



final report

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Biological control of Giant Rat's Tail grass utilising *Nigrospora oryzae*

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Executive Summary

Giant Rat's Tail grass (GRT) (*Sporobolus pyramidalis* and *S. natalensis*) has established itself as a significant problem to the profitability and sustainability of grazing environments across Eastern Australia. A combination of underutilisation by grazing animals due to unpalatability, resilience and persistence especially in poor soils, and an aggressively high seeding rate have seen GRT spread across many coastal and sub-coastal regions of Queensland.

In some areas, GRT infestations have dramatically decreased livestock producers' economic viability and lowered their land values. Current infestations are collectively costing the pastoral industry in the vicinity of \$60 million per annum through lost production and control (Bray and Officer, 2007). In many cases, the stocking rates have been halved on pastures heavily infested with GRT. In addition, the age of turn off of fat cattle on these pastures has been extended by up to 12 months (Bray 2008). The potential area of infestation in Queensland covers 108 million hectares or 60% of Queensland (Anon, 1999).

This project investigated the ability of the fungus *Nigrospora oryza* to cause die back in GRT and subsequent potential as a biocontrol agent of GRT. This fungus has been proposed as a pathogen of Giant Parramatta Grass (GPG) (*S. fertilis*), a closely related species to GRT.

Techniques were developed to produce large amounts of fungal inoculum for GRT plants in both field and pot trials. Dr Ken Goulter (University of Queensland, Gatton) used plate production methods to produce *N. oryzae* spores for the field trial inoculations. Dr Diana Leemon developed methods for the mass production of spores of *N. oryzae* and two *Fusarium* species using liquid and solid state media. This inoculum was used in the pot trials.

Field trials were carried out across three properties with high GRT infestations in the Mackay Whitsunday region to investigate the establishment of *N. oryzae* die back disease. The trial sites on two properties were prepared by fencing and slashing boundaries. These trial sites incorporated a replicated plot trial design to investigate the introduction of *N. oryzae* to GRT under different physiological states of stress. Therefore, pre-treatments of burning and slashing were carried out prior to inoculation. A range of methods of introducing *N. oryzae* to GRT plants was carried out at the third site. *N. oryzae* was introduced to the trial plots through transplanting diseased plants; splitting stools; applying agar based inoculum and overdosing.

A pot trial was conducted in glasshouses at the Ecosciences Precinct, Dutton Park to evaluate the pathogenicity of *N. oryzae*, *Fusarium chlamydosporum* and *Fusarium proliferatum* towards mature, field-sourced GRT plants. The trial was conducted under conditions conducive to the establishment of fungal infections in plants to provide data to support the interpretation of the Mirani field trial results and to further the work carried out at RMIT University by Professor Anne Lawrie under MLA project B.NBP.0716. The results of the RMIT research suggested that the *Fusarium* species may also be causing die back in potted and seedling GRT plants. However, the results reported in this project were inconclusive due to insufficient replication and further work in this area was recommended.

As recommended in B.NBP.0716, potted GRT plants were inoculated with *N.oryzae*; *F. proliferatum* and *F. chlamydosporum* applied on their own and in combination.

No evidence for a pathogenic relationship between GRT and *N. oryzae*, *F. proliferatum* or *F. chlamydosporum* was found despite thorough and stringent field and glasshouse investigations in this project. Assessments of the three field trials over a two year period failed to show any symptoms of disease in GRT that could be linked to *N. oryzae* inoculation. Isolations from plants sampled from the Mirani site failed to yield *N. oryzae*. Dry matter yields at four and eight months post inoculation in the pot trial failed to show any significant variation indicating that the inoculated plants were suffering from a disease. However, after eight months most treatment groups inoculated with *N.oryzae* on its own or in combination with the *Fusarium* species appeared to show a significantly higher dry matter yield suggesting this fungus has a positive effect on GRT growth.

A workshop was organised to facilitate a discussion between research and extension officers from different agencies on the current state of knowledge of GRT management. A range of issues were identified and factors contributing to the holistic management of GRT were highlighted. Key recommendations for future action including areas in which more research is needed were made and documented. An important outcome was the re-focussing of GRT management and control as a priority for a wide range of stakeholders and the identification of the need to update written extension material available to landholders.

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1 Background

The history of Giant Rat's Tail (GRT), (*Sporobolus pyramidalis*) grass in the Mackay Whitsunday region dates back to the mid-1960s when it was introduced into the region in contaminated pasture seed. Since then, it has become widespread and abundant in the grazing country within the region. A preference for higher rainfall, resilience to tree cover and high seeding rate has assisted in its rapid spread. Subsequently, it was given a priority 1 status by the Mackay Regional Pest Management Group following a study done within the Region by Mackay Whitsunday natural resource management (NRM) group (Folkers and Field, 2011).

The proximity of GRT infestation to the outlying Brigalow regions west of the Great Dividing Range, which resides within the climatic parameters which are optimal to its spread, are a concern. These areas represent excellent beef breeding and fattening country and significantly contribute to feedlots and meatworks on the Eastern coast and could be potentially devastated.

Many techniques have been developed for the control of GRT grass, including sowing with competitive pasture species, chemical management with selective chemicals (fluproponate), and fire. However, many of these options are high input and results can be variable depending on rainfall, soil type and follow-up management.

Recent research has been conducted on the use of a biological control for weedy *Sporobolus* in the Three Valleys region of northern NSW. The majority of the trials have been targeting Giant Parramatta grass (GPG) with a fungus *Nigrospora oryzae*. The *Nigrospora* fungus is generally a saprophyte (uses dead plant material for nutrients), or is occasionally a secondary cause of disease in other pasture grasses. In the case of GPG, it is believed that the *Nigrospora* manifests itself as crown rot. The disease has been reported by David Officer of New South Wales Department of Primary Industries (NSW DPI) to kill GPG and reduce GPG infestations to non-economic levels over a couple of years.

Sampling for *N. oryzae* by David Officer was carried out in the Mackay region in 2011, Subsequently, *N.oryzae* was found to already be present among the endemic fungi in GRT pastures in the Mirani area.

The purpose of this research has been to gauge the effects of the *Nigrospora* on Giant Rat's Tail grass host plants of within the Mackay Whitsunday region and to make an assessment of its role as a potential form of biological control.

2 Project Objectives

- Assessment of the potential of the fungus *Nigrospora oryzae* as a biological control agent for GRT.
- Investigate the relationship between pre-inoculation treatments (burning and slashing) and the rate of *N. oryzae* disease establishment and spread.
- Improved knowledge and understanding of grazing land management principles for control of GRT and promotion of productive pastures.
- Draft guidelines for use of *N. oryzae*, if it proves an effective and practical control option.

3 Methodology

3.1 Effect of *Nigrospora oryzae* on Giant Rat's Tail plants in the field

The aim of the field trials was to assess the potential of *N. oryzae* to establish, survive and spread through a large infestation of GRT as a significant plant pathogen.

3.1.1 Materials and methods

3.1.1.1 Selection of trial sites

Two trial sites were initially identified within the Mackay region for testing the biological control.

Site one, known as Gargett, was located on "Palmyra Downs", 5km east of the Gargett township. Site two, known as "Mirani", was located on "Wandobah" 5km south of the township of Mirani. These sites were chosen for the following reasons:

- Both sites were consistent for slope and soil type and were easily accessible.
- The owners of both sites were committed to collaborating with the project.

A third site, located on "Olympus", 20km south of the township of Clairview, was chosen for further investigation into application strategies for the biological control.

3.1.1.2 Trial site layout and preparation - Mirani and Gargett Sites

The Mirani and Gargett sites were used to investigate the effects of management practices (burning and slashing) on the rate of spread and efficacy of the control agent. The trial was developed to study the efficiency of *N. oryzae* in GRT grass across a number of pre-inoculation treatments. It has been hypothesized from work done on GPG in Northern New South Wales that the rate of spread and efficiency of control by *N. Oryzae* can be further enhanced by carrying out two main pre-treatment techniques. They are:

- Burning - *N. oryzae* is classed as a saprophytic fungus. This means that it is mainly found living in dead material. Burning GRT reduces the levels of dead material in and around the base of the tussock and may influence the disease to focus more on the plant itself as a host. In addition, the point of infection for the disease is normally at the base of the stem, by burning rank tussocks a fresher, less resilient point of infection is available for the disease.
- Slashing – Previous work carried out on fungal die backs in grass pastures has shown that it is most active during the wetter, warmer periods of the year. Optimal temperatures for growth of *N. oryzae* are around 22-28°C. Other requirements include adequate moisture within the base of the tussock and for the grass tussocks to be actively growing. This is why it is hypothesized that rather than allowing the grass tussock to grow to

capacity and become rank, continued slashing and residual growth of the plant throughout the optimal period of the year may help to increase infection. These blocks were treated with slashing once in December 2012, once prior to inoculation in January 2013 and again in January 2014 in order to maximise fresh growth in the growing season.

Four treatments were incorporated into a random block design with four replications of each. These treatments were:

1. Burn + *N.o* (B)
2. Slashed + *N.o* (S)
3. No pre-treatment + *N.o* (NT)
4. Control (C)

The Mirani and Gargett trial sites were pegged in September 2012. Due to a consistent, heavy population of GRT, the Gargett blocks (Fig. 1) were arranged together, whereas at Mirani the blocks were distributed across the paddock based on a consistent population of GRT (see Appendix 9.1 A and 9.2 B). Each of the 16 treatment plots consisted of a 20 metre by 20 metre square. The perimeters of each block were slashed and offset for easier identification and in the case of the burn treatments for fire control. The perimeters of the entire trial area were graded to bare dirt or offset as further insurance against the unpredictable and intense nature of GRT fires.

In October 2012, both the Mirani and the Gargett sites had their burn and slash treatments applied (Fig. 2). A follow-up slash treatment was carried out in February 2013 prior to inoculation. The Mirani trial was fenced to exclude cattle so they could graze the remainder of the paddock.



Fig. 1: Gargett trial site post slash and burn treatments.



Fig. 2: The intensity of GRT fires removes all organic cover. Forty-eight hours post fire and shoots are already appearing on GRT tussocks.

3.1.1.3 Trial Site inoculation – Mirani and Gargett

An isolate supplied as *Nigrospora oryzae*, was provided by Mr David Officer, Grafton Primary Industries Institute, NSW DPI. Sub-cultures of this isolate were stored in darkness under water at 25°C. Recovery after several months' storage was excellent.

In a preliminary experiment, a number of media were tested as to which would allow maximal spore production. These included potato dextrose agar, V8 juice agar, malt agar, oatmeal agar and two media based on banana concoctions. Inoculated plates were incubated at 25°C under a 12 hour photoperiod that included light from blacklight tubes. Plates were observed regularly for two weeks. Several media supported strong ("luxuriant") growth of hyphae with little spore production while others supported less hyphal growth and some sporulation. Oatmeal agar was chosen as the best candidate and a further experiment was conducted where full strength (1x), half strength (0.5x), quarter strength (0.25x) and eighth strength (0.125x) oatmeal agar were prepared with decreasing oatmeal extract but similar agar concentration (2%). Oatmeal agar (1x) produced strong hyphal growth and low sporulation. Oatmeal (0.125x) agar produced neither strong hyphal growth nor good sporulation. The other two concentrations produced good sporulation without excessive hyphal growth. This indicated that there is a balance between level of nutrition and spore production. Whether dilutions of other media would provide superior spore yields is worthy of further testing.

In a second modification it was found that spreading a spore suspension over the surface of a 0.5x plate produced good sporulation in as little as four days whereas plates inoculated with a single fungal disc took up to 7-10 days to reach a similar level of sporulation.

Inoculum for these trials was produced on 200 oatmeal (0.5x) plates that had been inoculated with spore suspensions produced from master plates. Suspensions were produced by washing the master plates with sterile water to which a drop of Tween 20 was

added. Spore concentration was adjusted to 1×10^5 /mL and an aliquot of 100 μ L was spread over the agar surface of production plates using sterile "hockey stick" plate spreaders. Ten plates were sealed within each of 20 plastic ziplock bags. These were incubated at 25°C. Due to space constraints the bags needed to be stacked therefore they were circulated daily, with the top bag going to the bottom of the stack.

Plates were incubated for two weeks by which time they were virtually black with sporulation. Spores were harvested by flooding the plates with 100 mL of sterile water with drop of Tween 20 and scraping with the edge of a microscope slide. Suspensions were filtered through fine mesh to capture pieces of hyphae. The hyphae was added to more water and shaken vigorously to liberate more spores. Spore concentration was assessed by haemocytometer counts and adjusted to 1×10^6 /mL with sterile water. This suspension was then used in the field trials.

Although a large number of spores were harvested it was obvious that many more spores were retained on the filtered mycelium even after vigorous shaking. Future work might look at the use of more extreme disruption methods (e.g. use of a blender) to increase spore yields.

Both the Mirani and Gargett sites were inoculated in the same day. Each treatment block had two separate inoculation transects (see 9.3 Appendix C) dissecting them. Ten plants were inoculated along each of the transects making a total of 20 treated plants per block. Each treated plant was marked with a peg so that re-assessment could be carried out on those plants individually. Viability plates were inoculated morning and night on the day of inoculation. These plates were left to grow out for a period of 10 days before being treated with cotton blue. The plates were then sent to the University of Queensland to confirm spore viability. Spore viability was confirmed for the entire inoculation process.

On the day of inoculation the field temperature varied from 23-29°C with humidity maintained in the mid to high 80% range. There were zero wind gusts for the duration of the day. On the day before inoculation, 56mm of rain fell providing for wet but not waterlogged conditions. The environment inside the inoculated stools was cool and moist. The burnt block exhibited very fresh green shoots removed of any dead matter (Fig. 4). The slash blocks also had a good population of green shoots although the base of the stools was covered by the dense dead matter that resulted from the slashing. Both the no pre-treatment + N.o and control treatments had high levels of dead matter present in the base of the stool but all plants were actively growing. Around 5mm of rain fell in the late afternoon just as treatment concluded.

