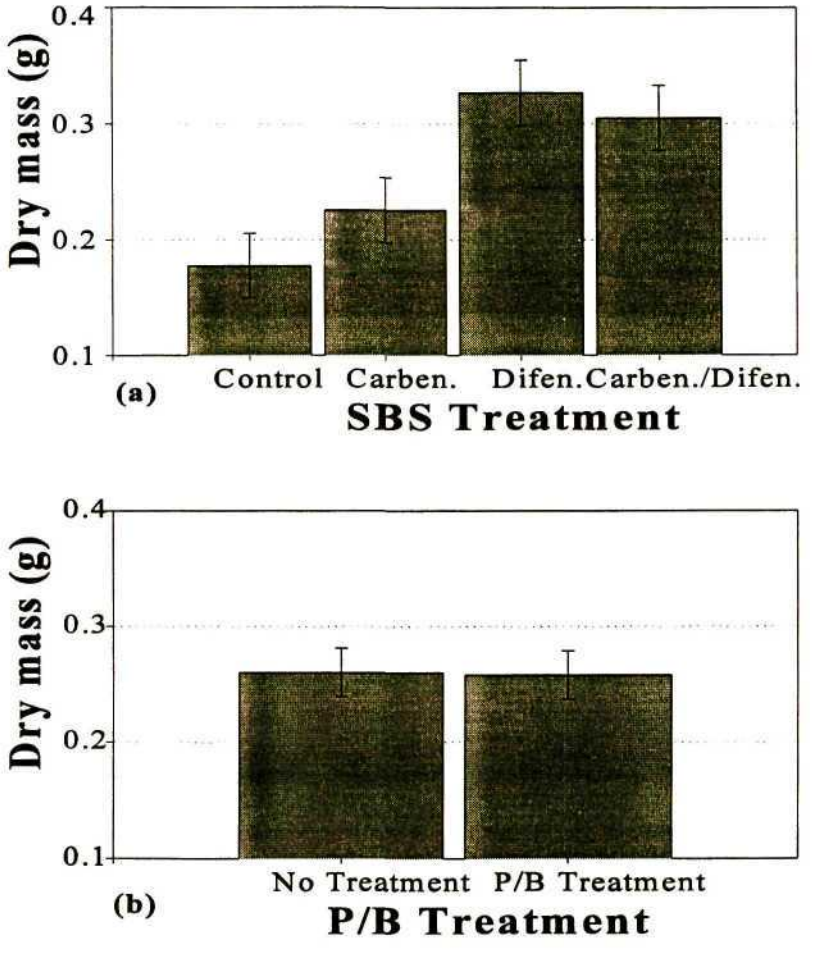


Figure 8.27 Effect of single-budded sett (SBS) treatment (a) and propamocarb-HCl/benomyl (P/B) treatment (b) on dry mass of the varieties NCo376 and N12. Means whose 95% comparison intervals do not overlap are significantly different.

The dry mass results were consistent for all varieties and SBS treatments as indicated by the lack of significant interactions between SBS treatment and P/B treatment, and SBS treatment and variety (Table 8.24). Although fungicide treatment did not significantly affect germination of P/B treated SBS, all fungicides increased dry mass, difenoconazole (+71%) and carbendazim+difenoconazole being most effective (Figure 8.28a). Treatment of untreated SBS with carbendazim+difenoconazole and difenoconazole significantly increased dry mass (+94%, $P<0.01$) and treatment of both P/B-treated and untreated SBS with carbendazim slightly increased dry mass.

Dry mass of both varieties was significantly affected by all SBS treatments. Dry mass of N12 was significantly increased by treatments with carbendazim (+50%, $P<0.05$), difenoconazole (+58%, $P<0.01$) and carbendazim+difenoconazole (75%, $P<0.01$). Dry mass of NCo376 was increased by treatment with carbendazim (+17%), and significantly increased by treatment with difenoconazole (+104%, $P<0.01$) and carbendazim+difenoconazole (+74%, $P<0.01$) (Figure 8.28b).

To summarise, both carbendazim and difenoconazole significantly increased germination, and difenoconazole significantly increased plant growth. Treatment of the SBS with the P/B solution before and after the fungicide treatment had no effect on the mean germination or plant growth, indicating the minor role of systemic infections and soil pathogens in germination failure. Therefore, the significant responses induced by carbendazim+ difenoconazole were due to either plant growth regulator activities or control of late infections. However, the increase in germination after treatment with these fungicides was observed soon after planting, indicating that germination failure was not caused by late infections, but by the plant growth regulator activities of carbendazim and difenoconazole, and a lesser extent to their fungicidal activity.

