Horticulture Innovation Australia

Final Report

State of the art hydro-sprigging technology to expand the Australian Turfgrass industry

Christopher Lambrides The University of Queensland

Project Number: TU13026

TU13026

This project has been funded by Horticulture Innovation Australia Limited with co-investment from The University of Queensland and funds from the Australian Government.

Horticulture Innovation Australia Limited (Hort Innovation) makes no representations and expressly disclaims all warranties (to the extent permitted by law) about the accuracy, completeness, or currency of information in *State of the art hydro-sprigging technology to expand the Australian Turfgrass industry*.

Reliance on any information provided by Hort Innovation is entirely at your own risk. Hort Innovation is not responsible for, and will not be liable for, any loss, damage, claim, expense, cost (including legal costs) or other liability arising in any way (including from Hort Innovation or any other person's negligence or otherwise) from your use or non-use of *State of the art hydro-sprigging technology to expand the Australian Turfgrass industry*, or from reliance on information contained in the material or that Hort Innovation provides to you by any other means.

ISBN 0 7341 3836 9

Published and distributed by: Horticulture Innovation Australia Limited Level 8, 1 Chifley Square Sydney NSW 2000 Tel: (02) 8295 2300 Fax: (02) 8295 2399

© Copyright 2016

Contents

Summary	3
Keywords	4
Introduction	5
Methodology	6
Outputs	11
Outcomes	12
Evaluation and Discussion	13
Recommendations	21
Scientific Refereed Publications	22
IP/Commercialisation	23
References	24
Acknowledgements	25
Appendices	26

Summary

Research was undertaken to develop a state of the art hydrosprigging protocol to facilitate additional market opportunities for turfgrasses and therefore greater profitability for Australian Turf producers. A broad range of research was undertaken in the field, glasshouse and laboratory on a panel of 12 bermudagrasses selected from a wide range of geographical locations across Australia. Because hydrosprigging relies on propagating new turfs from stolons a large part of the research activity investigated the physiological basis of sprouting in Australian bermudagrasses. An excellent understanding of the physiological basis of sprouting potential and the factors that influence sprouting in bermudagrass was achieved.

Sprouting percentage for stolons sampled in spring and summer (about 70%) was higher than that for winter and autumn (about 60%) suggesting that the warmer months of the year would be ideal for hydrosprigging. Sprouting percentage among genotypes was strongly associated with stolon diameter. When sprouting percentage was expressed on a per node basis, it was highly correlated with water soluble carbohydrate and crude protein. This observation suggested that the size and/or maturity of the axillary buds at nodes and the assimilate supply available to these nodes (related to photothermal quotient) was underlying the sprouting potential of the bermudagrasses. Research confirmed that the base temperature for growth in bermudagrass is 7.7 °C and significantly this sets the seasonal boundaries for when hydrosprigging may be occur.

Plant growth regulators (PGR's) were trialed to assess their effect on sprouting. Surprisingly, most hormone treatments (ethylene and various auxins) were effective on all grasses tested. At the optimum concentration of hormone, the sprouting percentage for any one grass could be elevated substantially. Ethylene was delivered in a microencapsulated dextran coating and packaged in powder form and would appear to have great potential in hydrosprigging as a safe and efficient method of delivering hormone to the sprigs.

Based on research findings and consultation with industry a hydrosprigging protocol for bermudagrass was developed and successfully implemented in field trials at Darra QLD on a sodic-saline soil. This protocol was found to be suitable for use with a range of organic media. Appropriate site characterisation and preparation is the foundation to a successful operation. A 5-15 cm scarification of the soil surface is recommended to allow for an incorporation of lime and/or gypsum if necessary, promote water capture and encourage root growth in the established grass. A two-pass method of hydrosprigging is strongly recommended. Pre-planting irrigation to fill the soil profile and sufficient

irrigation to match evaporation during establishment are key to successful hydrosprigging.

One of the important outcomes of this project is that there is now a technology package available that can be used with confidence to solve some of the most important environmental issues in Australia. There are enormous areas of denuded landscapes at mine sites, road verges, cuttings, CSG well heads, creek and riverbanks and now these have the potential to be vegetated minimising the loss of Australia's precious soil resources. The environmental benefits will extend beyond this with less dust in the atmosphere, less siltation and nutrient loads in waterways and less fire hazards around important infrastructure.

New laws that govern urban developments in Queensland and elsewhere in Australia require that 5% of land be allocated for storm water drainage and flood mitigation. Consequently, these parcels of land that can potentially be quite large would be highly suited to hydrosprigging with bermudagrasses. In these situations bermudagrasses have additional benefits in that they are drought resistant, water logging tolerant and can be landscaped for recreational use.

One major opportunity is to minimise siltation and nutrient runoff onto the Great Barrier Reef 'lagoon' thereby reducing the environmental impact of agriculture and urbanisation. The kilometres of waterways protected with narrow riparian zones could be widened by hydrosprigging bermudagrasses to act as a biological filter to separate the sugar cane production from the streams that drain into the Reef.

Keywords

hydrosprigging, bermudagrass, re-vegetation, sodic-saline soil, hostile soils, turfgrass

Introduction

An opportunity for the Australian Turfgrass Industry: Soil erosion and stabilisation are huge problems facing Australian land users including those working in agriculture, mining, road construction and urban sports and community landscapes. For example, soil erosion from river basins containing intensive agriculture has been estimated to be 1.26×10^9 t/yr (www.anra.gov.au/topics). While there are no published reports of soil losses from mining sites, road works, sports-fields and community parks they are likely to be significant as they are particularly susceptible to areas of de-vegetation resulting the potential for soil movement. There is a prospective opportunity for the Australian Turfgrass industry to play a role in providing solutions to Australia's land stabilisation issues. If ten per cent of these denuded landscapes can be vegetated by turfgrasses the Turfgrass industry as a whole can benefit greatly particularly in the current economic climate where the hangover from the global financial crisis has been persistent. Collectively, the areas to be stabilised are enormous and to date the methods used to revegetate these land masses are limited. The solution: Hydrosprigging is an inexpensive, easy method of delivering grass sprigs (stolons) with a hydraulic pump to large areas of bare soil. Sprigs are usually applied in a slurry of water, mulch (e.g. wood fibre, bagasse, paper pulp), fertilizer and tackifier/binder. The operator has full control of where the sprigs are applied which makes this approach highly efficient particularly in cases where the landscape is uneven and has major obstacles e.g. large rocks and sticks or contains electricity poles, drains, communication towers and perennial vegetation. Hydrosprigging is the method of choice particularly where the target areas are too large to plant using sod.