The inoculant was delivered to each plant via a vaccination gun so that consistent amounts were easily measured out (Fig. 3.). The spore concentration of the inoculum was 10^5 spores per ml of inoculant having been diluted 1:10 from the original solution that morning. Each plant received 12 ml in 6 doses (2ml per dose) to the base of the plant. This represents approximately 1.2×10^6 spores being delivered to each plant. The spores were kept evenly distributed in the inoculum through regular agitation.



Fig. 3: Inoculating plants at the Mirani site using the vaccination gun.



Fig. 4: Fresh GRT shoots in the burn treatments at the Mirani site.

3.1.1.4 Trial site layout and preparation – Clairview

The Clairview site was constructed to assess the efficacy of a range of inoculation strategies. The site was broad scale burnt in September 2012 (Fig. 5) and was heavily grazed until February 2013. This allowed grass to be kept short and in a vegetative phase and in good condition for introduction of the disease. Cattle were given access to the paddock to continue to utilise the grass and extend the period of the growth phase in the

GRT. The treatments utilised in the trial are described below:

1. Planted plants - Previously diseased GPG plants taken from disease sites in Rathdowney, trimmed and washed and replanted. This treatment compared the effectiveness of introducing diseased plants to an area against inoculating plants on site.
2. Overdose - 30 ml standard aqueous inoculant with 10^5 /ml fungal spores. This represents around 3000000 spores per plant.
3. Damaged stool - 1 ml standard water based inoculant with 10^5 /ml fungal spores, inoculated into a damaged stool (smashed with a chipping hoe). Damaged stools may have an increased likelihood of disease which may aid the initial spread.
4. Agar Slurry - 3 ml agar based slurry inoculant with 10^5 /ml fungal spores. This treatment assessed the ability of the left over agar base, containing a low level of spores, to illicit disease in the treated plant.

The site was pegged out in January, prior to inoculation in February. A strip trial design was adopted with each of the treatments being replicated four times (N= 16). The strips were 80m long and were spaced 10 metres apart perpendicular to a fence line (See 9.4 Appendix D).



Fig. 5: The Clairview trial site two weeks after fire treatment.

3.1.1.5 Trial site inoculation – Clairview

The trial site at Olympus was different in terms of preparation for treatment as it also involved introducing the disease through planted plants rather than solely utilising the spore inoculum. Diseased plants were sourced from 'Gunadoo' located roughly 40 km south east of Murwillumbah in Northern New South Wales. Approximately 120 plants were chipped, seeds removed then bagged in heavy duty plastic bags for transportation. The plants that

were sourced were mostly 'stress rating 3' when sourced (refer to table 1). The plants were transported back to Clairview the following day for planting. One plant was planted every four metres along the strip. This equates to 20 plants per replication and with four replicates in the trial giving a total of eighty plants, the rest were destroyed.

The remainder of the treatments were inoculated two days later after the Mirani and Gargett site. The temperature varied during the day of inoculation from 23-29 degrees, relative humidity maintained for most of the day at around 70%. This dropped mid-afternoon with a small shower just after inoculation. The site received 27mm and 47mm of rain in the two days prior to inoculation. This made conditions extremely wet but due to the elevation of the GRT stools the inoculation point was not immersed. A further 12mm fell the next day. These wet, cooler conditions were commonplace at the Clairview site for the next four months to June. Due to the persistent wet, rust was present in significant amounts within the block. The presence of rust made an accurate judgement on stress levels more difficult and may have increased the number of stress level ratings of 1 in the June 2013 assessment. The GRT grew out of the rust later in the year and was not affected by rust in the May 2014 assessment.

3.1.1.6 Trial Site Assessments at Mirani and Gargett

The first rounds of assessments were carried out at the Mirani and Gargett sites in July 2013. The emphasis was in assessing the known diseased plants in order to see if there were any initial symptoms of disease. Disease assessments took into account a number of elements. These elements were:

- Plant Diameter – A measure across the base of the plant
- Stress ratings – The criteria for assessing stress is below

Table 1: Plant stress ratings with descriptions

Disease Stress Rating	Description
Rating 0	No disease, healthy plants with healthy growing points.
Rating 1	Plant has visual effects to its productive capacity. There may be a reduction in the size or robustness of the plants when compared to healthy plants. Stool may have a 'grassy' appearance to the tussock. This means there is a high population of short thin leaf coming from a very squat tussock. There may also be a reduction in the number and/or health of seed heads. The plants have general stress indicators that may not be solely related to disease.
Rating 2	Plant is clearly reduced in its growth. There is a clear reduction in stool diameter when compared to other plants. Yellowing of the third leaf may be evident on some of the stems. Necrosis is present at the base of these stems. Seed head populations are significantly reduced. Seed heads present may be reduced in length or visually unhealthy.
Rating 3	There is clear evidence of crown rot present in the plant. Necrosis is significant evident in the base of the crown with areas of die off also evident. There is a significant reduction or absence of seed heads. The plants are generally unhealthy and lack competitiveness.

Disease Stress Rating (Cont.)	Description
Rating 4	The plants are being seriously impacted by disease. There are large areas of die-off within the crown of the plants. Necrosis is clearly evident. The plant looks likely to die.
Rating 5	Death, senescence

Seed head counts were not carried out in the initial July assessment as fire treated and slashed plants were still yet to produce a full yield of seeds, whereas the control and no pre-treatment + *N.o* replicates had already produced and distributed seeds making it hard to evaluate.

The second rounds of assessments were carried out 11 months later in May 2014. These assessments re-evaluated the same plants for any symptoms of disease. Seed head populations were taken on these known diseased plants for comparisons across treatments. Plant diameters and stress rating were also recorded.

In addition to the assessments carried out on known inoculated plants, assessments were carried out on the areas adjacent to the inoculation transects. Each side of the disease transects were evaluated for stress symptoms, plant diameter and seed head number to determine any potential spread of disease and to identify areas to sample for disease isolations. If a disease presence was identified these assessment would have also gauged any changes in GRT population density over time.

3.1.1.7 Field trial assessments at Clairview

The assessments that were carried out at the Clairview site were very similar to the Mirani and Gargett sites. At the first three month assessment the emphasis was placed on assessing the known diseased plants for diameter and stress ratings. At the May 2014 assessment, seed head populations were also assessed by treatment.

Due to the trial being a strip trial, each of the 20 known inoculated plants on each strip were assessed individually for stress and plant diameter in June 2013. This was repeated on those same plants in May 2014. In addition to this, four plants directly adjacent to each inoculated plant were also assessed for stress rating and seed head populations.

It had been observed that since the burn treatment that had been carried out in September 2012 there had been a considerable increase in the gilgae present in the site (Fig. 6). The June 2013 assessment identified that the high intensity burn in combination with the gilgae areas that had remained waterlogged had produced considerable stress on the GRT (Fig. 7). During the May 2014 assessment, each plant assessment was given a waterlogging score (1-3) to see if there was a relationship between waterlogging and plant diameter, seed production and stress ratings in GRT.

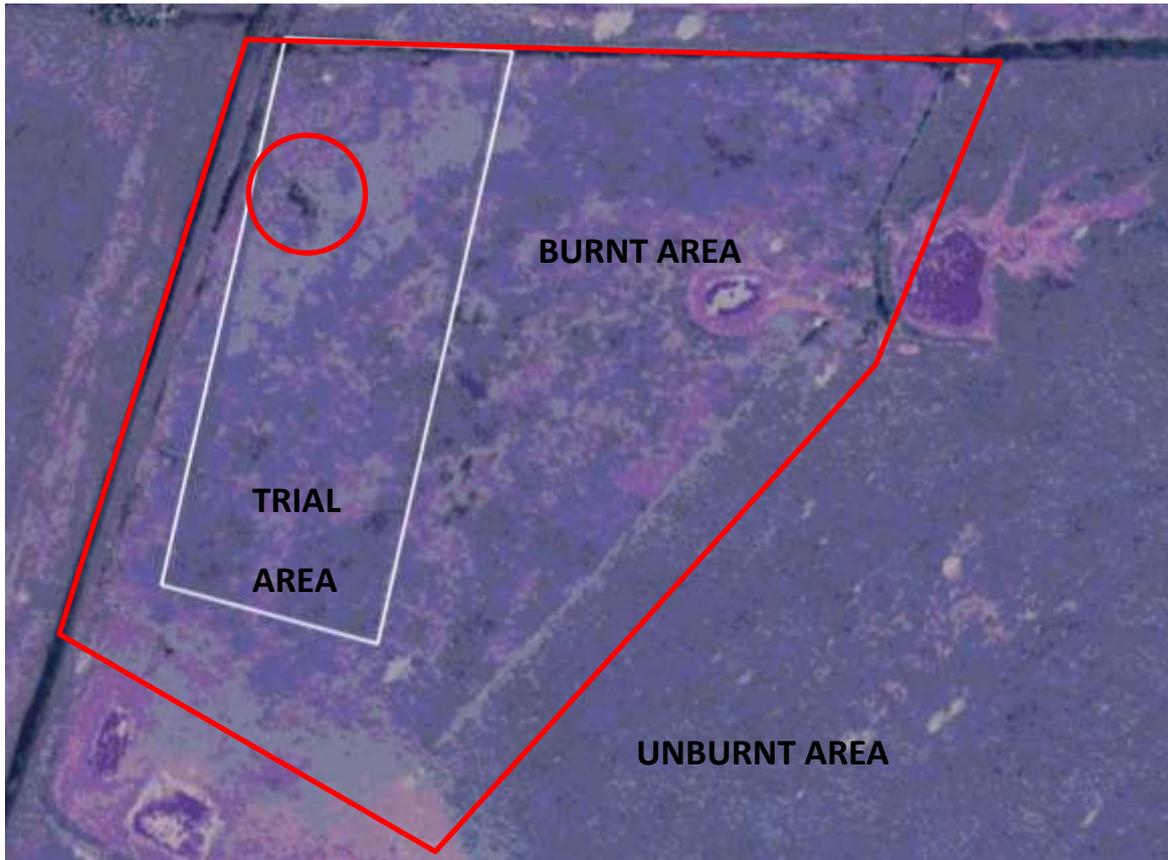


Fig. 6: Satellite image of the Clairview site contrasted to show the gilgaing which has resulted from the fire treatment in 2012. Note the deeper gilgae in the circle.



Fig. 7: The gilgae highlighted in the previous image. Note the GRT in the waterlogged area has died out. Closer inspection reveals *Pangola* (*Digitaria eriantha*) recolonising the area.

3.1.1.8 Laboratory isolations from field trial plant samples

After the May 2014 assessments were completed and areas of potential infection were identified, isolations were carried out on plants sampled from the Mirani site. This follows a previous recommendation made due to the difficulty encountered in visual identification of crown rot in GRT. In order to get the best scope of potential disease agents four samples were taken from each of the 16 plots (Table 2).

Table 2: Plants sampled from each treatment plot at the Mirani site for laboratory isolation of fungi

Treatment Plot	Inoculation	Stress	No Stress
Control	Uninoculated	4	4
<i>N. oryzae</i>	Uninoculated	4	4
<i>N. oryzae</i>	Inoculated	4	4
Slash + <i>N. oryzae</i>	Uninoculated	4	4
Slash + <i>N. oryzae</i>	Inoculated	4	4
Burn + <i>N. oryzae</i>	Uninoculated	4	4
Burn + <i>N. oryzae</i>	Inoculated	4	4

These samples were taken from the following:

- Sample A: Known inoculated plants with stress symptoms
- Sample B: Known inoculated plants without stress symptoms
- Sample C: Uninoculated plants showing stress symptoms
- Sample D: Uninoculated plants showing no stress symptoms

Symptoms of stress included:

- Chlorosis of the leaf sheath
- Reduced growth in comparison to other plants of the same diameter
- A large number of fine narrow leaves reduced in size (grassy stool)
- A reduction in seed head production
- Once sampled a large amount of dead, necrotic material around the base of the stem.

In the untreated control blocks, only samples C and D were taken as there were no inoculated plants. Only two samples were taken from *N. oryzae* Block 2 (samples B and D) as there were no plants within that block exhibiting any stress symptoms.

Plants were sampled by removing a section of the outside of the plant (<5cm diameter). The sample was then placed in an individual bag with ID number. A total of 54 samples were collected from the Mirani site. These samples were sent overnight to Grow Help at the Ecosciences Precinct (ESP).

At the laboratory all dead tissue and the outer layers of the leaf sheath were removed from the samples. The crowns were washed and sprayed with a 70% ethanol rinse and left to dry. Fifteen chip samples were taken from the crown of each of the samples and placed on Agar (50% PDA + Streptomycin) and incubated for five days, some samples had to be further sub-cultured to produce clean samples.

3.2 Effect of *Nigrospora oryzae* and *Fusarium* sp. on Giant Rat's Tail Plants in pot trials.

3.2.1 Background

A pot trial was conducted in the glasshouses at ESP, Dutton Park from April 2014 until February 2015 to evaluate the pathogenicity of *N. oryzae*, *F. chlamydosporum* and *F. proliferatum* towards GRT plants under controlled conditions which were conducive to the establishment of fungal infections in plants. The trial was conducted to first provide data to support the interpretation of the Mirani field trial results which indicated that *N. oryzae* may not cause crown rot in GRT; and second, to further the work carried out at RMIT under MLA project B.NBP 0716. The results of the RMIT research suggested that the *Fusarium* species may also be causing die back in potted and seedling GRT. However, the results were inconclusive due to insufficient replication and further work in this area was recommended. The primary aim of this trial was to assess the variation in plant health of potted GRT plants in response to a number of treatments of *N. oryzae* and two *Fusarium* species (*F. proliferatum*, *F. chlamydosporum*) applied on their own and in combinations as recommended by Professor Lawrie in her work at RMIT.