These results provide evidence that germination failure was mainly brought about by an inappropriate hormonal balance within the SBS. Therefore, increases in germination and plant growth can be obtained by treatments that affect the hormonal balance in the SBS, and by the use of good quality seedcane.

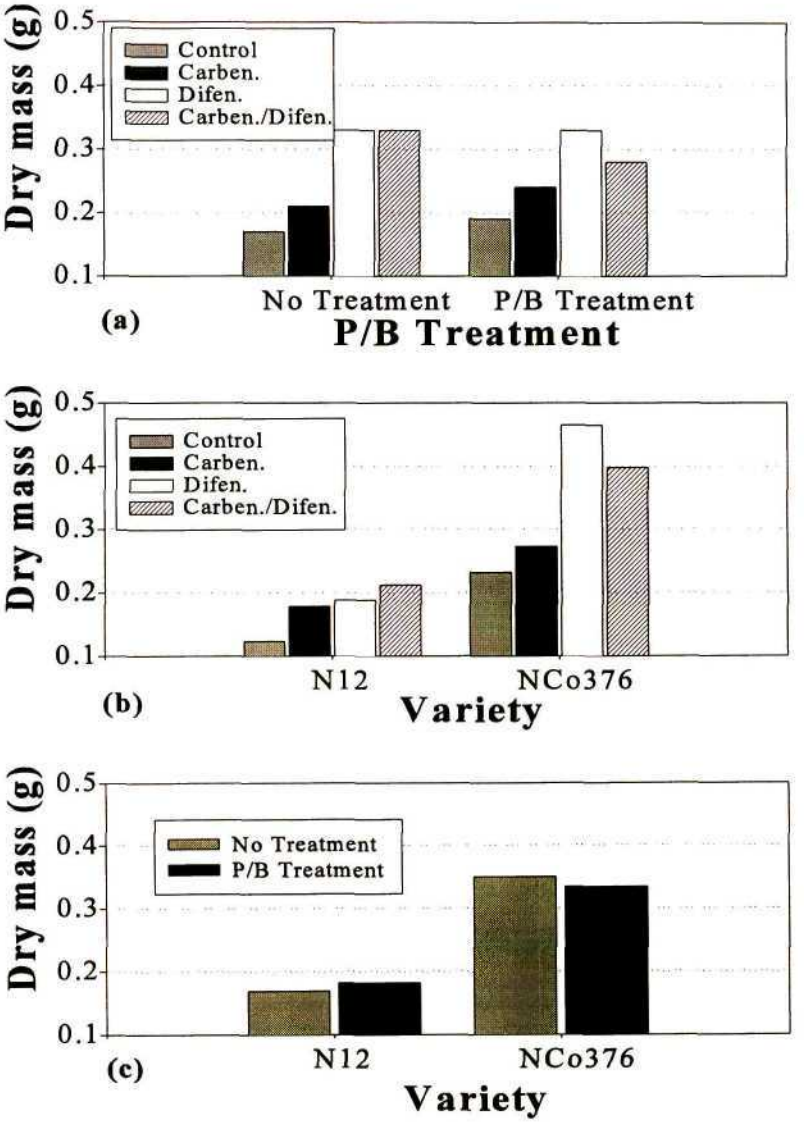


Figure 8.28 Effect of single-budded sett (SBS) treatment and propamocarb-HCl/benomyl (P/B) treatment on the mean dry mass of varieties NCo376 and N12. The interactions between SBS treatment and P/B treatment (a) and SBS treatment and variety (b) were non-significant. There was a significant interaction between P/B treatment and variety (c).

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8.7 Appendix 1

P_5ARP

The antibiotics (5 mg pimaricin, 250 mg ampicillin, 10 mg rifampicin and 100 mg PCNB) were added to autoclaved cornmeal agar (17 $g.\ell^{-1}$) when it had cooled to 50°C in a water bath.

9. GROWING MEDIA

Abstract

Germination and growth of the varieties N12, N17, N19 and NCo376 were affected equally when single-budded setts were planted in composted bagasse, composted pinebark and mixtures of the two.