The limitations of hydrosprigging and hydro-seeding: Hydrosprigging and hydro-seeding have grown in popularity particularly for mine-site rehab and roadside vegetation projects where hostile soil types including sodic/saline soil profiles are common. However, the success rate of hydrosprigging and hydro-seeding in terms of establishing a grass sward is relatively low. The reasons for the low establishment success are not clear although poorly applied and inadequate planting/follow-up irrigation, hostile soil profiles and unsuitable grass feedstock are likely to be major contributors.

The available plant materials for vegetating landscapes where abiotic stresses are multiple and severe are limited. For example, the mining industry has a requirement to vegetate sodic and sometimes saline batters in environments where rainfall is low. A further requirement of vegetation for these situations is short growth habit to improve visibility over engineered landscapes, e.g. dam walls, and reduce the fire

hazard during dry conditions. Currently there are no successful ways for vegetating these profiles. Hydro-seeding has often failed because seed germination is poor due to the elevated levels of salt in the upper layers of the soil profile; consequently, traditional revegetation options using seed blends of C₄ grasses have been unsuccessful. Rhodes grasses had potential but these grow over a 1 m tall during wet seasons reducing visibility and increasing the fire hazard during dry seasons both highly undesirable for mine-site landscapes. In addition bunch grasses such as these leave sections of the soil surface exposed allowing for tunnel erosion to proliferate.

Methodology

This project included a range of field and laboratory studies and in some cases involved multiple experiments. A brief description of only the major studies are provided.

Physiological basis of sprouting potential in bermudagrass

The aim of this part of the project was to gain an understanding of the physiological processes that underpin stolon sprouting in bermudagrass. Twelve bermudagrass genotypes (Cynodon spp.) including 11 ecotypes and a commercial cultivar Wintergreen as a check were grown in a field trial under optimal water and fertilizer conditions at the Allenview (27.54°S, 152.56°E), Production facility of The Jimboomba Turf Group in SE Queensland, Australia. Each bermudagrass genotype had been established with a plot size of $6m \times 5m$, and the gap between each plot was 2m to avoid cross contamination. Genotypes were arranged in a latinized row column design with three replications. Plots were managed as for commercial turfgrass cultivation with regular mowing (clippings retained), irrigation and fertilization. Above ground materials in each plot were sampled on clear sunny days from 9:30am to 11:30am across seasons including winter 2013 (26/08/2013), winter 2014 (26/08/2014), spring 2014 (26/10/2014), summer 2015 (16/01/2015) and autumn 2015 (20/03/2015) using a steel coring tube (6 cm diameter \times 4 cm deep) and then washed free of soil. Half the turf sample of each genotype was used to determine sprouting percentage of stolons and the remaining half was used to determine total nodes per unit area (Nds), stolon diameter (SD), total above ground biomass (TaB), and water soluble carbohydrate [WSC], [starch], non-structural carbohydrate [NSC = WSC + starch] and [crude protein] (CP) in the above ground dry matter on a dry weight basis. (N.B square brackets [] denote concentration). The stolons (hereafter includes tillers and leaves as they are all used for hydrosprigging) of each genotype were imbibed in tap water for five minutes and then placed in germination trays (20 imes10 cm rectangle) on filter paper moistened with distilled water; 72 trays were prepared. Trays were incubated in the dark for 15 and 21 days at two different constant temperatures, 20 and 30°C,

respectively. Three replications were placed at each temperature.

[WSC], [starch] and [CP] in TaB was determined on a dry weight basis. About 25-30 mg of dry sample was ground and sieved through a 0.5 mm mesh then weighed into 10 ml centrifuge tubes. Each sample was extracted with 80% ethanol at 80°C in a water bath for 15 minutes before being centrifuged for 5 minutes at 2500 rpm. This process was repeated 2 times using water instead of ethanol to extract the WSC. WSC was measured on a spectrophotometer using glucose as a standard. The residue from the WSC extraction was then used to determine starch by using total starch assay kit (AA/AMG) from Megazyme supplied by Deltagen Australia. An additional 100 mg of ground tissue was used for determining the total N. The samples were extracted using the Kjeldahl method to determine the total N and [CP] was determined as N × 6.25. Data from [WSC] and [starch] was added to provide an estimate of [NSC].

Growth of bermudagrasses in sodic-saline soil

This experiment using a dispersive, saline-sodic soil was conducted under well-watered and controlled conditions to compare the growth of Australian bermudagrasses (MED1 and Wintergreen) with a traditional seed mix used in revegetation projects. Two soil profiles used in the experiment were supplied by Origin Energy from their Condabri Central, SE Queensland, installation; Two samples of soil were provided: (i) moderately saline and non-sodic topsoil (A horizon: ECe = 7.5 dS m⁻¹, ESP = 5.9%) and (ii) a sodic and highly saline subsoil (B horizon: ECe = 8.4 dS m⁻¹, ESP = 10.5%). These soil types were selected because of the hostile nature and can be considered typical of the types of soil to be vegetated in hydrosprigging projects. All grasses were planted in 10 cm x 40 cm PVC columns. The grasses were established vegetatively from either stolons (10% sod), 50% or 100% sod or seed mix, giving seven planting options in all. Three profiles were studied, A horizon soil, B horizon soil and the third A (10 cm) and B horizon (30 cm). Consequently, the seven planting options by 3 soil profiles gave a total of 21 treatments.

Pots were placed inside a controlled environment glasshouse set at 20 - 25°C. Finely ground gypsum (< 0.5 mm maximum particle diameter) was incorporated into the surface 3 to 5 cm of soil at a rate corresponding to 5 tonnes per hectare before planting or sowing. All pots were fertilized with Flowfeed EX7 (20 - 3.5 - 16.6 + TE) at the rate of 50 kg N ha⁻¹ month⁻¹ (Snyder *et al.* 2008) and maintained at close to field capacity. Total aboveground biomass (clipping and verdure), roots and rhizome biomass were harvested twice for each treatment: 0-5 weeks (Run 1) and 0-11 weeks (Run 2). For each run, three replicates were harvested. The grasses were cut into two sections: aboveground and belowground (roots and rhizomes). Rhizomes were separated from the belowground section. Once separated, aboveground, root and rhizome sections were washed free of soils, oven dried at 65°C for 72 h and

weighed them. Data from aboveground biomass, roots and rhizome were combined to provide an estimate of total biomass.

Screening for salinity tolerance in Australian bermudagrasses

This part of the project included several experiments that adopted a flood and drain sand culture system to screen for salt tolerance among a set of Australian bermudagrasses. In all, including check varieties and species 80 grasses were tested in glasshouse experiments conducted at Redlands Research Station and UQ St Lucia.