3.2.2 Plants

Plants were sourced from a widespread established infestation of Giant Rat's Tail Grass (*Sporobolous pyramidalis*) located near the Gargett Trial site (Latitude 21°10'4.49"S, Longitude 148°47'13.92"E). To ensure that clean plants were sampled, the source block was approximately one kilometre from the Gargett trial site. The two sites are separated by a large dam (Fig. 8) and there was no prior history of stock or vehicle movements between the two locations. Only *S. pyramidalis* was sampled as this was the only GRT variety that could be accurately confirmed at the time from the large side branches that typify the species, and only large healthy plants over 30cm in diameter with seed head population >15 were chosen. Plants were chipped with a grubbing hoe and placed in large heavy duty plastic bags for transportation. Around 50 large (>30cm diameter) plants were removed from the site. Plants were chipped during the morning with the majority of soil being removed from the root system and then placed in plastic bags in large sealed cardboard boxes for transport via Toll overnight express to ESP arriving the next morning. All seed heads were removed from plants prior to transport.



Fig. 8: Satellite image showing the location of the sampling site for the pot trial in relation to the Gargett field trial

Upon arrival at ESP, the cardboard boxes were opened to air the plants. Processing into pots occurred the next day. Larger (>30cm diameter) plants were separated into smaller 12cm diameter plants and plant stems were trimmed to 15cm above the crown to maintain an even size before potting into black 200mm (4.5L) plastic pots (9). The potting substrate was a blended mixture of pine bark chips and river sand sourced from an accredited landscape garden supplier. Black saucers were placed under all pots. Potted plants were arranged randomly on free draining aluminium benches in a climate controlled glasshouse and watered twice weekly. Each month plants were randomly redistributed across the benches in order to remove any possibility of edge effects in the glass house. Survival rate of plants into pots was 100% from transport to potting to growth in the glasshouse. Plants were allowed to grow under glasshouse conditions for one month before they were assigned to a treatment group and treatments applied.



Fig. 9. Preparation of plants for pot trial at the EcoSciences Precinct

3.2.3 Inoculum preparation

A culture of *Nigrospora oryzae* was supplied by Goulter University of Queensland (UQ), Gatton, this was the same isolate originally supplied by David Officer (NSW DPI), for the GRT field trial (see section 3.1). Cultures of *Fusarium proliferatum* and *Fusarium chlamydosporum* were supplied by Professor Ann Lawrie (RMIT), these were the isolates used in the GRT pot trials of MLA project B.NBP.0716.

All three cultures were first subcultured onto a range of media including V8 agar with and without CaCO₃, oatmeal agar, and Sabouraud's dextrose agar to optimise the initial growth conditions. These cultures were then used to inoculate 250 ml flasks with 150 ml of sterile broth of either glucose peptone broth or fructose peptone broth. (Glucose peptone broth: 1% yeast extract; 1% peptone; 2% dextrose. Fructose peptone broth: 1% yeast extract; 1% peptone; 2% fructose). Liquid cultures were shake incubated for 5 days at 27 °C and 120 rev/min. The liquid inoculum was used to inoculate 500 ml flasks of different types of sterilised solid to find the best medium for spore production for each isolate. *Nigrospora oryzae* grew well and sporulated on all solid media while the *Fusarium* isolates just formed a solid mass in the flasks. Therefore, the liquid inoculum from the shake culture was used to inoculate the GRT plants with *Fusarium*. Fructose yeast peptone broth inoculated with either *F. proliferatum* or *F. chlamydosporum* was shake incubated as described above for 5 days to produce 900 ml of inoculum of each *Fusarium* isolate. The inoculum was checked under the microscope, *F. proliferatum* had a very high concentration of macro and micro conidia while *F. chlamydosporum* had a high concentration of chlamydospores. The *Fusarium* inoculum was prepared by blending 400 ml liquid culture with 450 ml distilled water.

The *Nigrospora oryzae* inoculum was prepared by inoculating sterilised oats (300 g moistened with 30 ml water) in mushroom spawn bags with 60 ml liquid inoculum prepared as above and 120 ml sterile 0.1% Tween 80. Bags were heated sealed and incubated for 14 days. Bags were examined under the stereo-microscope and the bag with the highest spore density was chosen. Sporulating *N. oryzae* on oats (300 g) was blended with 900 ml of sterile distilled water, filtered and made up to 1 litre.

3.2.4 Inoculation

To achieve a stratified randomisation of pot assignment to treatment groups, pots were measured by plant circumference, pot weight and number of green tillers. This allowed for the variation in the plants to be identified. The measured plant diameters were affected by the amount of dead matter present and not all plants were same shape, so circumference rather than diameter was chosen. Pot weight was affected by the amount of plant material and soil, as well as the water content with some plants dry and others quite wet. In addition the number of green tillers was multiplied by circumference to compensate for the observation that often when the number of tillers was high the average size of the tillers would be smaller. Conversely, pots with low numbers of tillers often had much bigger tillers. However, it was decided that tiller number best represented plant variation and the tiller ranking was divided into groups of eight (for the eight treatments). The top eight and bottom eight were removed to reduce the variability in the pool of plants to be allocated to the main treatment groups. These 16 plants were used for four additional treatment groups of four replications to test some extra fungal isolates. Plants in the middle 12 groups of 8 were randomly allocated to one of 8 treatment groups with 12 replicates per group (Fig. 10).

Each treatment group was photographed prior to inoculation for referral later, and then inoculated by applying 30 ml per plant of the relevant treatment (Fig. 11). The liquid inoculum was poured into the centre of the plant then watered in with a count of five to thoroughly wet the inoculum, plants and soil. See Table 3 for amounts of each fungus used in each treatment. The four extra fungal treatments are described later. Post watering all plants had liquid running through to the saucers. In order to provide maximum humidity to the plants and the inoculum, 60 cm bamboo stakes were added to each pot and a large plastic bag (85 x 50 cm) placed over the top and secured to the pot with an elastic band (Fig. 12). The bags were left over the plants for the initial 72 hours, and then removed. Plants were then randomly allocated to benches in the western wall of the glasshouse. The glasshouse temperature was maintained between 20°C and 30°C which is ideal for fungal growth. Plants were watered twice weekly with each plant given a timed watering of five seconds. Every month plants were moved around the benches in the glasshouse to re-randomise their position in the glasshouse.

Table 3. Amount (ml) of liquid inoculum of each fungus applied to GRT plants for each treatment. (N.o. = *N. oryzae*; F.p. = *F. proliferatum*; F.ch. = *F. chlamydosporum*)

TREATMENT	<i>Nigrospora oryzae</i>	<i>Fusarium proliferatum</i>	<i>Fusarium chlamydosporum</i>
1 (Control)	0	0	0
2 (N.o.)	30	0	0
3 (F. p.)	0	30	0
4 (F.ch.)	0	0	30
5 (N.o. + F. p.)	15	15	0
6 (N.o. + F.	15	0	15
7 (F.p. + F.ch.)	0	15	15
8 (N.o. + F.p. + F.ch.)	10	10	10

The extra treatments applied to the 16 “outlier” plants rejected from the main treatment group above comprised three different *Fusarium* isolates (ESP.F.02; ESP.F.03; ESP.F.04) and another *Nigrospora oryzae* isolate (UQ.N.o.003). These isolates were selected from a large number of fungi isolated from “diseased” GRT plants supplied by David Officer (NSW DPI). The *Fusarium* treatments were applied as 30 ml of liquid inoculum prepared in a similar way to *F. proliferatum* and *F. chlamydosporum*. The *N. oryzae* inoculum was prepared similarly to the *N. oryzae* isolate used in the main treatments above.



Fig. 10: Pots of GRT allocated to a treatment group before the first inoculation



Fig. 11: Inoculum of *F. chlaydosporum*; *N. oryzae* and *F. proliferatum*



Fig. 12: Plastic bags secured to GRT plants for 72 hrs post inoculation to provide an atmosphere of very high relative humidity conducive to fungal growth.

3.2.5 Additional treatment post four month assessment

After the four month assessment, the extra treatments (9, 10, 11 and 12) were removed because no treatment effects were observed and the available glasshouse space was reduced. Each remaining treatment group (containing 12 repetitions) was divided into three separate sub treatments to mimic the field pre-treatments applied in the GRT field trial at Mirani and Gargett (see section 3.1.1.2). These sub treatments included: No Treatment, Slash and Burn. The slash treatment had all biomass removed from the plant down to 10 mm from the base of the crown. Burn treatments were burnt in the pot to remove biomass (Fig. 13). The “no treatment” plants had no pre-treatment prior to inoculation. All plants minus the control group were re-inoculated four weeks after the “field” pre-treatments were applied. The inoculum and plant inoculation procedures were the same as for the initial inoculations except that twice the volume of inoculum per replicate plant was administered than that shown in Table 3 (i.e. total of 60 ml inoculum per replicate plant).

The rationale for the “field” pre-treatments of slash and burn was to add stress to the plants, thus increasing the potential for disease development.



Fig. 13: Burn pre-treatment before re-inoculation of pots after the four month assessment

3.2.6 Treatment Assessment

Assessments were made on plant health across all treatment groups after four months and then after eight months. Tiller numbers, dry matter yield (g) and plant circumference (cm) were recorded to assess any potential disease effects on plants. To measure dry matter yield, all plants were cut back to 15 cm above the plant soil interface, the green biomass from each plant was individually bagged in a brown paper bag (4 month assessment: 15 × 37.5 cm with a 4.5 cm gusset; 8 month assessment: 26 × 35 cm with a 4.5 cm gusset) and dried for 24 hrs at 60 °C in an fan forced dehydrating oven (Thermoline Scientific, model TD-78T-2-D). After cooling to ambient temperature, bags were weighed on a Mettler PC 4400 balance and the weights recorded. A sample of 10 bags without plant biomass were similarly dried, then weighed so that the average bag weight could be accounted for when calculating the dry matter yield for each plant.

3.2.7 Statistical Analysis

The four month trial data was analysed in two ways: i) The eight main treatments were analysed separately to the four extra treatments using ANOVA and ii) All 12 treatments were analysed together with ANOVA allowing a comparison of the main and extra trial treatments. For the eight month data, the inoculation treatment and field pre-treatment effects on dry matter weight and circumference at four and eight months for the main trial were assessed using Residual Maximum Likelihood (REML) with “size” grouping as a blocking factor. REML is used to account for an imbalance with the “field” pre-treatment and the blocking factor. The increase in bag weight and circumference from four to eight months was restricted to pots with a “field” treatment level of “none” and were analysed with ANOVA. Both ANOVAs and REML were carried out by David Reid, DAF using GenStat Release 16.1.

3.3 The Development of Strategies for ongoing Research, Development and Extension in Weed *Sporobolus* control

On Tuesday 7 July 2015, a meeting was convened to discuss opportunities for further research, development and extension into control of weedy *Sporobolus* grasses (WSG). The attendees list was as follows:

- Jim Fletcher (Beef Extension Officer DAF, Mackay)
- Diana Leemon (Senior Scientist DAF, ESP)
- John Hughes (Agronomist DAF, Mackay)
- Joe Vitelli (Principal Weeds Scientist DAF, Ecosciences Precinct)
- Graeme Elphinstone (Senior Extension Officer DAF, Gympie)
- John Reeve (Senior Biosecurity Officer DAF, Rockhampton)
- Lalith Gunasekera (Biosecurity Officer DAF, Mackay)
- Nicole Restelli (Biosecurity Officer DAF, Mackay)
- Wayne Vogler (Senior Weeds Scientist DAF, Charters Towers)
- Eric Dyke (Senior Land Protection Officer, Bundaberg Regional Council)
- Brett Cawthray (Rural Lands Officer, Gladstone Regional Council)
- Paul Tippet (Heritage Seeds, Mackay)
- David Officer (Weed research Officer NSW DPI, Grafton)

The meeting incorporated discussion on a number of factors associated with the management of WSG with a focus on two overarching outcomes:

- Outcome 1: To develop a basic understanding of the issues underpinning effective management of weedy *Sporobolus* grasses in Australia.
- Outcome 2: To develop a number of pathways to further research, developing current research knowledge and creating better extension strategies for the industry.

Weedy *Sporobolus* Management was broken down into a number of sub-topics for more detailed discussion. These sub-topics were;

- Grazing Land Management
- Soil Health and Nutrition
- The Social implications to extension
- The Impacts of Fire on WGS
- Grass selection for competitiveness
- On-farm biosecurity
- Chemical management
- Seed population reduction of WSG
- WSG research gaps

4 Results

4.1 Results from Field Trials

4.1.1 Plant Diameter and Seed Head Production

The Mirani site had only a slight variation in plant diameter between the controls (11.4 cm) and the No pre-treatment + N.o and Slash + N.o (12.9 and 12.2 cm) at the three month post inoculation assessment (Table 4). The fire treatment resulted in significantly smaller plants (9.2 cm). Burning also reduced plant diameter initially at the Gargett site (Table 5).

Although the control replicates were significantly larger in diameter (13.0cm) than the No pre-treatment + N.o and Slash + N.o treatments which were the same (11.3 and 11.0 cm). In the 11 months to the next assessment growth varied between treatments and also between treatments by site.

Table 4. Increase in GRT plant diameter (cm) from June 2013 to May 2014 and seed head populations for May 2014 at the Mirani site.

	Plant diameter (cm)			Number of seed heads	
	Jun-13	Apr-14	Inc. 2013-14	Jun-13	Apr-14
Treatment	***	***	***	N/A	***
Control	11.4 b	15.4 a	4.1 a		6.1 b
N.o only	12.9 a	15.0 a	2.0 b		7.5 a
Slash	12.2 ab	13.5 b	1.1 bc		6.6 ab
Burn	9.2 c	9.5 c	0.3 c		2.9 c
ave. s.e.d.	0.5	0.5	0.6		0.5

N/A - not available

Values followed by the same letter within columns, are not significantly different. * P<0.05; ** P<0.01; *** P<0.001

Table 5. Increase in GRT plant diameter (cm) from June 2013 to May 2014 and seed head populations for May 2014 at the Gargett site.