9.1 Effect of Growing Media on Germination and Growth

9.1.1 Introduction

A medium with good drainage, adequate air-filled porosity and sufficient water-holding capacity must be used in the transplant nursery because quality of the medium greatly influences plant growth. The growing media commonly used in nurseries for the production of sugarcane transplants include mixtures of composted bagasse (CB), composted pinebark (CPB), composted filtercake (FC), sand and vermiculite. Commercial CPB mixtures, available from local compost factories, are stable, well-drained and aerated. Both filtercake and bagasse, available from sugar mills, can be composted at the planting site using the turned-pile method. Previous experiments at SASEX showed that germination and shoot development were good when transplants were grown in CB mixtures (Anon., 1992). Frequent problems have been encountered with the use of FC because it is saline and can be phytotoxic. Furthermore, FC must be mixed with vermiculite or sand to aid drainage and strict water management must be employed because it has a high water-holding capacity. The aim of this experiment was to determine the effect of CB, CPB and two mixtures of these media on germination and plant growth of sugarcane transplants. The CPB was kindly donated by Gromed® at Cramond and the FC and CB were composted at SASEX.

9.1.2 Materials and methods

Single-budded setts of varieties N12, N17, N19 and NCo376 were heat treated at 50°C for 120 minutes, soaked in 800 ppm guazatine for five minutes and planted into trays containing the

following media:

a. Coarse screened CPB with < 12 mm particle size
b. CB
c. 1CPB:1CB
d. 1CPB:3CB

Each treatment was represented by a total of 60 SBS, divided into three replicates of 20 SBS each. Germination and dry mass results were recorded 28 days after planting, pooled and evaluated using two-way analysis of variance (ANOVA). Means were separated using least significant differences.

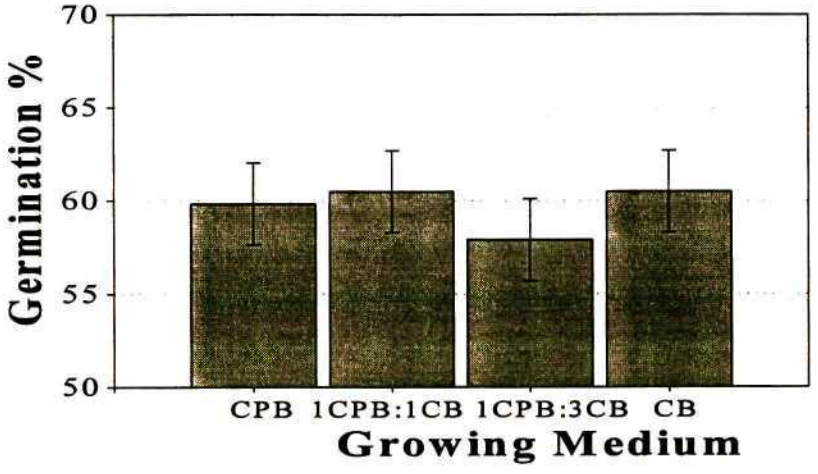
9.1.3 Results and discussion

Statistical analysis of the data indicated that growing medium had no significant effect on the mean germination and dry mass of the four varieties (Table 9.1). Therefore, germination and growth were affected equally when SBS were planted in CB, CPB and mixtures of the two (Figure 9.1). Costing exercises comparing the use of CB with CPB need to be conducted. Currently, CB appears to be the more economically viable medium because bagasse is relatively inexpensive and easy to compost. However, costs such as transport, machinery and labour to turn the compost need to be taken into account.