Genotypes were planted as vegetative sod in plastic pots (10.5 cm square \times 12.5 cm deep, 1.38 L capacity) filled with a coarse sand. The potted vegetative material was grown for 2 months using a 92 L basal nutrient solution containing 5.1 g of Flowfeed EX7. During this 2-month period turf was clipped weekly to 25 mm. The pots were arranged in a split-plot design, with different salinity treatments allocated to main plots and genotypes allocated to sub-plots.

After establishment salinity treatments up to 40 dS/m were applied to the root system of the plants dominated by sodium chloride (NaCl), with additional calcium to each treatment to avoid sodium-induced calcium deficiency. The calcium activity ratio (CAR) of solution was maintained at \geq 0.035. Salinity treatments were increased by 1.5 dS m⁻¹ daily until the desired level was obtained. Each sand culture system consisted of a reservoir containing 92 L of solution and a submersible electric pump connected to a growing tray (1 × 1 m square, 0.18 m deep). Pots were supported by parallel steel rails across the top of the tray. Solution from the reservoir was daily pumped into the tray around the pots (but not completely submerging them) for a period of 10 minutes, every 4 hours. Treatments were ceased after at least 11 weeks.

Data were collected on green cover (GC), clipping yield (CY), verdure biomass (VB - the amount of shoot and stolon biomass aboveground remaining after clipping) and root biomass (RB, including rhizomes). GC refers to the percentage of leaves that remain green despite the salinity stress and was measured after salt treatments were imposed using digital image analysis. Immediately after the final clipping, grasses were removed from the pots, washed free of sand and then separated at the crown into two parts: VB and RB. Once separated, VB and RB were washed, dried and weighed as described above. Data from CCY and VB was combined with RB to provide an estimate of total biomass (TB).

Plant Growth Regulator trials

These experiments were designed to test the effect of plant growth regulators (in this case plant hormones) on the sprouting potential of bermudagrasses. Four bermudagrass genotypes (MED3, MED2, Wintergreen and 659) were chosen to represent a range of different levels of sprouting in stolons based on previous experiments. These bermudagrasses grown in a field trial under optimal water and fertilizer conditions at the Allenview (27.54°S,152.56°E), Southeast (SE) Queensland, Production facility of The

Jimboomba Turf Group were used to test various commercial and non-commercial hormone formulae. Based on a pilot study where the application rates were based on the recommendation by the manufacturer, the hormone/hormone combinations Auxinone (Barmac Pty Ltd, Blackstone, Qld, Australia), ESI-Root (Kippax Packaging, Harbord, Australia) and 2,4-D amine 625 (referred to as 2,4-D) (Kendon Chemical & Mnfg. Co. Pty Ltd, Fairfield VIC, Australia) were applied at various concentrations from 0.15 to 625 mg L⁻¹, while the concentrations of ethylene were applied from 0.79 to 787.50 mg g⁻¹ (Run 1: 25/03/2015). Ethylene used in this study was encapsulated in the form of inclusion complexes with a-cyclodextrin to form ethylene complex powder which can dissolve in water to release ethylene gas and ethylene concentration in this product was about 1.5% w/w.

To determine the concentrations of the hormones that maximize sprouting percentage, a second study (Run 2: 26/05/2015) was conducted using the same bermudagrass genotypes as above at concentrations from 0.015 to 4.8 mg L⁻¹; i.e. Auxinone (0, 0.24, 1.2, 2.4, 4.8 mg L⁻¹), ESI-Root (0, 0.015, 0.075, 0.15, 0.30 mg L⁻¹) and 2,4-D (0, 0.0625, 0.3125, 0.625, 1.25 mg L⁻¹); while the concentrations of ethylene were applied at 0, 0.79, 3.94, 7.88 and 15.75 mg L⁻¹.

Stolons without sprouts of each genotype were placed onto two filter papers in 85 mm diameter plastic petri dishes and dipped for 15 seconds before being dunked with 6 ml for each concentration of Auxinone, ESI-Root and 2,4-D. For the ethylene treatment stolons were soaked in 200 ml solutions in 250 ml beakers for 4 hours at 25°C. After soaking, stolons were transferred to an 85 mm diameter plastic petri dish containing filter paper wetted with 6 ml of the respective ethylene solution.

Petri dishes were sealed with parafilm, placed in aluminium foil laminate bags and incubated at $25 \pm 1^{\circ}$ C in the dark until scoring. A replicate was placed in one petri dish and each treatment had three replicates. Each node of each stolon was scored for sprouting every three days. A node had sprouted if there was evidence of a root or shoot or root + shoot.

The effect of gibberellin inhibitor Primo on growth and quality of stolons for sprouting

These trials were designed to test the effect of the commonly used growth inhibitor Primo on the sprouting potential of bermudagrass. Twelve bermudagrass genotypes (*Cynodon* spp.) grown in a field trial under optimal water and fertiliser conditions in SE Queensland were used to test if differences between varieties for sprouting of stolons are affected by applications of the commercial gibberellin inhibitor Primo (Syngenta Crop Protection Pty Ltd, Macquarie Park, NSW, Australia). Primo was tested at four different rates 0, 0.5, 1 and 2 L ha⁻¹ applied with a portable boom spray to each of the 12 grasses grown in the field. One month after treatment, the stolons in each plot were sampled using a steel coring tube (6 cm diameter \times 4 cm deep). Each core was washed free of soil and oven dried to provide estimates of total above ground biomass (TaB). At the time of coring tiller height (canopy height) was determined for each treatment. Tiller height was measured from the base of the stolon to the top of the

shoot at harvest. The stolons harvested from these treatments were tested for sprouting percentage as described previously.

Hydrosprigging trials

We established hydrosprigging field trials at Darra QLD on a sodic-saline soil using a range of organic media including 1. Coconut coir (CC), 2. Enviro-Straw (ENV), 3. Flex Terra (Flex), 4. Hydromulch 2000 (HY) and 5. Sugar Cane mulch (a blend of sugar cane, paper and Lucerne in ratio 3:1:1) (SC). The aim of these replicated trials was to test if the hydrosprigging methodology that evolved during this project could successfully vegetate a sodic-saline soil. Prior to planting the soil surface was scarified to 10 cm, 3 t/ha gypsum was broadcast, lightly cultivated and 30 mm irrigation applied to bring the profile to field capacity. Treatments were applied to 1.5 m x 2 m plots. Sprigs of bermudagrass MED2 were treated with 2.4 mg L⁻¹ Auxinone overnight. Each hydrosprigging blend was applied in two passes. The first pass consisted of the stolons mixed with a slurry of organic matter (2t/ha), complete fertilizer (300 kg/ha Amgrow NPK fertilizer 16-3-11) water and binder. Stolons were applied at the rate of 2t/ha (fresh weight). This first layer was then overlain with a second pass of organic medium mixed with water and binder applied at 4 t/ha. All blends were mixed by hand in 10 L buckets and applied to the plot manually by broadcasting. The total water applied at planting with the blends was equivalent to 6 mm of irrigation. Irrigation was applied at the rate of 4 mm a day in dry periods and not at all during rainfall events. The number of viable shoots and the area of spread of the grasses was monitored over time as a measure of the success of the hydrosprigging methodology.