	Plant diameter (cm)			Number of seed heads	
	Jun-13	May-14	Inc. 2013-14	Jun-13	May-14
Treatment	***	***	***	N/A	***
Control	13.0 a	16.1 a	3.3 a		8.9 b
N.o only	11.3 b	16.0 a	4.5 a		11.1 a
Slash	11.0 b	11.5 c	0.6 b		5.5 c
Burn	9.7 c	13.7 b	4.5 a		7.9 b
ave. s.e.d.	0.4	0.6	0.7		0.5

N/A - not available

Values followed by the same letter within columns, are not significantly different. * P<0.05; ** P<0.01; *** P<0.001

There was significantly higher growth across both sites in the controls and also for the No pre-treatment + *N.o* blocks (Tables 4 and 5). The increase in plant diameter in the Burn + *N.o* treatments at Mirani were significantly lower for that period compared to other treatments (+ 0.3 cm), although this is of contrast to the Gargett site where Burn + *N.o* exhibited high growth in comparison (+ 4.5 cm). The Slash + *N.o* treatments had lower comparative growth to other treatments at both sites which were significant. This may be due to the additional slashing placing nutritional strain on the plants.

Due to wide scale fire, the plant diameters for the Clairview site were more even with the exception of the 'Planted' Plants treatments which were smaller initially (Table 6). All treatments grew significantly in the eleven months to May 2014 (+ 6.4-7.2 cm) with the exception of the 'Wounded' Plant treatment which still grew (+ 3.6 cm) but to a lesser degree. This may be explained by the damage carried out to the plant during inoculation and correlations might be drawn to the stresses placed on the slashed plant in other locations.

Table 6. Increase in GRT plant diameter from June 2013 to May 2014 and seed populations in May 2014 at the Clairview site.

	Plant diameter (cm)			Number of seed heads	
	Jun-13	May-14	Inc. 13-14	Jun-13	May-14
Treatment	*	***	P=0.103	N/A	**
Planted plants	12.2 b	19.4 a	7.2 a	7.5 b	
Overdose	14.1 a	20.5 a	6.4 a	9.8 a	
Agar Slurry	14.1 a	20.5 a	6.4 a	8.6 ab	
Wounded plants	13.7 a	17.3 b	3.6 b	7.4 b	
ave. s.e.d.	0.7	0.8	1.3	0.7	

N/A - not available

Values followed by the same letter within columns, are not significantly different. * P<0.05; ** P<0.01; *** P<0.001

No treatments across any of the sites experienced a reduction in plant diameter.

Seed head populations across treatments were strongly associated with plant diameter. Treatments with larger diameter increases over the 13/14 period tended to have larger seed head counts. The Burn + *N.o* treatments at Mirani had the smallest diameter increase and therefore a significantly smaller population of seed heads. At Gargett, where post burn plants grew more rapidly over the 2013/14 period there was a higher population of seed heads, significantly more so than the Slash + *N.o* and in similar populations to the controls.

At the Clairview site there was also a significant reduction in plant diameter and seed head productions for plants growing in areas rated '3' for waterlogging as compared to a '1' or '2' (Table 7).

Table 7. GRT plant diameter (cm) and seed head number in June 2013 and May 2014 at the Clairview site – accounting for effect of waterlogging

	Plant diameter (cm)			Number of seed heads	
	Jun-13	May-14	Inc. 2013-14	Jun-13	May-14
Treatment (T)	*	***	N/A	N/A	P=0.097
Planted plants	13.5	19.4	a	5.9	6.7
Overdose	15.1	20.0	a	4.9	8.1
Agar Slurry	15.3	20.6	a	5.2	7.9
Wounded stools	15.0	17.6	b	2.6	7.0
ave. s.e.d.	0.7	0.8			0.7
Waterlogging (W)	n.s.	***			***
Score 1	17.8	21.1	ab		8.7 a
Score 2	13.5	20.1	a		9.6 a
Score 3	12.9	17.0	b		4.0 b
T x W	n.s.	n.s.			n.s.

N/A - not available

Values followed by the same letter within columns, are not significantly different. n.s. - not significant; * P<0.05;

** P<0.01; *** P<0.001

4.1.2 Disease Stress Ratings and Disease Spread Transects

Across Gargett and Mirani there was a significantly higher population of burn treated plants rating a disease stress score of 1 across both the June and May assessments (Tables 7 and 8). Slashed plants also showed significantly higher proportions of stress ratings of 1 in the 2014 assessments at both locations. It is important to understand that in comparing stress scores across treatments that over the period of assessment very few plants exhibited a stress score higher than 1. At the Mirani site there was no significant variation across treatments for plants rating higher than 1 for stress. At the Gargett site no plants rated higher than 1 for any of the assessments.

When plants adjacent to known diseased plants were assessed the population of plants with disease ratings >0 decreased. No plants rated higher than 1 for stress in these assessments at both the Mirani and Gargett sites.

At the Clairview site there was a significantly higher proportion of the planted plants rating higher than 1 for stress and this is indicative of plants being transplanted into a new environment combined with the wet conditions that were observed at that site for four

months after planting. Plants adjacent to the planted GPG had the lowest incidence of stress of any of the treatments.

4.1.3 Disease Spread Transects

Table 8. Percent of GRT plants in spreading assessments exhibiting stress in May 2014 at the Gargett site.

Treatment	% field assess. plants with stress score		% adjacent plants with stress score	
	0	>0	0	>0
		n.s.		n.s.
Control	95	5	96	4
<i>N. o</i> only	87	13	94	6
Slash	90	10	95	5
Burn	87	13	97	3

n.s. - not significant; * P<0.05; ** P<0.01; *** P<0.001

There was no relationship (chi-square) between treatment and stress ($p>0.10$) for uninoculated plants at the Gargett site, although there was some slight evidence of a lower percentage of controls with stress greater than 0.

Table 9. Percent of GRT plants in spreading assessments exhibiting stress in April 2014 at the Mirani site.

Treatment	% field assess. plants with stress score		% adjacent plants with stress score	
	0	>0	0	>0
		***		***
Control	97	3	97	3
<i>N.o</i> only	100	0	100	0
Slash	82	18	88	12
Burn	60	40	74	26

n.s. - not significant; * P<0.05; ** P<0.01; *** P<0.001

4.1.4 Isolations

The results of the isolations indicate that overall the samples were collected from plants in good health. There was very little evidence of any crown rot symptoms in the interior tissues to be seen once the outer layers of dead material were removed (Fig. 14). Of those samples that did exhibit crown rot symptoms no one pathogen was consistently isolated. No

overarching trends could be observed between treatments and no one pathogen was isolated in high frequency. With 15 chips taken from the crowns of each of the 54 grass samples there were 810 plant tissue isolations. Of these, 258 chips had no fungal growth. The three genera of fungi most frequently isolated were *Trichoderma spp.*, *Penicillium spp.* and *Rhizopus spp.*, none of which are known to be significant pathogens of grasses.



Fig. 14: Typical example of stems from sampled plants.

Eleven of the 54 samples from the Mirani site yielded a *Fusarium* species. Although some *Fusarium* species are known pathogens and *Fusarium* was isolated at moderate to low levels it could not be definitively linked to the presence of crown rot.

Nigrospora oryzae spores were observed on the decaying plant tissue of one sample taken from a post slash treatment (Slash R3) at the Mirani site. However, *N. oryzae* was never isolated from the crown tissue of any sample.

Three additional as yet undetermined fungi were isolated from the plant samples. One *Helminthosporium* like fungus, one *Cladosporium* like fungus and a peach coloured *Fusarium* species with short monophialide conidiophores. The first two fungi were found on samples that did exhibit some signs of dieback, but were found in relatively low populations. Larger quantities of the peach coloured *Fusarium* were isolated but did not appear to correlate to any obvious symptoms of die back. There did not appear to be any correlation between stressed and unstressed plants and populations or combinations of the various fungi (Table 10.).

Table 10. Average number of fungi isolated from plants sampled in the Mirani treatment plots in June 2014

Treatment Plot	Inoculation	Stress	No Stress
Control	Uninoculated	5.5	5.75
<i>N. oryzae</i>	Uninoculated	14	13.5
<i>N. oryzae</i>	Inoculated	12.7	11.5
Slash + <i>N. oryzae</i>	Uninoculated	5.75	3.75
Slash + <i>N. oryzae</i>	Inoculated	4.0	7.5
Burn + <i>N. oryzae</i>	Uninoculated	5.5	8.75
Burn + <i>N. oryzae</i>	Inoculated	5.5	4.75

4.2 Ecosciences Precinct Pot Trial

After four months, there was no significant difference in dry matter production (Fig. 15) between any of the treatments including the additional *Fusarium* and *Nigrospora* treatments of the outlier plants. In addition there was low variability, as shown by the standard errors across the eight main treatments (Fig. 15). The variability in the additional treatments was slightly higher, but this most likely reflects that there was only four replicates per treatment and these plants were from the outlier group with greater initial variability. However, there was a significant difference in tiller number and circumference between treatments although this difference did not appear to relate to any meaningful treatment effects. In plants with a big increase in tiller number, the tillers were noted to be mostly small “grassy” tillers. Other plants with a smaller increase in tiller number often had larger individual tillers.

The fungal inoculum showed good growth at the base of the GRT plants when the plastic bags were removed three days after inoculation (Fig. 16). Thus the inoculum clearly showed initial vigorous growth under these conditions, even if not pathogenic.

When the plants were re-inoculated after four months with the *N. oryzae*, *F. proliferatum* and *F. chlamydosporum* and grown for a further four months (eight months post the first inoculation) a significant difference ($p < 0.05$) in the dry matter production occurred between treatments (Fig. 17). However, three of the treatments with *N. oryzae* had a significantly higher dry matter production ($p < 0.05$). The control group had the lowest average dry matter production (70 g) over this four month period. It was difficult to see any difference across the treatment groups through a visual inspection at eight months (Fig. 18). When the fungal treatments are separated out into the pre-treatments of burn, slash or no treatment there is little variation across the fungal treatments in both the no pre-treatment and slash pre-treatment groups (Fig. 19). There was some variation across the fungal treatments burn pre-treatment group (Fig. 19). However, this variation was due to the unevenness of the burning of the GRT. It was difficult to control the burn damage to each plant as the burning was influenced by the amount of dead matter in the centre of each plant. A couple of plants were burned so badly that they did not recover, thus reducing the number of replicates and further influencing the variation. The limited control over the burning of GRT plants in pots reflected the experience with burning GRT plants in the field.

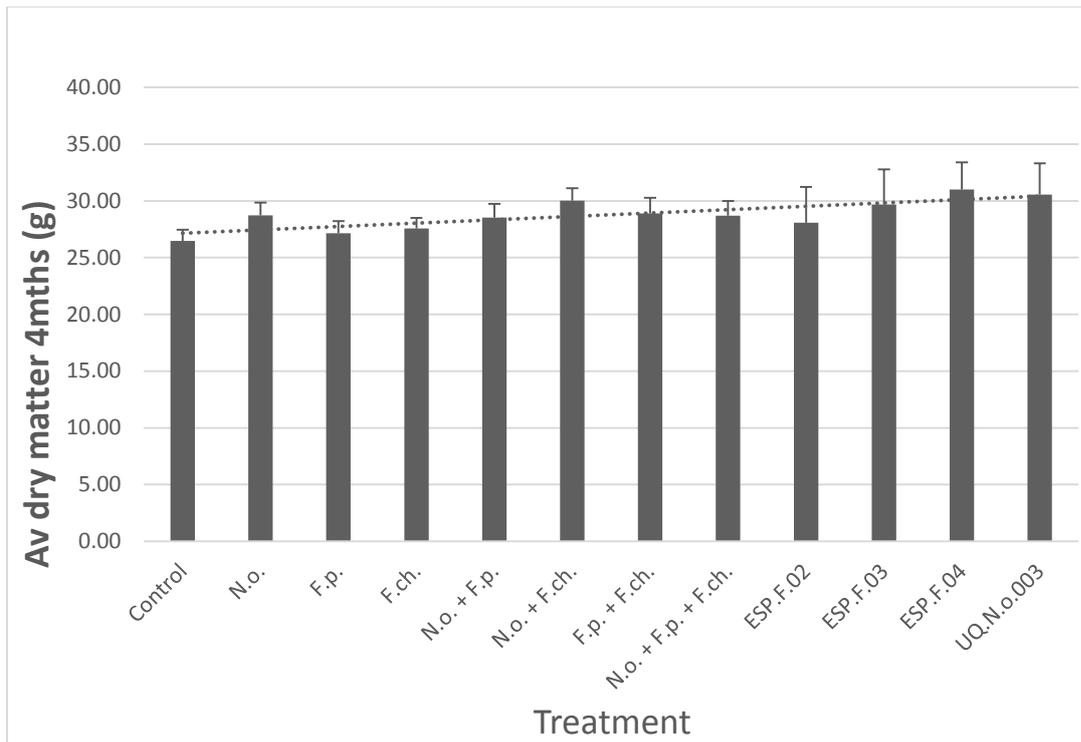


Fig. 15: Average dry matter production (g) four months post inoculation with different fungal treatments, there was no significant difference between treatments. (N.o. = *Nigrospora oryzae*; F. p. = *Fusarium proliferatum*; F. ch. = *Fusarium chlamydosporum*. ESP.F.02; ESP.F.03; ESP.F.04 = new *Fusarium* species, UQ.N.o.003 = new *N. oryzae* from GRT plants). Standard error bars shown.



Fig. 16: Fungal growth from the inoculum in treatment 8 (all three fungi) evident after the plastic bags were removed 3 days post inoculation. The pink growth is *Fusarium chlamydosporum*.