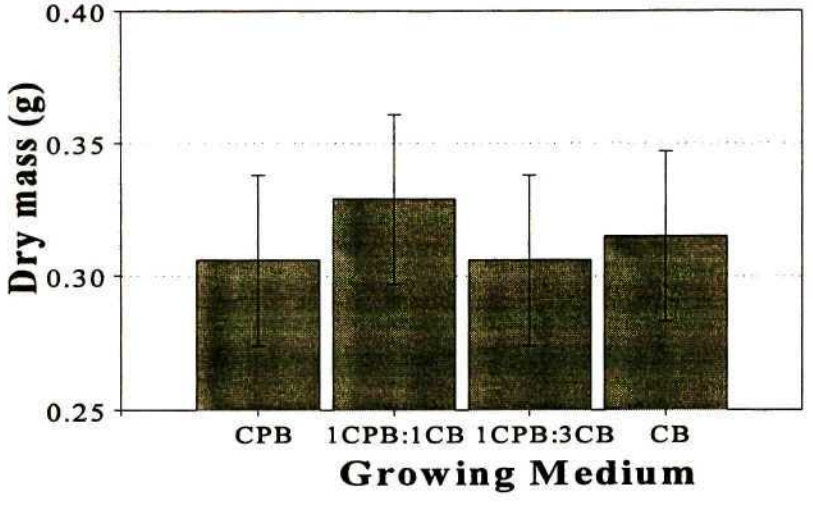
The germination results were not consistent for all varieties as indicated by the significant interaction between medium and variety (Table 9.1). The germination of N19 and NCo376 was not significantly affected by the growing medium. However, germination of N12 was significantly higher in CB than in the other media ($P<0.01$), and germination of N17 was significantly higher in a mixture of 1CPB:1CB than in CPB or CB alone ($P<0.05$) (Table 9.2).

Table 9.1 Two-way ANOVA of the mean germination and dry mass of varieties N12, N17, N19 and NCo376.

Factor	df	Germination		Dry mass	
		Variance-ratio	P-value	Variance-ratio	P-value
Medium	3	0.568	0.6402	0.250	0.8609
Variety	3	228.418	0.0000	30.577	0.0000
Medium x variety	9	5.471	0.00001	1.094	0.3940



(a)



(b)

Figure 9.1 Effect of growing medium on the mean germination and dry mass of N12, N17, N19 and NCo376. Means whose 95% comparison intervals do not overlap are significantly different (CPB=composted pinebark, CB=composted bagasse).

The dry mass results were consistent for all varieties as indicated by the lack of a significant interaction between medium and variety (Table 9.1). The type of medium used had no effect on the dry mass of all varieties (Table 9.2). These results suggested that both CB and CPB are good growing media that have little effect on overall germination and growth. Therefore, the use of either medium is recommended for transplant production.

Table 9.2 Effect of growing medium on the germination and dry mass of varieties N12, N17, N19 and NCo376.

Medium	Germination %				Dry mass (g)			
	N12	**N17**	**N19**	**NCo376**	**N12**	**N17**	**N19**	**NCo376**
CPB	39 b	80 b	75	45	0.20	0.30	0.20	0.52
1 CPB : 1 CB	33 b	93 a	85	30	0.34	0.32	0.16	0.50
1 CPB : 3 CB	34 b	87 ab	70	42	0.27	0.33	0.16	0.47
CB	50 a	81 b	75	36	0.30	0.32	0.22	0.42
LSD (P=0.05) LSD (P=0.01)	7.50 10.90	9.08 13.21	NS	NS	NS	NS	NS	NS

Means in a column with a letter in common are not significantly different at the 5% level.

9.1.4 Literature Cited

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GENERAL DISCUSSION AND RECOMMENDATIONS

This study has been successful in that practical methods have been found to improve germination of single-budded setts (SBS) planted in trays. As a result, the transplant production process is quicker, easier and more economically viable. Firm recommendations cannot be given with respect to control of *Clavibacter xyli* subsp. *xyli*, but the results gave an indication of the research required in this area.

Bud position on the stalk and length of seedcane storage strongly influenced germination. The lower buds on old seedcane germinated poorly, indicating the importance of using young seedcane (8-10 months) of good quality for transplant production. When old seedcane must be used, more seedcane will be required, the lower internodes should be discarded, and the SBS sorted to remove those damaged or visibly infected with *Eldana saccharina* or the red rot pathogen *Glomerella tucumanensis*.

Seedcane storage for three and eight days between collection and transplant preparation adversely affected germination and growth. *E. saccharina* larvae already present in the stalks had bored into adjacent internodes, damaging buds and infecting the cane with *G. tucumanensis*. Furthermore, the sugars in seedcane stored for eight days fermented and the stalks became dehydrated. Therefore, to obtain optimum germination, seedcane stalks used for transplant production should not be stored for three days or longer after collection, and should preferably be cut and planted the same day they are collected.

Topping the seedcane stalks three days before collection slightly improved germination and growth, particularly when seedcane was subsequently heat treated. However, this practice is not recommended because results were not conclusive and further experiments are necessary to determine the effect of topping stalks more than three days before seedcane collection. This longer standing period before collection would allow lateral buds to germinate while still on standing stalks because of the removal of apical dominance. Planting of SBS that already have swollen buds should stimulate faster germination and reduce infection by soil pathogens when the SBS are planted in growing medium.