Outputs

- 1. A state of the art hydrosprigging methodology for the Australian Turfgrass Industry. From almost no published literature on hydrosprigging in Australia we have conducted a number of experiments and have now compiled a methodology that has been successfully applied to field conditions.
- 2. A detailed understanding of the physiological basis of stolon growth in bermudagrass has been developed. This achievement was based on a set of experiments where non-structural carbohydrates tracked across seasons in a range of bermudagrasses giving greater insight into how stolons sprout when harvested from a turf sward.
- 3. A best practice hydrosprigging manual for the turf industry has been written. This manual combines outcomes from our latest research findings and best practice knowledge from our industry collaborators.
- 4. Better management strategies for Turf producers. Our experimentation with plant growth regulators can potentially give turf producers more tools to grow higher quality turfgrasses. The hydrosprigging technology package could be adapted on farm by Turf producers in the situations where there is a need to multiply particular varieties. The knowledge of seasonal changes in non-structural carbohydrates in turf swards may help growers predict seasonal variation in sod strength.
- 5. Microencapsulated ethylene was shown to improve the sprouting potential of bermudagrass. The normally gaseous form of ethylene can now be handled as a powder and stored indefinitely. This product has great potential for use in hydrosprigging.
- 6. Australian Turfgrass Management Journal article on the UQ hydrosprigging project was written and published in Sept 2014.
- 7. A hydrosprigging manuscript was written and published in the proceeding of the International Horticultural Conference Brisbane in 2014.
- A scientific manuscript "Physiological basis of sprouting potential in Australian bermudagrasses (Cynodon spp.)" has been submitted to the Agronomy Journal for publication and presentation at the International Turf Research Conference in the USA 2017.
- 9. Potentially the methodologies and approaches developed and adopted in this project could be applied to other vegetatively propagated horticultural species.

Outcomes

This Hydrosprigging project has the potential to expand the Australian Turfgrass industry returning greater profits to Turf producers and to those companies involved in establishment of large areas of turf, revegetation of disturbed lands and exposed subsoil and the nutrient and sediment capture along riparian zones in environmentally sensitive areas. Due to the global financial crisis many Australian Turf producers had seen the need to diversify their enterprises by investing in revegetation projects and therefore, the new technology package will greatly benefit them. As there is a demand for cost-effective, reliable hydro-sprigging technology, growth in bermudagrass sales are anticipated within the first 2 years after the completion of the project provided there are clear lines of communication to the turf and environmental services industries to make them aware of the opportunity. UQ has published one trade magazine article on the project but will follow up with additional published materials and a field day at the recently established hydrosprigging site at Darra in suburban Brisbane. A best practice manual on hydrosprigging has been written and will be distributed to the turf, environmental services and related industries.

One of the important outcomes of this project is that there is now a technology package available that can be used with confidence to solve some of the most important environmental issues in Australia. There are enormous areas of denuded landscapes at mine sites, road verges, cuttings, CSG well heads, creek and riverbanks and now these have the potential to be vegetated minimising the loss of Australia's precious soil resources. The environmental benefits will extend beyond this with less dust in the atmosphere, less siltation and nutrient loads in waterways and less fire hazards around important infrastructure.

One major opportunity is to minimise siltation and nutrient runoff onto the Great Barrier Reef 'lagoon' thereby reducing the environmental impact of agriculture and urbanisation. The kilometres of waterways protected with narrow riparian zones could be widened by hydrosprigging bermudagrasses to act as a biological filter to separate the sugar cane production from the streams that drain into the Reef.

New laws that govern urban developments in Queensland and elsewhere in Australia require that 5% of land be allocated for storm water drainage and flood mitigation. Consequently, these parcels of land that can potentially be quite large would be highly suited to hydrosprigging with bermudagrasses. In these situations bermudagrasses have additional benefits in that they are drought resistant, water logging tolerant and can be landscaped for recreational use.

Evaluation and Discussion

This project has successfully developed a state of the art hydrosprigging protocol designed primarily for bermudagrasses, however, the basic principles outlined here can be applied to many perennial C4 turfgrass although we strongly recommend that practitioners carry out their own practical field trials to validate our methodology, regardless of turfgrass species used. The field research has been conducted in SE QLD in sub-tropical environments and therefore there are likely to be other considerations for applying this technology package elsewhere in Australia. The manual that was prepared for this project, wherever possible, highlights parts of the protocol that may require alternative practices to be used. The manual was put together from two sources of information, current practitioners and our own research. There was no published scientific literature to be found on hydrosprigging and as such much of the research areas investigated in this study were novel.

Important considerations for hydrosprigging

Physiological basis of sprouting in bermudagrasses

The research conducted here gave us an excellent understanding of the physiological basis of sprouting potential in bermudagrass. We investigated the genotypic and seasonal variation and the physiological basis of sprouting from nodes of all above ground shoots among 12 Australian bermudagrasses grown on a commercial Turf Farm (Allenview, QLD - Jimboomba Turf Group). Sprouting percentage, total nodes per m² (Nds), stolon diameter (SD), total above ground biomass (TaB), and the concentration of water soluble carbohydrate (WSC), starch, non-structural carbohydrate (NSC) and crude protein (CP) were determined in different seasons including winter 2013, winter 2014, spring 2014, summer 2015 and autumn 2015. Sprouting percentage for stolons sampled in spring and summer (about 70%) was higher than that for winter and autumn (about 60%) suggesting that the warmer months of the year would be ideal for hydrosprigging (see Appendix 1). However, the trade off with hydrosprigging in the warmer months is the higher evapotranspiration rates likely to be experienced at planting making supply of water to the sprigs more important. Interestingly, large genotypic variation for sprouting was observed ranging from 44.1 to 80.2% of all nodes when averaged across seasons. Sprouting percentage among genotypes was strongly associated with stolon diameter, TaB and Nds, and weaker associations with concentrations of photoassimilates such as WSC, starch and CP in the above ground biomass. However, sprouting percentage was highly correlated with WSC (r = 0.80), CP (r = 0.82) and WSC + CP (r = 0.88) when calculated as an amount per node basis. These data suggested that the size and/or 13

maturity of the axillary buds at nodes of stolons and the assimilate supply available to these nodes was underlying the sprouting potential of the bermudagrasses. We then measured the size of the axillary buds and found that this trait was positively correlated with stolon diameter. A corollary to this research was that the sprouting process was highly temperature dependent and different experiments could be compared by converting the time scale to thermal time (see Appendix 2) by using 7.7 °C as a base temperature a value obtained from the literature. Our research on sprouting confirmed that this base temperature was accurate. The significance of this base temperature should not be overlooked as this value represents the lowest temperature at which bermudagrass will grow and sets the boundaries for when hydrosprigging may be curtailed during winter months.