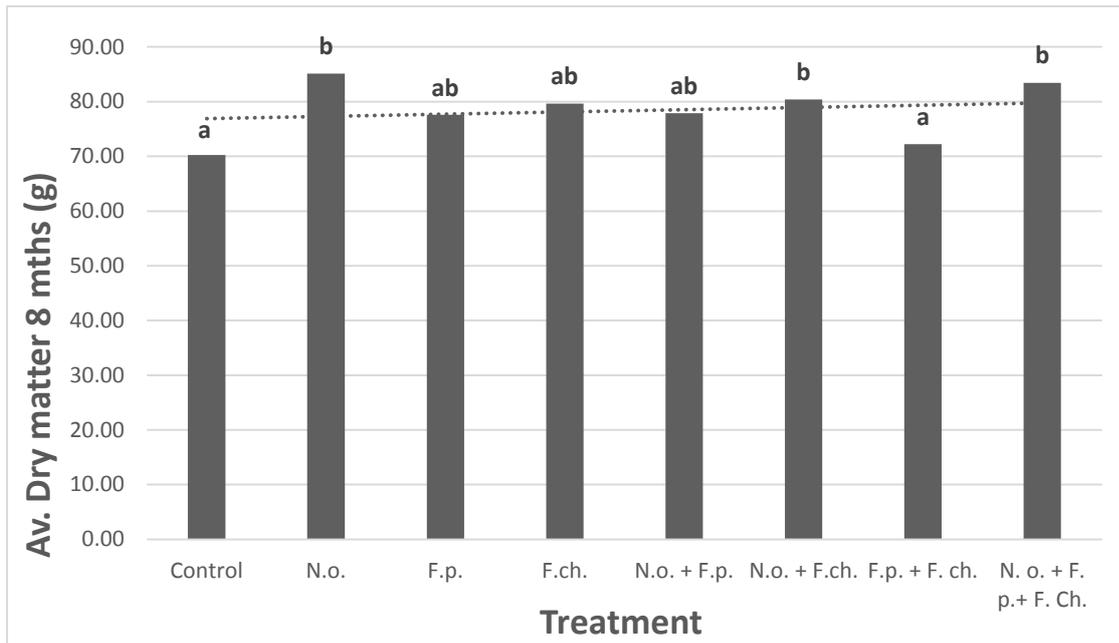


Fig. 17: Average dry matter (g) production from 4 months until 8 months across all fungal treatments, the field treatments are grouped within each fungal treatment. Bars with different letters are significantly different ($p < 0.05$).

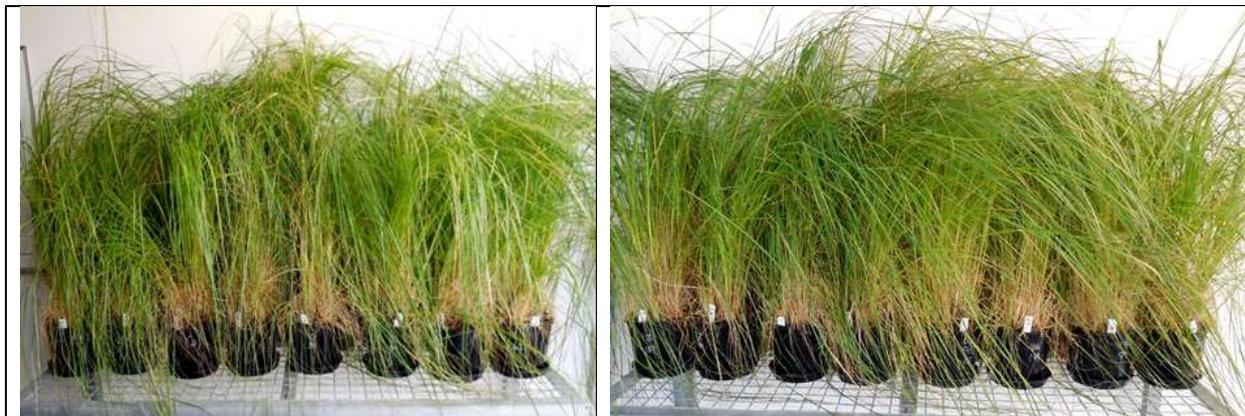


Fig. 18: Little visible difference between any of the treatment groups after 8 months. Treatment groups arranged from the far left (control) to the far right (all three fungi combined: *N. oryzae* + *F. proliferatum* + *F. chlamyosporum*).

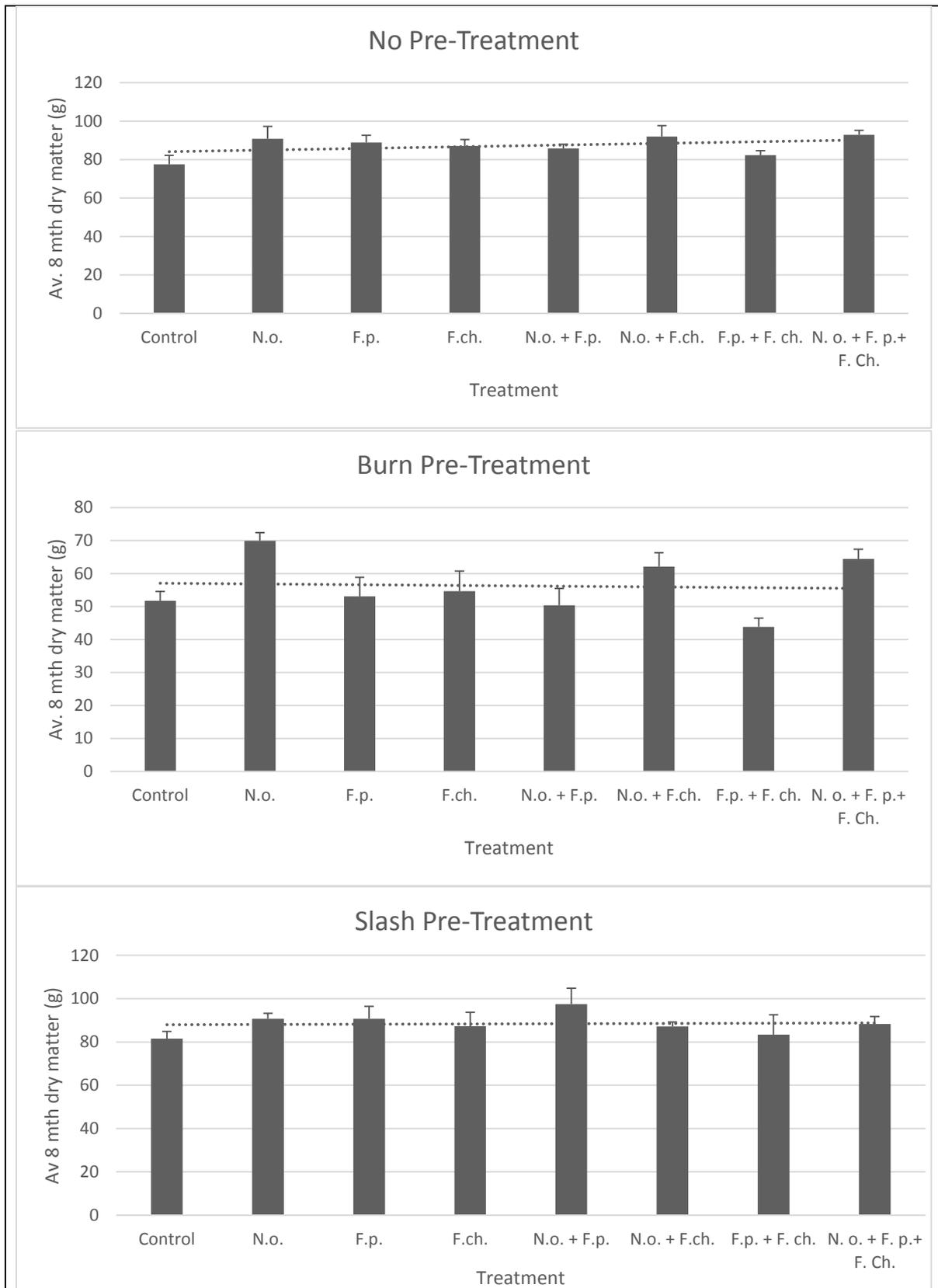


Fig. 19: Average dry matter (g) production from 4 months until 8 months with the pre-treatments separated (Top = No treatment; Middle = Slash; Bottom = Burn). Standard error bars shown.

Post the four month assessment, three GRT plants that were to be discarded from the first part of the trial were re-potted in some *N. oryzae* production medium. These were grown next to and under the same conditions of three other plants that were discarded from the first part of the trial. After four months of growth, the plants repotted in the *N. oryzae* growth medium appeared greener than the other plants (Fig. 20).



Fig. 20: GRT grown in *N. oryzae* medium from 4 – 8 months was greener (plant on the left) than GRT grown in potting mix without added fertiliser (plant on the right).

5 Discussion

This project has expanded on the research into the potential of *N. oryzae* as a biocontrol agent for GRT reported in MLA project B.NBP.0716 ("Giant Rat's Tail grass susceptibility to fungi effective in biological control of Giant Parramatta Grass") through the conduct of both field trials at three sites and a large pot trial under glasshouse conditions.

Neither the field trials conducted at Mirani and Gargett nor the pot trial conducted at ESP has provided data to support *Nigrospora oryzae* as a causal agent of crown rot in Giant Rat's Tail Grass (*Sporobolus pyramidalis*). However, results of the pot trials at ESP would suggest that *N. oryzae* has a slightly beneficial effect on the growth of potted GRT plants under glasshouse conditions.

5.1 Field trial results

The primary objective of the research was to establish whether there is any evidence of a pathogenic relationship between *N.oryzae* and GRT. In order to do this the field trials at Mirani and Gargett looked at any variation in stress ratings, plant diameter and seed production between three treatments (No pre-treatment + *N. oryzae*, Slash + *N. Oryzae*, Burn + *N.oryzae*) and Controls.

Plant stress ratings of 0 and 1 made up a vast majority of the assessments of both the Mirani and Gargett trials. This means that plants were rated as either being healthy or exhibiting symptoms of stress that were mild and could not be directly attributed to any one cause. Plants exhibiting a stress rating of 1 could be found individually in a block or in large populations together. Several factors can be contributing to this:

- Rainfall Pattern – Variation in rainfall events and also rate of fall can affect soil moisture levels but also growth patterns over the growing season. Prolonged wet conditions may also adversely affect plant growth.
- Plant Maturity – Both younger and older plants can be more susceptible to adverse seasonal conditions and also common forms of disease.
- Variation in soil nutrient availability - Nutrient deficiencies can reduce plants tolerance to adverse environmental conditions.
- Soil moisture – This can occur across very short distances. Stress can be caused from lack of moisture or too much moisture.
- Genetic variation in individual plants – There is a genetic variation in plant survival and efficiency in a specific environment.
- Common disease – Such as brown rust was present in all the trial sites during the 2013 and 2014 growing seasons. Its distribution was fluid across the block during that time and despite attempts to differentiate it from the stress ratings it would have some impact on the assessments.
- Competition – From other species of grass. Particularly in areas that have been excluded from grazing by cattle.

In order to try and remove these environmental factors from the assessment of potential disease a comparison was made on plants rating 0 and >1. This was done to identify the proportion of healthy plants as well as plants exhibiting symptoms more closely associated with crown rot. Unfortunately, due to the low number of plants recording that level of stress

the results were not significant. There were a smaller population of higher stressed plants across treatments at the Mirani site for the 2014 assessment. These plants were given a score of 2 because they exhibited a lack of seed heads and a yellowing of the leaf sheath consistent with crown rot. The locations of these plants were recorded and later sampled for further assessment of disease, where it was found that the plant culms were relatively healthy and well preserved. *N. oryzae* was never isolated from any of these plants sampled. It was later identified through scrutiny of the uninoculated plant data that the higher stress scores may be attributed to competition from two stoloniferous grass species, that being *Brachiaria humidicola* (Tully grass) and *Digitaria eriantha* (Pangola grass) (Fig. 21). This does provide some insight into a potential lack of competitiveness by GRT with stoloniferous species in an ungrazed environment.



Fig. 21: GRT plants in burn plots excluded from grazing for eighteen months shows an increase in stoloniferous *Digitaria eriantha* from what was 100% GRT.

When looking at both inoculated and uninoculated plants across treatments there is little evidence to show a clear relationship between the mild stress symptoms observed across the trial sites and inoculation with *N. oryzae*. This form of stress was clearly correlated to the previously mentioned environmental factors and physiological differences in GRT.

The biggest influence of plant diameter across the trial work was fire as a pre-treatment. This can be seen in the work at both Gargett and Mirani where plant diameters in the burn treatments were significantly smaller than the other treatments. How those plants grew post-fire varied between the two sites, with the plants at Mirani growing significantly less in the 18 months after fire and those plants in the Gargett site growing slightly more than the controls, although the difference was not significant (Fig. 22 and 23). The contrast in growth between

the two sites is consistent with work done by Vogler (Pers. Comm.), who suggests that the growth response by GRT after fire can be influenced by a number of factors including soil fertility, rainfall, plant maturity and the nature of the fire. Rainfall for the two sites was similar, as was the size and age of the plants and the intensity of the fire treatment. The sites did not vary by soil type being both sandy loams but did vary significantly by soil depth with the Mirani site having shallow soils over a sandstone escarpment. The reduction in growth by plants in the burn treatments at Mirani may have been a result of reduced moisture content in these shallow soils as a result of intense fire and removal of plant biomass. This explains the reduced growth in both inoculated and uninoculated plants in the burn treatments across that site.



Fig. 22: GRT plants in the burn treatments at the Mirani site, which had reduced plant diameters and seed populations to the Gargett site.



Fig. 23: Post Burn treatment at the Gargett site. Note the seed head production.

The “slash” treatments showed similar increases to plant diameter and seed populations across both the Mirani and Gargett sites. Plant diameter was significantly reduced from the controls across both sites, with a slight reduction in seed heads at the Gargett site. This decrease in plant growth affected both inoculated and uninoculated plants and was likely the result of repeated slash treatments. Seed production at the Mirani site was similar to the controls and only slightly less at Gargett. From a control perspective a reduction from nine seed heads to six would not be seen as significant. As with the other treatments, diameter increased across both sites in the period June 2013 to May 2014. Once again no *N.oryzae* was isolated from samples taken at the Mirani site.

Plants from the no pre-treatment + *N.o* only treatment had plant diameter increases consistent with the controls but grew more seed heads at both trial sites. One of the basic understandings of crown rot is that it reduces the plants ability to transport nutrition and water up the stem for seed head production (Smiley et al. 2005). This places further doubt against *N. oryzae* having any effect on GRT.