The major problem encountered in most commercial sugarcane transplant nurseries was poor germination of SBS after heat treatment of seedcane stalks or SBS at 50°C for 120 minutes. Low germination leads to high production costs, increasing labour, preparation time, nursery space and seedcane requirements. Since most farmers require *C. x. xyli*-free transplants for their nursery fields, this treatment cannot be avoided. However, steps can be taken to alleviate this problem. Germination results were comparable when both whole setts (WS) and SBS were heat-treated at 50°C for 120 minutes and control of *C. x. xyli* was more effective after heat treatment of SBS than that of WS. Therefore, treatment of SBS is recommended as it increases the quantity of material that can be treated in a single-batch tank, reducing preparation time and electricity costs. Nurseries that presently have the capacity to heat treat WS may continue to do so, but should avoid rough handling after heat treatment. These nurseries should also store the heat treated WS for a few hours in the germination room until the buds have hardened, making them more resistant to mechanical damage that often occurs during the cutting process.

The heat treatment period could possibly be shortened for SBS, because their small size enables more efficient penetration of the tissues by heat. In the first experiment with heat treatment, treatment of SBS at 52°C for 30 minutes either significantly increased or did not affect germination compared with the control. However, in subsequent experiments, the same treatment had adverse effects on germination. Results showed that the response to heat treatment not only depended on variety, but also on stalk width, cane quality, cane age and growing conditions. Treatment of SBS at both 50°C for 120 minutes and 52°C for 30 minutes adversely affected germination although germination was significantly lower after treatment at 50°C than after treatment at 52°C. Treatment of SBS at 52°C for 30 minutes controlled *C. x. xyli* in six commercial varieties but not in the variety N14. Therefore, the effects of treatment of SBS at 52°C for 30-60 minutes on germination and control of *C. x. xyli* requires further investigation.

Results showed that germination of SBS treated at 52°C for 60 minutes and 50°C for 120 minutes was comparable. Although both treatments adversely affected germination, the former may be useful since it shortens the treatment time and increases the number of batches of cane

that can be heat treated in a single-batch tank. This would expedite the production process, enabling the grower to produce more transplants when higher demands are placed on the nursery. Moreover, labourers would not be required to work overtime, and the nursery manager would have greater control over the heat treatment process, through regular checks of the water temperature, preventing overheating with consequent total germination failure.

When long hot-water treatment to control *C. x. xyli* is not required, short heat treatments can be used to increase both germination and plant growth. These treatments do not control *C. x. xyli,* but change the hormonal balance of the buds to one favourable for germination and probably control systemic fungi in the SBS. Treatment at 52°C for 10 minutes significantly improved the germination of SBS prepared from poor quality seedcane and the growth of plants prepared from good quality seedcane. This treatment is highly recommended and would be easy to carry out in nurseries that already have heat-treatment tanks; it can also be used to promote the activity of most fungicide treatments. Where heat treatment tanks are not available, the use of hot tap water in large plastic containers is possible. During the present study, treatment of SBS in 10 *l* buckets in hot tap water that was initially 50° to 52°C and dropped to 45° to 48°C after 10 minutes induced significant increases in germination of commercial varieties. The use of the SHWT has been used successfully by other departments at SASEX for both commercial and unreleased varieties. Some unreleased varieties were sensitive to the treatment, indicating that all new varieties must be tested before large-scale treatments are carried out on new varieties (MG Keeping, 1997, pers. comm.[1])

Fungicide treatments had a marked effect on germination and growth, particularly the fungicide Eria® (carbendazim+difenoconazole, Novartis). Eria® improved germination and plant growth when used as a hot treatment at 52°C for 10 and 30 minutes, and was more effective than the other fungicides when used as a cold treatment. Eria® can also be successfully used as a treatment in cold water after heat treatment at 50°C for 120 minutes. Eria®-treatment increased germination soon after planting, indicating that the increase in germination was due either to its plant growth regulator activity or control of early infections.

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[1] Dr MG Keeping, SASEX, Private Bag X02, Mount Edgecombe, KwaZulu-Natal

Plant dry mass was significantly higher when SBS were treated with difenoconazole than when treated with carbendazim. Therefore, it was the activity of difenoconazole, rather than that of carbendazim, that contributed towards improved germination and plant growth after treatment with Eria®.