Our original intention was to apply the concept of photothermal quotient an environmental indicator of the amount of radiation available to the plant relative to the growing temperature. This quotient has been successfully used to understand the yield potential of cereal crops in Australia. Applying this concept to turfgrasses was met with some success in this project although additional research will be needed. One of the difficulties in applying the concept of photothermal quotient to turfgrass was that the turfgrass is clipped on a regular basis and this biomass remains unaccounted when correlating photothermal quotient and biomass accumulation. In addition, the biomass estimates we determined did not account for investment to underground parts including rhizome and roots which we found varied greatly among genotypes. Nevertheless, we were still able to derive relationships between photothermal quotient is a concept that still has great potential in turfgrass systems and other horticultural crops.

Plant growth regulators including microencapsulated Ethylene to improve sprouting potential

Plant growth regulators (PGR's) were trialed to assess their effect on sprouting percentage of 12 bermudagrasses grown in the field. These treatments were applied to field grown stolons and tested for sprouting under controlled laboratory conditions. No attempt was made to sterilize the surface of the stolons prior or during the sprouting process. Surprisingly, most hormone treatments were effective on a panel of 12 bermudagrasses selected from a wide range of geographical locations across Australia. The concentrations of the hormones to maximize sprouting were obtained with 7.88 mg L⁻¹ ethylene, 2.4 mg L⁻¹ Auxinone, 0.15 mg L⁻¹ ESI-Root and 0.625 mg L⁻¹ 2,4-D. At these concentrations the sprouting percentage for any one grass could be elevated substantially (e.g. from 50% for controls to 70% with PGR treatment) (see Appendix 3). The advantage of these hormone treatments is that they are inexpensive and easy to apply. The ethylene which was delivered in a microencapsulated dextran coating, is packaged in powder form and can be added to the hydrosprigging blend prior to planting. Ethylene is a gas and packaging this hormone in a powder overcomes all of the safety issues of transporting gas cylinders. 2,4-D is a synthetic auxin and used as a herbicide in the turfgrass industry but because the concentration needed to stimulate sprouting is low, it has the potential to be used as a

cheap plant growth regulator. Auxinone and ESI-root are both registered for use in turfgrass so there appears to be a range of options available to turf producers to improve the quality of stolons for hydrosprigging. For our field test (described below) we applied Auxinone to our sprigs and were highly successfully in achieving excellent plant stands with a high proportion of stolons sprouting. We also tested BAP (a cytokinin) but were unable to find a concentration that increased sprouting percentage. To the contrary, our treatments inhibited sprouting to levels below the control. Seasol enhanced sprouting in preliminary experiments and is worthy of further consideration.

We also evaluated the effect of a gibberellin inhibitor Primo (Trinexapac-Ethyl) on growth and quality of stolons for sprouting in bermudagrass. We hypothesised that application of Primo would retard growth but enable the plant to accumulate greater WSC that might enhance sprouting. There were clear differences between Primo treatments among 12 genotypes in terms of sprouting, tiller height and total above ground biomass. Stolons harvested from turf treated with 0.5 L ha⁻¹ Primo gave slightly higher sprouting percentages compared to the control treatment, while treatment of 1 and 2 L ha⁻¹ Primo gave sprouting levels lower than the control treatment. Clearly, more work is needed to identify application rates that might give greater increases in sprouting percentage. We suspect that Primo applications in the range of 0.3 to 0.7 L ha⁻¹ may be worth pursuing.

The list of registered products and permits for turf production are available on the Australian Pesticide and Veterinary Medicine Authority (AVPMA) website (http://www.apvma.gov.au).

Land preparation is key

Appropriate site characterisation and land preparation is key to any agricultural pursuit and equally so for hydrosprigging projects where the profile to be sprigged may be sodic-saline, compacted and/or littered with rocks and dead vegetation. Depending on the slope and stability of soil, we would recommend at least a 5-15 cm scarification of the soil surface to allow for a pre-planting irrigation and application of lime and/or gypsum if necessary. The amount of irrigation needed will depend on the moisture status of the profile, but waiting for a rain event or 20-50 mm of pre plant irrigation will ensure the profile will be able to sustain growth of the stolons over a prolonged period. Scarification of slopes is more problematic and shallower depths would be required, but any scoring of the surface along the contours that will allow the retention of moisture will greatly assist the sprouting, establishment and growth of the grass. Polymer enhanced hydromulches can be used on sloping grounds and steep slopes can be reinforced with artificial mesh-like materials (e.g. like those supplied by Geobrugg) to stabilise the slope but also allow vegetation to penetrate. Fertilizer recommendations are based on soil tests and both macro and micro nutrient applications may be necessary particularly if planting into subsoils where there is likely to be little or no organic matter and nutrient stores. Follow-up fertiliser applications may be necessary to optimise the supply of nutrients to match growth demands thereby reducing the risk of nutrients making their way into water ways.

Which organic amendment should be used?

We established a hydrosprigging field trial at Darra QLD (see Appendix 4) on a sodic/saline soil using a range of organic media including 1. Coconut coir (CC), 2. Enviro-Straw (ENV) a blend of wheat and lucerne straw, 3. Flex Terra (Flex) a combination of pine wood and polypropylene fibres, 4. Hydromulch 2000 (HY) pine wood fibres and 5. Sugar Cane mulch (SC) a (3:1:1) blend of sugar cane, lucerne and paper. We attempted to obtain a sample of Ecoblanket but it was not available at the time of planting, however, this product is used in the market place and is worthy of further consideration.