The Clairview site showed fairly consistent plant growth across the treatments with the exception of the damaged plant treatments. This lower plant growth can be explained by the damage received to the stool pre-treatment. There was no variation in seed production across the treatments. Although no relationship was seen between waterlogging score and stress rating, comparisons of plants rating scores of 2 (extended wet conditions) and 3 (waterlogged) found a reduction in plant growth between the 2013/14 seasons and also a significant reduction in seed production. Plants growing in areas rated 1 for waterlogging were also significantly bigger than those in wetter areas. These results pose more questions regarding the competitiveness of GRT in prolonged wet conditions. Additional work might also look at the viability period for GRT seed in waterlogged areas.

It was observed that although some of the planted GPG plants used at the Clairview site died between the February plant and the June assessment, many survived and improved in

condition. This was also observed at ESP with GPG plants sampled from northern New South Wales recovering in the glass house. This may provide some limited evidence that environmental parameters make a significant contribution to the control effects seen in GPG in NSW.

5.2 Evaluation of the ESP pot trial with GRT

The pot trial was conducted to provide data to support the interpretation of the results from the Mirani field trial and to further the work carried out at RMIT under MLA project B.NBP.0716. Thus, the primary aim was to assess the variation in plant health of potted GRT plants in response to fungal treatments applied to the GRT plants under controlled conditions conducive to the development of fungal infection.

This pot trial tested both *N. oryzae* and two *Fusarium* species (*F. proliferatum*, and *F. chlamydosporum*) identified as having potential to cause disease in GRT (Lawrie, 2014). The trial used a large number of replicates (12), large amounts of inoculum (30 ml; 60 ml), post inoculation procedures to favour the establishment of fungi (bagging plants), controlled glasshouse conditions favourable to fungal growth and took account of any natural background variation in the GRT plants when assessing any disease symptoms (through a stratified randomised allocation of treatments to plants). Despite these procedures there was no data to support that either *N. oryzae*, *F. proliferatum* or *F. chlamydosporum* applied individually or in combination could induce die back in potted GRT plants.

The results from the pot trial are entirely consistent with the findings in the Mirani field trial in which inoculations with *N. oryzae* failed to induce disease symptoms in the GRT plants. The majority of GRT plants exhibited few stress symptoms and *N. oryzae* was never isolated from any of the sampled plants.

In the pot trial a range of measurements were taken to best assess any change in plant health. It was found that increase in dry matter provided a reliable quantifiable measure. Lawrie (2014) also did not find any significant difference in the amount of live biomass present four months after inoculation with *N. oryzae* in two experiments with potted GRT plants.

The results reported here for the pot trial are consistent with research reported by Widmer et al (2006) in which they were unable to induce disease symptoms in *Arundo donax* with spore suspensions of *N. oryzae* applied to the whorl of leaves surrounding the apical tip. Although they had been able to cause infection and death in the flag leaf of some plants under greenhouse and field conditions when *N. oryzae* spores were injected into the flag leaf. Disease induction only under such conditions does not suggest that *N. oryzae* is a practical candidate for the biological control of *A. donax*.

In a recent study investigating the diversity of fungal endophytes in the semi evergreen vine thickets of the southern brigalow belt bioregion (Mapperson, 2014) *Nigrospora oryzae* was the most commonly isolated endophyte from a diverse range of plant taxa. Endophytic fungi are endosymbionts that live within the plant tissues for at least some of the plant life cycle without causing apparent disease (Rodriguez et al, 2008). They are ubiquitous and have been found within all species of plants studied to date. Although most plant/endophyte relationships are not well understood, it is thought that many confer benefits to the host plant, while a small number can induce disease under stress conditions. This research

supports the possibility of *N. oryzae* being a potential beneficial endophyte of GRT rather than a pathogen with potential as a biocontrol agent.

5.3 Evaluation of the efficacy of *Nigrospora oryzae* as a biological control agent for Giant Rat's Tail Grasses.

The Australian Pesticides and Veterinary Medicines Authority (APVMA) details the criteria to which potential biological agents must adhere for regulation. Section 5.8 of the guidelines relates to assessing the efficacy of biological agents and the following must be addressed:

- advantages of the product
- laboratory assays
- field experiments under practical conditions in representative areas of Australia
- Preliminary range finding tests.

The advantages of utilising die-back pathogens to control grass weeds in grass pastures are clear. Die-backs which are host specific would allow for the steady reduction of GRT giving preferred species time to repopulate infested areas of the paddock. Utilising control agents that can be broadcast applied into paddocks as well as introduced through distribution of diseased plant material assists in the efficiency of its distribution.

Defining the range of potential control of a die back for GRT is difficult. In order to achieve this the modes of action and environmental constraints of the die back need to be fully understood. The population distribution of the two species of GRT (*S. pyramidalis* and *S. natalensis*), as well as the other species which make up the group weedy *Sporobolus* grasses, must also be better defined.

The European and Mediterranean Plant Protection Organisation (EPPO) is an intergovernmental organization responsible for European cooperation in plant health. As a Regional Plant Protection Organization, EPPO has produced a large number of standards and publications on plant pests, phytosanitary regulations, and plant protection products. One of these publications, (EPPO 2012), discusses the principles of efficacy evaluation for microbial plant protection products. Many of these principles mirror the guidelines laid out by the APVMA, but include additional insights.

EPPO identifies that “*a statistical effect on the target population relative to the untreated population may not in itself be sufficient justification for the authorisation; control should be of sufficient magnitude to deliver a worthwhile agronomic benefit*”.

Firstly, there has been no consistent statistical evidence showing a pathogenic relationship between *N. oryzae* and GRT. This has been confirmed in both the pot trials and also in the field trial work. Secondly, no evidence of *N. oryzae* was seen post inoculation in the field trials through the isolation work.

Both the APVMA and EPPO detail the requirement for a good understanding of the how the pathogen interacts with the target species. EPPO stipulates that “*where effects are observed the symptoms should be accurately defined*.” The APVMA also requires information on “*the nature and extent of control*” of the agent. Despite the fact that the general symptoms of crown rot are known, these symptoms or others specific to GRT are yet to be witnessed in plants inoculated with *N. oryzae*.

The EPPO document also highlights that “*the applicant should attempt to elucidate the mode of action.*” There is no defined mode of action or changes in the micro environment or interactions with other microorganisms that would explain or contribute to the understanding of how an organism like *N. oryzae*, which is normally endophytic on a broad range of grass species and endemic to many of the regions GRT already infests, might become pathogenic on GRT.

5.4 Findings of the Mackay Giant Rat's Tail Research Development and Extension meeting

Facilitated discussions on management of GRT between research, extension officers, weed contractors, local government officers, biosecurity officers and natural resource management (NRM) bodies was initiated through the GRT RDE meeting held in Mackay in July 2015. Since then, further discussion into the nature of further work into GRT has been ongoing. Below is a brief overview of the topics discussed in the initial meeting, with further recommendations provided later. One of the key findings of these discussions was that there is a large amount of useful information on the topic of GRT management available. This knowledge has been developed through a number of avenues including producer experiences, demonstration sites and detailed trial work. A need exists to gather this information and develop it into useful extension messages. This forms one of the primary recommendations from the project.

5.4.1 Grazing Land Management

A commonplace misconception is that GRT is an unbeatable burden sweeping the landscape unchecked and there is nothing people can do. However, there are options available that will contribute to effective management of GRT although the main issue that seems to hinder people in GRT management is a lack of understanding of the underlying issues that contribute to its spread. Landholders need to ask themselves ‘why’ GRT is having a competitive edge over their preferred pastures, rather than just treating the weed in exclusion. This is important if a management plan is to be developed.

There has been some Grazing Land Management research (unpublished) in which the effect of stocking rate on the rate of GRT spread was investigated. A lighter stocking pressure resulted in a reduction in the spread of GRT due to the higher competitiveness of other grasses. However, in low rainfall years grazing pressure is not managed well by many beef producers, giving GRT a competitive edge in establishment once the season does break.

When the impact of different grazing strategies on GRT is compared, there appears to be little difference between rotational, cell grazing and conservative grazing. The principal requirement for maintaining a competitive pasture is some form of spelling strategy during the year (five to seven weeks minimum).

The common practice by graziers to hold onto cattle too long in the hope of a response in the season increases the threat of quick establishment of GRT once conditions become favourable for growth. This practice in many cases can be profitable once the season breaks and demand grows for restocking cattle. Unfortunately, it is generally at the expense of land condition.

There are pasture management implications with the use of the selective herbicide, flupropanate. The use of flupropanate as a selective on GRT appears to have a “chemotherapy” type effect on the response of the more favourable grasses to the wet season and their subsequent growth and resilience during the following year. The period of this effect lasts longer than the required withholding period of the chemical (four months). Therefore, it is recommended that pasture that has been treated with flupropanate should be spelled for at least three to six months otherwise this pasture may not recover. A more conservative view suggests a full 12 months of spelling, where possible, to give a pasture the best opportunity to recover. It appears that poor grazing practices post treatment with flupropanate will result in the rapid return to large populations of GRT.

In general the effectiveness of grazing land management extension across the regions with high levels of GRT tends to be low. The typical landholders in these regions are smaller peri-urban blocks with off-farm income. These landholders are time poor and generally lack a background in agriculture. Consequently, they have less time to manage their grazing lands and are potentially less capable of monitoring stock densities and controlling weeds. A new approach needs to be taken with this demographic.

There is a marked decline in the palatability of GRT once plants transition from a vegetative state to a reproductive state. Some preliminary work by David Officer of NSW DPI showed that GRT kept in a vegetative state through slashing and burning exhibited much higher crude protein and digestibility. This suggests better palatability and utilisation.

In addition, anecdotal evidence suggests that the introduction of a nitrogen source to the ruminant diet through either feeding legumes or urea will also increase the utilisation of GRT. Unfortunately there are issues with long term survivability of legumes in GRT pastures. The only long term legumes to survive are tree legumes such as *Leucaena*. The rapid transition of GRT from a palatable vegetative state to an unpalatable reproductive state means that a reasonable response by cattle would occur much earlier in the year than with normal pastures if urea was fed.

5.4.2 Soil Health/Nutrition

Managing soil health and nutrition has been identified as a critical factor in controlling all forms of invasive weeds and also in maintaining good productive pastures. Over the past thirty years the application of fertiliser has declined as associated costs have increased. There is still a need for the nutritional support of sown grass species, particularly with nitrogen. It is not critical whether this is done through fertiliser application or legume establishment. However, in some of the wetter coastal areas long term successful legume establishment can be difficult.

GRT has proven to be very competitive on a range of soil types; however it grows particularly well on poor soils or soils that have been run-down. Through being aware of and promoting soil health and nutrition producers can have a significant effect on weed control. Maintaining good soil health and nutrition is a crucial part of a holistic plan that includes selecting competitive pasture species, carefully monitoring grazing and the timely control of weed incursions, with an overarching system of effective biosecurity.

It is also important to establish soil nutrition thresholds that can support better competitiveness of pastures to GRT. There is a potential to look at the effect of low level fertiliser applications on GRT control/spread.

5.4.3 Social implications to GRT Management

There are a variety of reasons why people would want to manage GRT on their properties. These reasons vary but are not specific to location and property size. There are a number of motivators:

- Maximising animal production through maintenance of 3P species
- Managing environmental diversity on the property
- Reducing risk of spread to other properties
- Compliance to control of Class 2 weed
- Preserving the overall visual aesthetic of the property.

All landholders would identify with these motivators in some sense but obviously how they prioritise them differs. Smaller scale landholders may have a lesser focus on animal production and be more motivated in preserving the visual aesthetics of their property. For larger more viable properties it would be important to control GRT from a production sense. Either way, it is important that these motivators be identified when planning extension strategies as regionally the proportion of small and large scale operations will differ.

A better understanding of the regional demographics may change the way extension approaches weed control. For example, Mackay Whitsunday has over 2200 registered PICs (property identification codes) located in a land area that is 0.5% of the Queensland landmass. Sixty-nine percent of these operations consist of less than 100 head and 36% are smaller than 50 ha. This small operation size dictates that the primary means of income will be off-farm revenue. Typically, the approach by the Queensland Department of Agriculture and Fisheries (DAF) to GRT extension has revolved around the negative effects GRT has on beef production. This approach may not resonate with the priorities and motivations of landholders of smaller land parcels.

Overall, extension efforts in GRT control need to assist landholders to gain a better understanding of what influences the spread of GRT or any other weeds on their land parcels. Although it is important to assist with developing a management plan, it is of critical importance that landholders identify why weeds are establishing on their properties in the first place.

Other issues arise when dealing with problem weeds in coastal areas, one being absentee ownership. As coastal land values create opportunity for investment, many blocks with GRT infestation are being bought by people who are not present to manage them. This gives further opportunity for GRT to dominate and creates weed pressure issues for adjoining properties.

5.4.4 The Impacts of Fire

Fire is not recommended as a management tool for GRT. There are several reasons for this:

- GRT is fire resistant. When plants come into contact with fire the plants with a larger diameter tussock burn but are replaced by a higher density of plants with smaller diameters, thus increasing the total plant population.
- Fire has been seen to increase seeding rates of GRT by 15-40%.
- Seedlings are particularly resistant to fire. Work done by Vogler (2002) has shown that even small seedlings, moisture stressed and non-moisture stressed and as small as 0.5-1cm basal diameter are able to survive fires with fuel loads of 1800 and 3600 kg/ha respectively. Seedlings as young as 1 week old (1 mm basal diameter and 1 cm tall) were able to survive a fire with a fuel load of 1200 kg/ha.
- Post fire GRT plants tend to be the first to reach their reproductive stage as compared to other pasture species.
- GRT fires tend to be very hot, which can kill off other species present in the pasture, especially desirable pasture species.
- GRT fires are dangerous and hard to control.
- Although fire will destroy a proportion of seed reserve, this tends to have little effect in the long term, as the overall population is so large and much of it is protected by the insulative ability of the soil.