The use of Eria® for transplant production is presently not recommended as it is not registered for use on sugarcane; registration trials have been planted for this purpose. However, since Eria® is readily available, some growers have already started using it, and report considerable increases in germination and plant vigour compared with the use of other registered fungicides, particularly when used as a cold treatment after setts have been heat treated at 50°C for 120 minutes. The positive response has lead to many enquiries about the product and a number of farmers are testing Eria® for commercial plantings.

The cost of using Eria® works out to be nearly double that of Benlate® and a third of that of Panoctine®. However, its positive effects on germination and plant growth will probably make it the most commonly used fungicide in sugarcane transplant nurseries in the future.

Treatment of both SBS and medium in trays with a solution of propamocarb-HCl and benomyl alone or before Eria®-treatment did not affect germination and growth, although benomyl is active against a wide range of fungi and propamocarb-HCl is active against Oomycetes. Thus, systemic infections and soil pathogens appeared to play a small role in germination failure and growth inhibition, suggesting that germination after treatment with Eria® was increased by the plant growth regulator activity of difenoconazole. To confirm these results, further experiments must be carried out in a pathogen-free environment, using sterilised trays, vermiculite and water to prevent fungal infection of the medium and SBS after planting. To clarify the role of both active ingredients, their activity needs to be compared with that of uniconazole and paclobutrazol, both triazoles with strong plant growth regulatory activity and little fungicidal activity.

The success of Eria® in transplant nursery trials requires confirmation through field trials to investigate the effect on germination and plant growth after treatment of seedcane with Eria®,

Score® (difenoconazole), Panoctine® and Benlate®. In these trials, four-budded setts should be treated before planting, and emerged shoot numbers and sugarcane yields determined. Since Eria®-treatment produced vigorous plant growth in transplants, it may also cause significant increases in yields of mature cane. Soil pathogens probably affect germination and growth of seedcane in the field more than that of SBS in growing medium in trays, therefore, pot experiments should also be carried out using field soil and various drenches to provide information on the presence of pathogenic soil microorganisms in field soils and their effect on germination and plant growth.

When SBS were planted in media containing composted bagasse or composted pinebark, germination and growth were similar, showing that both media can be used successfully in the nursery. Costing exercises comparing the use of composted bagasse with that of composted pinebark are necessary to determine which is the most economically viable medium for transplant production. At SASEX, it has taken up to a year to compost bagasse and each heap to be turned often before it is ready for use. Adjustments to the carbon:nitrogen ratio of the bagasse will expedite the composting process, making the use of composted bagasse more beneficial.

Results of all experiments strongly suggested that to obtain optimum germination and control of *C. x. xyli*, seedcane must be of the highest quality and free of *E. saccharina* and *G. tucumanensis*. The seedcane must be cut, treated and planted soon after harvest to prevent the spread of larvae and pathogens, the fermentation of sugars and dehydration of the stalks. Although less mechanical damage occurs to SBS than WS when heat treated, either can be used and treated, depending on the tank capacity and demands of the nursery. Recommendations cannot be made with respect to shortening the treatment period. However, results showed that treatment of SBS at 52°C for periods greater than 30 minutes may provide more effective *C. x. xyli* control than the standard treatment of 50°C for 120 minutes.

The costs of transplant production using these procedures need to be determined, and the costs of planting transplants must be compared with of the conventional planting method. Currently, some commercial nurseries are not profiting because of the inefficient use of seedcane.

However, production costs can be decreased and germination increased by using good quality seedcane, Eria® and the short hot water treatment. In areas where *C. x. xyli* is not prevalent, nurseries could establish their own seedcane plots or obtain certified *C. x. xyli*-free seedcane, that is frequently tested for the presence of *C. x. xyli*, from contracted growers. The long hot water treatment could then be avoided, alleviating germination problems and decreasing preparation time and labour requirements.

Transplant producers should ensure that mature, good quality, healthy seedcane is always available to promote good germination and vigorous plant growth. They could then provide sugarcane growers with good quality, disease-free, vigorously growing transplants at a price comparable with that of whole stalk seedcane used in conventional planting. Sugarcane growers would then be more willing to use transplants to establish their own first and second-stage nurseries, thereby ensuring that their subsequent crops are healthy, provide optimum sugar yields, and form part of an integrated plan to fight *C. x. xyli*.