At the time of writing this report we were able to successfully establish a stand of bermudagrass using each of the organic media without any no media showing a clear benefit over the other highlighting our finding that post-hydrosprigging irrigation is the key to successful establishment. We strongly recommend a two-pass method of hydrosprigging where stolons are applied to the soil surface first and then overlain with a second pass of media. In our experiments we applied 2 t/ha of media with the stolons overlain with 4 t/ha of covering media. This strategy is designed to reduce the likelihood of the bottom layer containing the stolons drying out. Effective follow up irrigations is also key to successful hydrosprigging. Our discussions with industry suggested that inadequate irrigation post planting was a major reason for poor establishment. In our trials only 6 mm/day of irrigation to match evaporation was necessary because the profile had been wet to field capacity prior to planting. Soil water release curves showed that the HY and Flex products held most water about 3.5 g H_2O/g media and the ENV and SC the least 0.7 g H_2O/g media with CC intermediate 2.5 H_2O/g media (see Appendix 5). These data suggest that products with a higher water holding capacity would be beneficial under conditions of high evaporative demand to maintain more water around the stolons in between irrigation events. Consequently, more frequent small irrigations per day would be optimal. In our experiments during the early establishment phase we applied 1.5 mm of irrigation 4 times per day. We strongly recommend wherever possible that temporary irrigation systems be installed particular during the first four weeks of establishment. Water-holding crystals did not appear to benefit establishment and soil water release curves with and without Stockosol water crystals showed that there was almost no increase in water holding capacity of the media (Appendix 5).

The stability of the organic media will be an important determinant of the nitrogen drawdown associated with microbial degradation of the organic matter. For example, experience with coconut coir suggests that this organic medium will degrade slowly, therefore, the nitrogen drawdown is not expected to be as severe as may be the case of other media that may degrade quickly. To date, we have no data on how the other media may degrade with time. Cost of the media will be an important driver of the profitability of hydrosprigging projects. At this point in time the cost of the media from cheapest to most expensive is Sugar Cane, Envirostraw, Coconut Coir, Hydromulch 2000 and Flex Terra. While we weren't able to

show any clear differences between the media in the establishment of a bermudagrass on a sodic-saline soil other factors should be considered e.g. the slope of the land and how well the media adheres to the soil surface, nitrogen draw down, and the ability of the media to supply nitrogen. For example, Envirostraw is an example of one medium that contains Lucerne straw which potentially can release N to the system. Blends of the above media were not tested but should be considered. For steep slopes stronger binders than the ones we tested can provide greater stability of the different media.

Why Bermudagrass for hydrosprigging?

From the outset it was decided that this research would be conducted on bermudagrass. Several important characteristics of bermudagrasses in Australia are worthy of discussion including; adaptation, supply of product (stolons), drought resistance. While these discussion points make it clear the advantages of this species the research undertaken in this project on sodic/saline soil profiles provided further evidence of the value of bermudagrass for hydrosprigging. This project has also identified a number of Australian bermudagrasses that have great potential for hydrosprigging.

Adaptation

Bermudagrasses have the broadest range of adaptation of all C4 perennial turfgrasses grown in Australia and consequently have naturalized across a wide geographical distribution across the Australian continent with herbaria specimens collected from over 30 degrees of latitude and 38 degrees of longitude. They can be found inhabiting a range of soil types (from sandy soils to heavy clays) and climatic zones that are characterised by large differences in temperature, humidity, rainfall and day-length. Therefore, they are likely to have a greater chance of succeeding in the many and varied environments that hydrosprigging options are being considered. While thought to be introduced at the time of European settlement (Groves 1991), recent molecular evidence suggests that some species of *Cynodon* may be native to Australia (Jewell et al. 2012). A recent collection of over 1000 bermudgrasses made by UQ has increased the germplasm base from which hydrosprigging bermudagrasses can be developed.

Supply of product

Bermudagrass in terms of square meters of sod sold is the most traded turfgrass in Australia. Bermudagrass production occurs in every state and territory and the supporting industries are well established. Because hydrosprigging invariably will involve vegetating large tracts of land e.g. hectares at a time rather than smaller areas associated with residential blocks a ready supply of stolons will be necessary. In warmer months of the year turf producers can expect to harvest stolons multiple times through the year from a dedicated section of their turf farm. Bermudagrasses regenerate quicker than non-rhizomatous species such as buffalo, Queensland Blue Couch and quicker than rhizomatous species such as Zoysias. In our experiments we harvested approximately 5t/ha of fresh stolons and applied these at a rate of 2t/ha, therefore a ratio of 2.5 ha of hydrosprigged land per ha of harvested stolons could be achieved. However, because we obtained an excellent strike of stolons we conservatively estimate that this ratio could be increased to 10 ha of hydrosprigged land per ha of harvested turf giving the Turf producer a cheaper an effective option. Further efficiencies are gained in the summer months as the yield of stolons is far greater because the average above ground biomass ranges from about 6 t/ha in the winter to 12 t/ha in the summer. The rates of stolons applied could be priced according to how quickly the client wanted to vegetate the land.

Drought resistance

Bermudagrasses are renowned as being among the most drought resistant turfgrasses available. Recently we published studies on drought resistance in Australian bermudagrasses (Zhou et al. 2012, Zhou et al. 2013 a, b, c). In one study (Zhou et al. 2012), we investigated the water use and water use efficiency among Australian turfgrasses grown in shallow soil profiles and found that the bermudagrass were more drought resistant than Queensland blue couches (*Digitaria didactyla*), seashore paspalums (*Paspalum vaginatum*) and the buffalo grasses (*Stenotaphrum secundatum*). The bermudagrasses were more drought resistant because they had lower stomatal conductance and less water loss during the early stages of water deficit.

Tolerance to sodic/saline soils.

The exposure of subsoil layers (B horizon) of the profile that are typically sodic and/or saline are a common occurrence during major construction works like those associated with roads/highways, mining, dam wall construction etc. Consequently, these hostile soils offer both chemical and physical constraints that present major limitations to vegetation projects that aim to stabilise the landscape after construction has been completed. For these soil types to be vegetated species need to be identified with high levels of salt and sodicity tolerance. In a series of studies conducted in this project using a flood and drain, sand-solution hydroponic system or direct exposure to sodic/saline soils we identified a number of bermudagrasses with excellent tolerance to salinity and sodicity. Leaf tissue analysis for important ions Na, Cl, K, showed that the salt tolerant grasses were able to exclude Na and maintain a higher K/Na ratio in their tissues. These experiments suggested that application of Ca in the form of gypsum (to exchange with Na on the Cation Exchange Capacity) would be an important soil amelioration measure but also K⁺ nutrition should also be considered when formulating fertilizer requirements for hydrosprigging projects involving hostile soils.