However, fire can be utilised effectively in GRT control as one step in a process to re-establish pasture. What happens 'next' after fire is very important. Fire will reduce the proportion of seed in the soil and also removes the biomass that can make working the ground difficult. It is important that the intensity of the fire in a GRT infested paddock be reduced to ensure other species survive. Burning therefore, is recommended only after a significant rainfall event at the end of the year.

Burning GRT late in the growing season (early autumn) can also extend the period of better feed quality. However, this practice is not recommended to be used repeatedly. Removing biomass through fire year after year can have detrimental effects on soil health.

5.4.5 Grass Selection

Grass mixes for re-establishing into GRT infested land need to address a number of criteria in order to be successful:

- Any mix used needs to contain a fast establishing pasture species that can provide competitiveness to GRT seedlings immediately.
- Where possible the mix must also incorporate a stoloniferous grass that can slowly fill in behind.
- Overly palatable species of grass tend to not be long term options for control of GRT as selective grazing places too much pressure on the better species.
- GRT is least competitive as a seedling, therefore grasses that can produce adequate biomass for shading and organic ground cover are preferable under well managed conditions.

Pre-preparation of good seed beds and minimising seed populations of GRT through fire and herbicide applications are critical to getting the best establishment. Once a higher

population of desirable grasses are present further selective control of GRT through spot spraying and fluproponate can be utilised.

5.4.6 Biosecurity

One of the biggest challenges confronting the Queensland beef industry at present is controlling the spread of GRT from the wetter coastal areas to the temperate rangeland areas in the West. There may be a need to develop a separate set of control measures for the regions west of the great divide due to different soil fertility, ground cover and competitive pasture options.

Although the optimal rainfall requirement for GRT is above 700mm per annum, it has the capacity to establish in drier areas during the years of good rainfall. Once established, GRT would be extremely difficult to eradicate in these areas due to reduced levels of ground cover and GRT's high seeding rates.

Biosecurity measures stand at the forefront of any good weed management plan; it is the easiest and cheapest form of control. It is imperative that strategies be put in place to reduce the risk of GRT establishment. Historically the ability of landholders to implement effective biosecurity plans on their properties is below standard. Many operations lack a formal process for inducting cattle and machinery onto their properties. In addition, there is room for improvement in how property owners negotiate access and monitor movements of outside parties on their land.

Cattle moving from coastal areas can be vectors for GRT with seed present in both gut contents and on their coats. Because of the high seeding rates of GRT, even if a high percentage of seed is destroyed by digestion, enough can still pass through into dung which will then provide an excellent seed bed for germination. Cattle being inducted onto the property need a minimum of seven days containment in a well monitored area in order to ensure decontamination.

Weed contamination of hay is also contributing to an increase in outbreaks of GRT. In dry years hay sources can be limited and hygiene compromised. Additionally, very few risk reduction practices are implemented to ensure that if hay is contaminated that a potential infestation can be identified, isolated and controlled. Currently, under Section 45 of the Land Protection Act (2002) it is an offence to knowingly distribute hay which has been contaminated with a Class 2 weed. The current Class 2 weeds which are prescribed in the act are as follows:

- American Rat's Tail Grass
- Giant Rat's Tail Grass
- Parramatta Grass
- Giant Parramatta Grass
- Parthenium
- Prickly Acacia

Under the new Biosecurity Act (2014), the landholder is responsible for developing their own biosecurity plan for their landholding. This has advantages and disadvantages for the industry. It places more power in the hands of the landholder for dealing with the management of access to their properties, but on the other hand it only heightens the

requirement for a detailed biosecurity plan specific to that property. Once again it is this formal documentation which has always been underutilised by the industry.

A significant cultural change is required by industry to become more concise in their management of biosecurity on-property. This change needs to be seen right through the industry and needs to include hay producers and saleyards etc. Producers in extensive grazing areas will be the most affected by the spread of GRT. There is an opportunity to provide wide scale extension to these producers to implement effective biosecurity plans now to reduce the potential of weed spread.

5.4.7 Chemical Management

In terms of the use of chemicals in the control of GRT there are two options. Those options are the use of knock down chemicals (i.e. Round-up) and the selective herbicide Fluproponate which is available in both liquid and granular form.

There are a number of management considerations in the use of fluproponate. These considerations are important in ensuring the chemical achieves a desirable kill. With fluproponate having a high application cost (varying between \$150-\$250 per hectare) and a significant withholding period it is important that it be used effectively.

Fluproponate is a highly soluble chemical which is prone to movement within the soil horizons. Three measurements critical to a residual effect and subsequent efficacy of fluproponate are:

- Moisture – Yearly rainfall and also water holding capacity (soil structure and depth)
- pH (Neutral to alkaline soils vary)
- Organic matter

Soils with higher clay content tend to hold onto fluproponate better and so the efficacy and residual effect is greater than in sandy or loamy soils. Lighter soils will lose this chemical from the soil surface faster meaning less protection from seedling germination. Even on better soils with good moisture and structure there is little evidence to suggest the residual period is longer than six months. High rainfall years can reduce this period further regardless of soil environment.

The efficiency of control with the selective herbicide is also dependant on follow-up treatments. It is important that when budgeting for GRT control with fluproponate that follow-up work also be accounted for in order to accomplish effective control in high infestation areas.

There is anecdotal evidence to suggest that treatment with fire after application of fluproponate can adversely affect the result. This may be a result of fire increasing the loss of the active that may be present within the tussock on the leaf or stem surface. A critical element to this occurring would be the lack of a rainfall event between application of fluproponate and a fire. In comparison, a fire event prior to the use of fluproponate may increase the efficiency of GRT control if timed well with seasonal conditions. This is will occur if fresh actively growing GRT plants are present to take up this selective herbicide.

The withholding period for paddocks treated with fluproponate is 40 days, with a 14 day hiatus between the grazing of treated pastures and slaughter. In soils where there is the

potential for fluproponate to remain longer the current withholding period may not be long enough, although no consistent evidence has supported this.

To date there has not been any work looking at the potential effect of fluproponate on ecosystems further downstream nor its presence in soil water tables. This represents quite a large knowledge gap which has the potential to significantly affect the use of this chemical in the future, particularly in reef catchment areas.

The collateral effect of fluproponate on other grasses varies with rate of application, seasonal conditions, soil type and species. A potential opportunity exists to screen for general fluproponate resistance across these grass species.

Research has been conducted into fluproponate resistance in serrated tussock grasses in New South Wales. Serrated tussock grass resistant to fluproponate was first identified on a Victorian property in 2002 and has since been confirmed in several locations from Armidale and Goulburn in NSW to Diggers Rest and the Rowsley Valley in Victoria. There is anecdotal evidence that some stands of GRT may also show fluproponate resistance, although this has not yet been quantified.

There is anecdotal evidence that utilising 'pasture topping' or light applications of glyphosate at the end of the growth phase may increase the palatability and utilisation of weedy sporobolous by cattle. Further research is needed to understand whether this technique produces an economically viable outcome.

Results from pot trials conducted by Vogler (2010) suggest that the herbicide Atrazine may also be an effective chemical for controlling sporobolous species. Currently however the chemical is not registered for use on sporobolous species. Atrazine is also extremely mobile depending on rainfall and issues with the impact on reef water quality could soon see Atrazine removed as an approved chemical for any application.

Another barrier to effective chemical management is boom spray efficiency. Many landholders do not take the necessary steps in calibrating their boom sprayer for optimal application of chemicals. In undulating country or variable soil types, an even rate of application of the selective chemical can be very difficult, even for skilled operators. Spray overlap is another issue although this can be reduced through the use of marking dyes and GPS.

5.4.8 Seed Reduction

Where there are dense stands of GRT there are huge seed banks present in the soil (up to 18000 seeds/m²). In comparison, many common pasture species such as Rhodes grass (*Chloris gayana*) can only produce soil seed banks of around 180 seeds/m². Therefore, it may not be advisable to expend too much time or resources trying to reduce the soil seed bank of GRT, with the exception of longer term options such as cropping rotation. It could be more efficient to establish a competitive pasture rather than trying to prevent new generations of GRT from establishing and setting seed. In higher rainfall areas, there is value in allowing GRT seedlings to germinate then removing them with a knockdown herbicide. In addition, enabling a healthier microbial environment through grazing management and soil nutrition may reduce GRT seed survivability.

5.5 Implications to Industry

The practical implications for industry are that work into bio-controls for GRT is ongoing. The resounding conclusion that has been drawn from the results of the field and pot trial has been that there is no pathogenic relationship between *N. oryzae* and GRT. It is important that this outcome be integrated into the extension message on GRT to landholders, as there is some evidence that landholders may opt to hold off on implementing other forms of control if they are of the understanding that a biocontrol may be available in the short term.

The draft extension message for GRT that has been generated from this work is that developing effective bio-controls for managing a weedy grass species in a grass pasture will always be very difficult. A line needs to be drawn between developing a control that is effective in the reduction of the target species without jeopardising off target species (Palmer et al. 2008). Problem grasses like GRT tend to have an ability to colonise and spread in a number of bio-regions, which adds to the difficulty of finding a control that will operate in all of those areas.

5.6 Meeting the Objectives

5.6.1 Assessment of the potential of *Nigrospora oryzae* as a fungal biological control agent for Giant Rat's Tail (GRT) (*S. pyramidalis* and *S. natalensis*).

Work conducted to meet Objective 1 included:

- Development, maintenance and assessment of three field trials within the Mackay-Whitsunday region. One located at Gargett, one at Mirani and one at Clairview.
- Development, maintenance and assessment of a two stage pot trial at the Ecosciences Precinct, Dutton Park Brisbane.

The two field trials (Gargett and Mirani) looked at comparing plant diameter, seed head production and stress between plants inoculated with the fungus *N. oryzae* and plants not inoculated (Controls).

The trial at Clairview compared inoculation techniques and efficiency of disease establishment.

The pot trial at ESP looked to re-evaluate Objective 1 in a controlled environment. Plants were given high doses of inoculation under ideal conditions. The pot trial also included combined treatments of *N. Oryzae* with two *Fusarium* (*F. proliferatum* and *F. chlamydosporum*) to see whether combinations of these fungi helped to illicit disease.

Assessment of field trial and pot trial data have concluded that there is no consistent evidence that shows a pathogenic relationship between *Nigrospora oryzae* and GRT.

5.6.1 Investigate the relationship between pre-inoculation treatments (burning and slashing) and rate of disease spread.

Treatments incorporated into the field trials at Gargett and Mirani included the use of burning and slashing prior to inoculation with *N. oryzae*. This was further assessed in the four to eight month period of the pot trial where plants were split into three treatment groups

(inoculation only, burn and slash) before being re-inoculated. Due to the fact that no disease symptoms or consistent reductions in plants growth were witnessed, there can be no linkages made between pre-treatment (burning and slashing) and disease spread. Monitoring how plants responded to the pre-treatments has provided some insights that contribute to the grazing land management principles that address Objective 3.

5.6.2 Improved knowledge and understanding of grazing land management principles for control of GRT and promotion of productive pastures.

There have been multiple avenues of work that have contributed to this objective. Firstly, the insights collated from the field sites shows the interaction of GRT to a number of environmental and management factors such as burning, slashing, waterlogging and competition from other grass species. All of which has been highlighted through the discussions. This is a somewhat smaller contribution to the information and insights developed through the GRT RDE meeting held in Mackay. The collation of information from a number of different fields within weeds research and extension has meant the development of document that includes all aspects of management. In addition to this, the discussion helped to generate a number of key recommendations for ongoing research into not only GRT but weedy *Sporobolus* grasses as a whole.

5.6.3 Draft guidelines for use of *Nigrospora oryzae*, if it proves an effective and practical control option.

Due to the fact that *N. oryzae* did not show any signs of being a potential bio-control for GRT, no guidelines have been developed for its use.

6 Conclusions

The work carried out under this project failed to show any pathogenic relationship between Giant Rat's Tail grass and *Nigrospora oryzae* or *Fusarium proliferatum* and *Fusarium chlamydosporum*. A number of recommendations have been developed for further work into biological control agents including fungi and insects.

Effective management of GRT starts with good biosecurity procedures by the landholder. Work over the last two decades has shown that good quarantine policies on vehicles and stock entering the property are critical to reducing the risk of infestation. The recently implemented Biosecurity Act 2014 details that it is the obligation of the landholder to manage and mitigate biosecurity risks to their land parcels.

For those properties that already have a significant presence of GRT or any of the weedy *Sporobolus* species, implementing good grazing land management practices that promote healthy and competitive pasture is critical.

The widespread distribution of GRT and its dense growth, often in locations with limited vehicular access, along with the issues associated with control through the use of selective herbicides or management practices such as burning, make this weed an ideal target for bio-control agents, especially agents that can be introduced and left to multiply and spread under optimal conditions. It is unfortunate that *N. oryzae* has not proven to be a strong die back pathogen. However, previous research (Palmer 2008) on a eurytomid wasp (*Tetramesa sp.*) should be revisited, along with a targeted search for potential endemic fungal pathogens that could be formulated and applied as biocontrol agents.

One important outcome of the workshop held in Mackay was the re-focussing of GRT management and control as a priority for a wide range of stakeholders. Subsequently, Biosecurity Queensland has now taken the lead in organising several agencies to submit a bid through Rural Industries Research and Development Corporation to access funds from the new Federal Government "R&D for Profit" grant funding program for a comprehensive GRT management and control program.

7 Recommendations

7.1 Recommendations for future field work on fungal biocontrols for GRT

One criticism of the trial design would be the absence of negative controls (burn and slash without inoculation). This would have helped to further remove the environmental effect of the pre-treatment. It was initially hypothesised that assessing disease symptoms in the treatment blocks and their absence in the controls would be a clear comparison. This has not been the case as no defining disease symptoms have been witnessed. This also highlights the need for future biocontrol assessments to focus on measurable parameters such as seed heads, dry matter yield and plant diameter and/or circumference. Attempting to identify disease symptoms may continue to be difficult in GRT due to its resilience. Also sampling of treated and untreated plants for laboratory scrutiny (i.e. plant dissection and isolations) must be included.