In many hydromulching (or hydroseeding) applications used for vegetating hostile soils there are limited choices of grass species available. In our research we tested a common commercial seed mix consisting of warm-season grasses, Bermudagrass (*Cynodon* spp.) and Japanese millet (*Echinochloa esculenta*)

and the cool-season grass, Annual ryegrass (Lolium multiflorum). We separated the seeds into the component species and germinated them in saline conditions. In all cases the germination of the seeds was severely reduced and reflected the experience of germinating these seeds under saline conditions in the field. Furthermore, the Japanese millet in particular cannot tolerate sodic/saline conditions even if germination is successful and a plant stand is established (see Appendix 6). It should be noted that the seeded bermudagrasses in commercial seed mixes are imported from the USA and are poorly adapted to Australian conditions. Invariably the seeded bermudagrasses have poor root systems and are generally not rhizomatous although they can offer rapid ground cover under pristine, non-saline conditions. The lack of rhizomes of seeded types give them limited potential to stabilize slopes and batters that are characteristic on road verges and mine-sites. By contrast, the Australian bermudagrasses used in the present research e.g. MED2 (see Appendix 7) are highly rhizomatous with a high potential of stabilizing slopes and batters made of subsoil layers of the soil profile. Our research with a sodic/saline soil provided by our industry partner from their Condabri Central installation in QLD showed that there was substantial rhizomatous growth from the bermudagrasses compared to almost no rhizome growth from the seed mix. Rhodes grass (*Chloris guyana*) is a salt tolerant C4 grass that has been used extensively in revegetation projects. However, Rhodes is a bunch grass that even when sown at high densities can allow for gully erosion on slopes and dam walls during heavy rainfall events. In addition, Rhodes under favorable conditions can grow well over 1.5 m tall, reducing visibility and in dry conditions providing a large fuel load and substantial fire hazard. Bermudagrasses are low growing, stoloniferous and rhizomatous and therefore form a solid sward to stabilize the soil surface and with a reduced fire hazard.

Invasiveness of bermudagrasses

Generally bermudagrasses are not aggressive invaders of wild habitats, rather they are common place on disturbed denuded landscapes. They are not shade tolerant and are frequently outcompeted by more aggressive grasses and other vegetation. In addition, many of the Australian bermudagrasses identified in this project with attributes amenable for hydrosprigging are also seedless.

The case for Bermudagrass

The preceding discussion makes a clear case for bermudagrass as the species of choice when hydrosprigging is being considered as a revegetation option. In some environments a case could be made for kikuyu (*Pennisetum clandestinum*) or Zoysia spp., however, neither of these species have the drought resistance of bermudagrass nor the salt tolerance in the case of kikuyu. One issue not discussed in great detail here is the value of bermudagrass as sod versus the value of other turfgrasses. Generally, bermudagrasses have the least value as sod and therefore make them more attractive for hydrosprigging purposes. Turf producers will no doubt be able to determine the price point at which it becomes viable to supply sprigs for hydrosprigging versus cutting it for sod.

Further research

The hydropsprigging trials established in this project will need to be monitored over a longer time period to fully assess the advantages and disadvantages of each organic mix used. Also over time the nutrient status of the grass system will be exhausted. The next phase of research should therefore look at situations where adding a legume to the system, that may provide a more sustainable nutrient base for the persistence of the grass.

Recommendations

- The results of this research and the hydrosprigging manual need to be disseminated widely to Turf producers, the broader Turf industry and other allied environmental services via media, field days and conferences.
- Further research is required to understand the physical attributes of organic media used in hydrosprigging particularly the relative merits of water holding capacity, rate of breakdown over time, ability to withstand severe weather events and nutrient drawdown particularly N.
- For long term sustainability of grass swards on disturbed soils, exposed subsoil such as mine spoil and road cuttings the introduction of a suitable N-fixing legume to the hydrosprigging protocol should be explored.
- While we were able to establish a benefit of pre-treating stolons with plant growth regulators (PGRs) additional work is required. We have preliminary results on the benefits of PGRs used individually but we have yet to establish their value in different combinations.
- The feasibility of microencapsulating PGRs inexpensively should be investigated.
- In partnership with industry operators, demonstration sites should be established in a range of variable environments across Australia differing in soil type, slope, and climatic conditions and end use. A range of locations would provide exposure and technology transfer to Turf producers and the broader Turf industry and environmental services.
- Strong correlations between sprouting percentage on a per node basis and water soluble carbohydrates implicate photothermal quotient as a concept that has great potential in turfgrass systems and therefore worthy of further investigation. In addition, we believe photothermal quotient is an underutilized concept in horticulture and research to investigate correlations with yield in a range of species is warranted.

Scientific Refereed Publications

Journal article

Tran TV, S. Fukai S, van Herwaarden AF, and Lambrides CJ (2016) Sprouting Variation Among Australian Bermudagrasses and Implications for Hydrosprigging Acta Horticulturae accepted for publication

Tran TV, Fukai S, Zhou Y, and Lambrides CJ (2016) Screening Australian Turf and Pasture Bermudagrasses (*Cynodon dactylon*) for Salt Tolerance: Association between Salt Tolerance and Drought Resistance Acta Horticulturae accepted for publication

Tran TV, S. Fukai S, van Herwaarden AF, and Lambrides CJ (2016) Physiological basis of sprouting potential in Australian bermudagrasses (Cynodon spp.)" has been submitted to the Agronomy Journal for publication and presentation at the International Turf Research Conference in the USA 2017.

Intellectual Property/Commercialisation

Microencapsulated ethylene has been patented by UQ but not for use in the Turf industry. Consequently, there may be IP discussions required should this product find a niche in the Turf industry.

References

Groves, R.H., 1991. A short history of biological invasions of Australia. In: Groves, R.H., Di Castri, F. (Eds.), Biogeography of Mediterranean Invasions. Press Syndicate of the University of Cambridge, Cambridge, pp. 59-63.

Jewell M, Frere CH, Harris-Shultz K, Anderson WF, Godwin ID, Lambrides CJ (2012). Phylogenetic analysis reveals multiple introductions of Cynodon species in Australia. Molecular Phylogenetics and Evolution 65:390-396

Zhou Y, Lambrides CJ, Kearns R, Ye C, Fukai S (2012) Water use, water use efficiency and drought resistance among warm-season turfgrasses in shallow soil profiles. Functional Plant Biology 39(2)116-125

Zhou Y, Lambrides CJ, Fukai S (2013a). Drought resistance of bermudagrass (Cynodon spp.) ecotypes collected from different climatic zones. Environmental and Experimental Botany 85: 22-29

Zhou Y, Lambrides CJ, Fukai S (2013b) Drought resistance of C4 grasses under field conditions: Genetic variation among a large number of bermudagrass (Cynodon spp.) ecotypes collected from different climatic zones. Journal of Agronomy and Crop Science 199:253-263

Zhou Y, Lambrides CJ, Fukai S (2013c) Temporal and spatial patterns of soil water extraction and drought resistance among genotypes of a perennial C4 grass. Functional Plant Biology 40:379-392

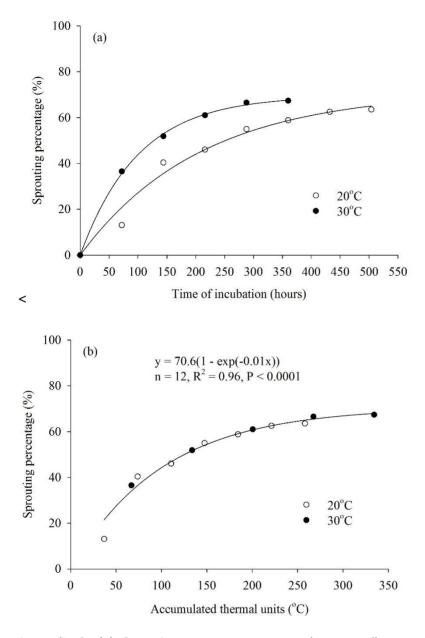
Acknowledgements

- Adam Christison APLNG Origin Energy
- Andrew McLaughlan, Paul Weldon, Darryl Pearce, Lyn Davidson from Jimboomba Turf Group
- Peter Carmichael, Hercules Strydom from EnviroStraw
- Technical assistance of Dr Harshi Gamage, Nahuel Pachas, Lisa Xian
- Soil Scientists at UQ; Dr Gunnar Kirchhof and Robert Banks
- Administrative support staff at UQ for helping with smooth operational admin support
- Professor Bhesh Bhandari UQ for supplying microencapsulated ethylene

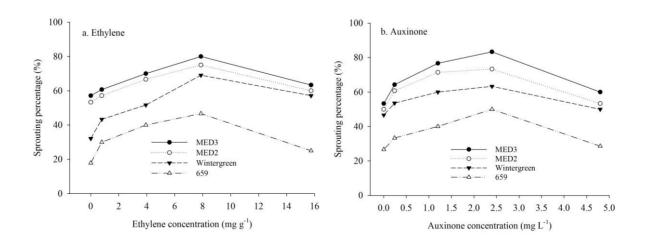
Appendices

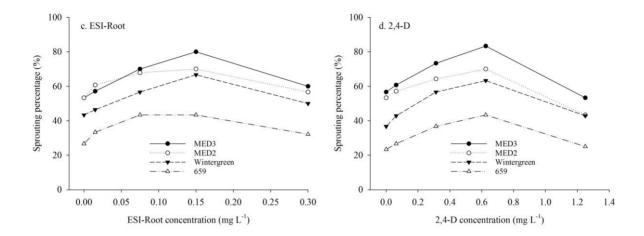
Appendix 1. Sprouting percentage of 12 bermudagrass genotypes harvested in different seasons (winter 2013, winter 2014, spring 2014, summer 2015 and autumn 2015) at Allenview, SE Queensland, Australia. Sprouting assays were conducted in the laboratory at $20/30^{\circ}$ C. Least significant difference (LSD) at P = 0.05 is for season (S), genotype (G) and their interaction (S × G).

Genotype	Group -	Season					Mean	
		Winter 2013	Winter 2014	Spring 2014	Summer 2015	Autumn 2015	genotype	
MED3	1	72.2	78.4	83.6	87.9	79.1	80.2	
MED2	1	65.9	76.9	83.1	86.3	73.8	77.2	
MED1	1	67.2	78.2	82.1	84.6	72.9	77.0	
Wintergreen	1	69.2	69.5	77.8	79.9	74.6	74.2	
MED4	2	60.7	64.7	72.7	73.8	68.0	68.0	
55	2	66.3	69.4	69.4	69.4	59.6	66.8	
40	2	61.7	66.8	68.2	70.0	65.0	66.3	
25a1	2	64.1	65.7	69.6	69.2	62.0	66.1	
394	2	57.9	60.3	64.9	76.1	68.3	65.5	
1033	2	42.9	49.1	59.8	61.4	56.0	53.8	
698	3	47.1	45.0	50.2	46.8	42.2	46.3	
659	3	43.0	39.4	52.4	46.7	39.2	44.1	
Mean season		59.8	63.6	69.5	71.0	63.4		
LSD _{0.05}	$S = 1.7, G = 2.7, S \times G = 6.1$							



Appendix 2. (a) Sprouting percentage averaged across all genotypes at two different temperatures *versus* time of incubation. (b) Sprouting percentage averaged across all genotypes *versus* accumulated thermal units.

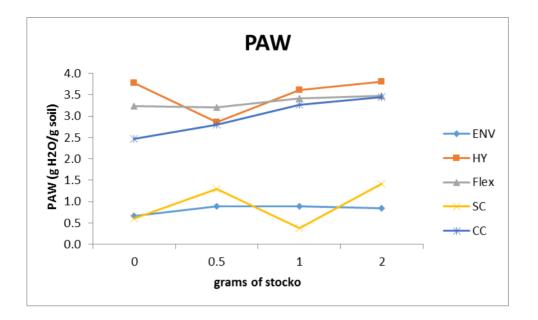




Appendix 3. Sprouting percentage after 6 days of four bermudagrass genotypes treated with four different hormone treatments at a range of concentrations (Run 2: 26/05/2015). Bermudagrass stolons were harvested from autumn grown field plants.



Appendix 4. Top - Hydrosprigging trial established on a sodic-saline batter on 20th April 2016 at Darra, Brisbane. Bottom - MED2 bermudagrass 37 days after establishment by hydrosprigging with Envirostraw



Appendix 5. Plant available water of five organic mixes typically used in hydrosprigging with different levels of Stocko (1:1 mix of Stockopam and Stockosol) water crystals. ENV – EnviroStraw, HY – Hydromulch 2000, Flex – Flex Terra, SC – Sugar Cane mulch, CC – Coconut Coir



Appendix 6. Japanese millet grown on a saline-sodic soil from Condabri Central, QLD. Plants show severe symptoms of salt stress.



Appendix 7. Left - Rhizomatous bermudagrass MED 2 (left) used in the hydrosprigging trial at Darra, QLD versus Wintergreen (right). Right – Close up of rhizomes and roots of MED2.