Looking at the variation that was found within the measurable data (plant diameter, seed heads) across treatments, it is recommended that future trial work utilise this statistical analysis in combination with long term species ground cover trends. Where possible replication should be maximised in GRT trials in order to try and smooth out the variation that can exist.

An opportunity exists to carry out a large scale survey of GRT infested pastures for other potential die-back pathogens. This work should be conducted in tandem with a study of the species and genetic diversity of weedy *Sporobolus* grasses.

7.2 Review of previous work on GRT control

One of the critical recommendations of this report highlighted through the GRT RDE meeting was the need for a thorough review of work previously carried out into GRT management. It was identified that there is already a wealth of information that can be utilised by producers to form an effective GRT management plan. Another important aspect of identifying this work is to circumvent future research efforts replicating what has already been done previously.

7.3 Region demographic study

There is an opportunity to define what the break-up of land parcel size, land use and producer motivations are by region. A better understanding of the ratios of peri-urban, semi-commercial and large scale enterprises in each region will help to better define a strategic pathway for control of GRT in those areas. This might also help in co-ordinating the work of local and state government employees who are involved in different aspects of GRT control and who also have differing requirements in their roles (i.e. extension, compliance).

7.4 Utilising molecular tools to better understand the genetic diversity of weedy *Sporobolus* grasses

Sampling of weedy and native *Sporobolus* species can be taken to identify species accurately through DNA microsatellite work. This will provide accurate information on the population distributions across the state of the various species. This is crucial to the

development of targeted biocontrol agents as well as developing effective management plans.

7.5 Skilling government staff

There is an opportunity to upskill DAF Biosecurity and FutureBeef staff in the identification and control of GRT through technical workshops for biosecurity on effective management practices. As GRT moves to new areas it is important that all departmental officers have the ability to recognise GRT and understand how to manage it. Therefore, any workshop skilling should be extended to local government officers and landcare agents.

7.6 Giant Rat's Tail Control Producer Demonstration Site

There is scope for a large scale medium to long term funded (>5 year) PDS looking at a variety of treatment options for GRT control. This PDS could involve the use of granular and liquid forms of the herbicide Task Force®, burning options, cropping, competitive pasture and fertiliser treatments in a statistically robust, replicated trial.

7.7 GRT utilisation Producer Demonstration Site

There is anecdotal evidence to suggest a significant increase in the utilisation of GRT if additional protein dietary support is available to maintain rumen efficiency. There is potential to develop a PDS to examine the effect of urea supplementation and/or a significant legume presence on the increase in utilisation of GRT.

7.8 Defining the issues with broadacre Flupropanate use

Work could be conducted to better define a number of issues relating to flupropanate use, including:

- Managing application better – achieving effective calibration, timing of application, and options for application (boom, helicopter, aeroplane).
- Understanding the factors that influence residual effect – pH, organic matter, soil moisture.
- Cost effectiveness and payback period for a number of levels of utilisation and repeat applications of flupropanate across a number of land types.

7.9 Accelerating uptake of good on-farm biosecurity

Investment into extension to assist producers in strategic areas to develop effective, practical biosecurity plans. In terms of investment this could be seen as the most effective investment in GRT control as prevention is the best form of control for GRT.

7.10 Economics of GRT control

To attract more investment into GRT control better economic definitions of the impacts of GRT infestation across a number of different land types are needed. These economics might also guide the parameters for control option particularly on low fertility soils where cost effective options may be limited.

7.11 Fluproponate resistance in sown pasture

A better understanding of the fluproponate tolerance thresholds of a variety of pasture species needs to be considered in the context of pasture selection. Off target control is a big issue in the use of the selective for GRT. Competitive species of grasses that have a higher tolerance to Fluproponate would be of great use to the industry.

7.12 Biodiversity impacts of GRT

Since the notification of GRT as a weed of national significance, the potential long term impacts of this weed have been linked to primary industries. There has been no research conducted into the effects of GRT on native biodiversity. Anecdotal evidence suggests a marked decline in species diversity in areas invaded by GRT which includes both flora and fauna.

7.13 Sediment management impacts of GRT

In areas where there is a significant GRT population, there is potential for ground cover to be severely reduced due to the overutilization of the preferred pasture species. Many of the larger areas of GRT infestation are in the Great Barrier Reef catchments. Therefore, investigations to identify if GRT contributes to issues impacting on run-off and reef quality should be considered. A potential method to investigate this could be through the use of rainfall simulators.

7.14 Assessing the chemical spectrum for controls

As noted earlier in this report initial work by Vogler has found that Atrazine has potential for use in controlling GRT. Further work needs to be done to investigate whether any other herbicides currently available might have potential for GRT control.

7.15 Re-assessment of Stem Wasp (*Tetramesa sp.*) as potential biocontrol

In 2004, the Department of Natural Resources and Mines, formerly the Department of Natural Resources, Mines and Water, undertook an assessment on the potential of leaf smut *Ustilago sporobli-indici* and Stem Wasp *Tetramesa sp.* as biological controls for GRT. Unfortunately, the leaf smut was able to infest a number of *Sporobolous* species including a number of native species. The stem wasp was abandoned after it proved too difficult to raise in a controlled environment. Since then there has been significant improvements in the techniques used to raise insects in a laboratory environment. This means that there could be potential for a controlled re-assessment of the stem wasp as a bio-control option.

7.16 Assessing pasture species for weedy competitiveness

There is potential to draw together a list of approved competitive pasture species by region and investigate if these species can fulfil criteria to be approved for planting as a competitive species to GRT.

7.17 Updating the Weedy Sporobolous Management Guide

The publication 'Weedy Sporobolous Grasses' was republished in 2007; it should be updated to include more current information. Some potential edits include;

- Remove the section on the background of the origin of this weed. It may be more important to note why GRT is an issue; especially that it is a symptom of land condition problems.
- Include grazing strategies to improve GRT competitiveness.
- Include strategies for better management of soil health to provide better nutrition for competitive pastures.

7.18 Targeting Low level fertiliser regimes

GRT has the capacity to become dominant on soils that cannot provide adequate nutrition to more productive species of grasses. It would be useful to have a better understanding of what sort of yearly fertiliser inputs might support significant competitiveness by sown species, particularly in marginal areas where high inputs are un-economical.

7.19 Utilising Spatial and Reflectance technology

Multi-spectral cameras are an effective tool for measuring species composition and yield in cropping situations. Although this technology is not yet well utilised in grazing systems it has the potential to become a very useful tool in the long term monitoring of research trials looking at controlling problem weeds like GRT.

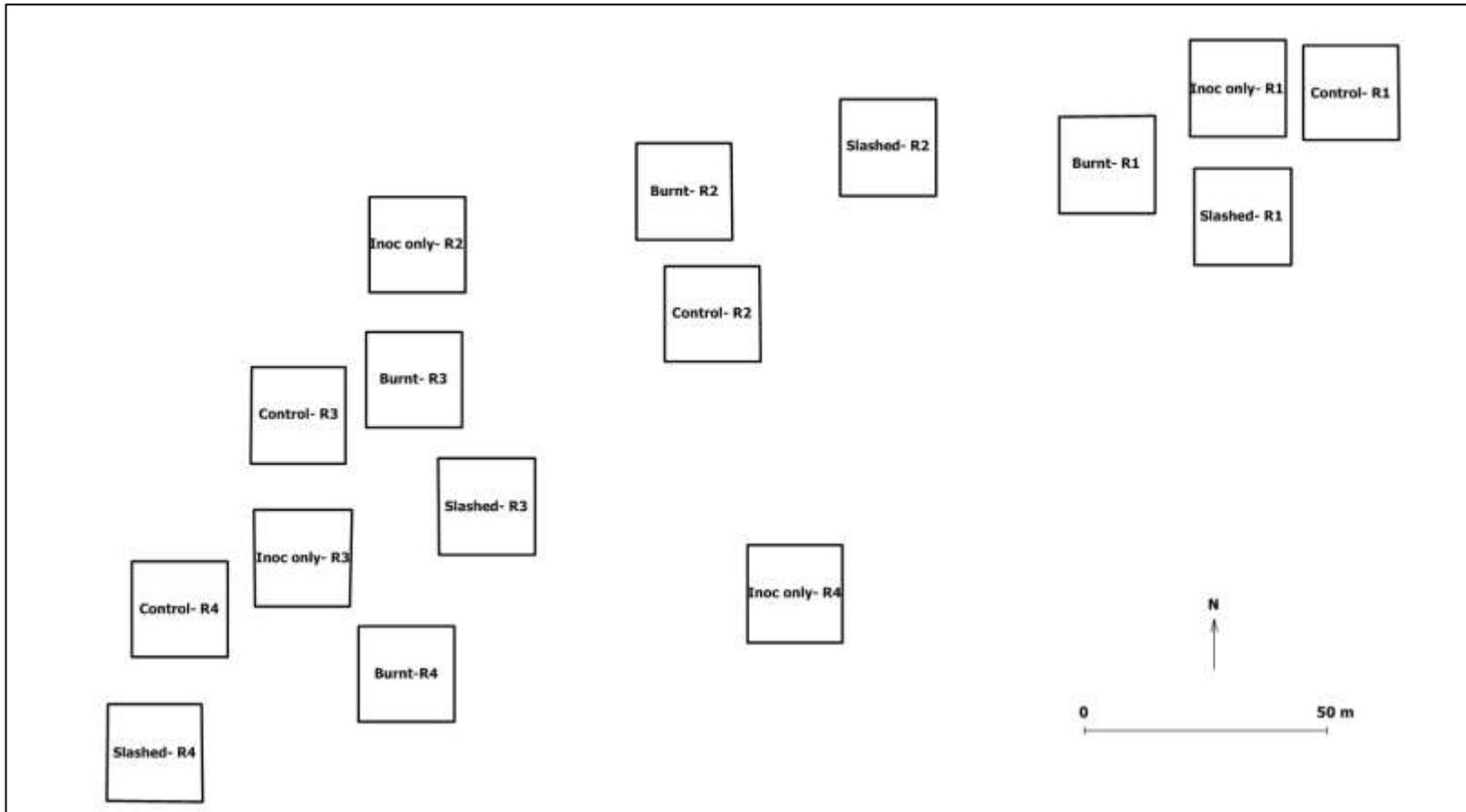
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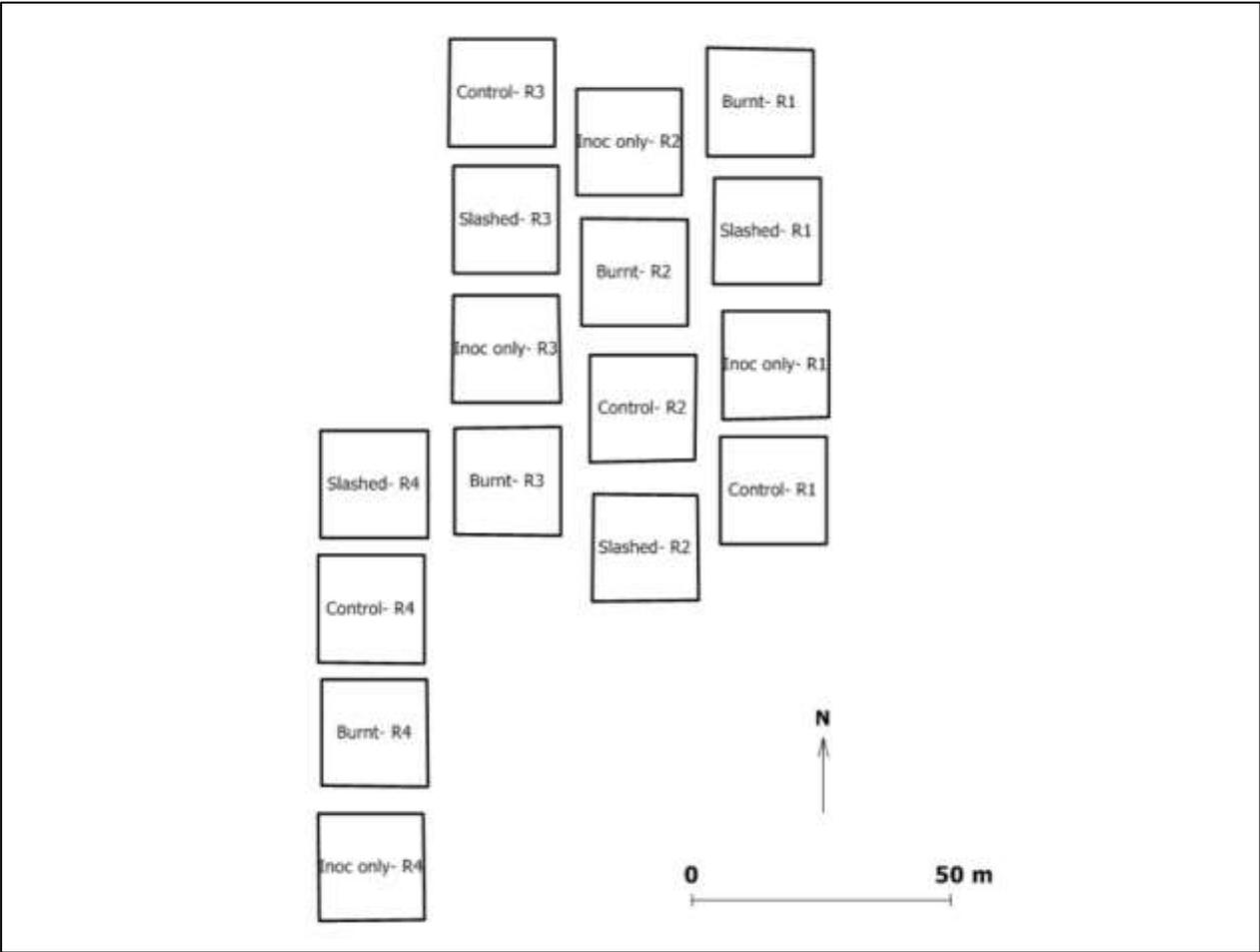
9 Appendix

9.1 Appendix A – Trial layout “Mirani site”



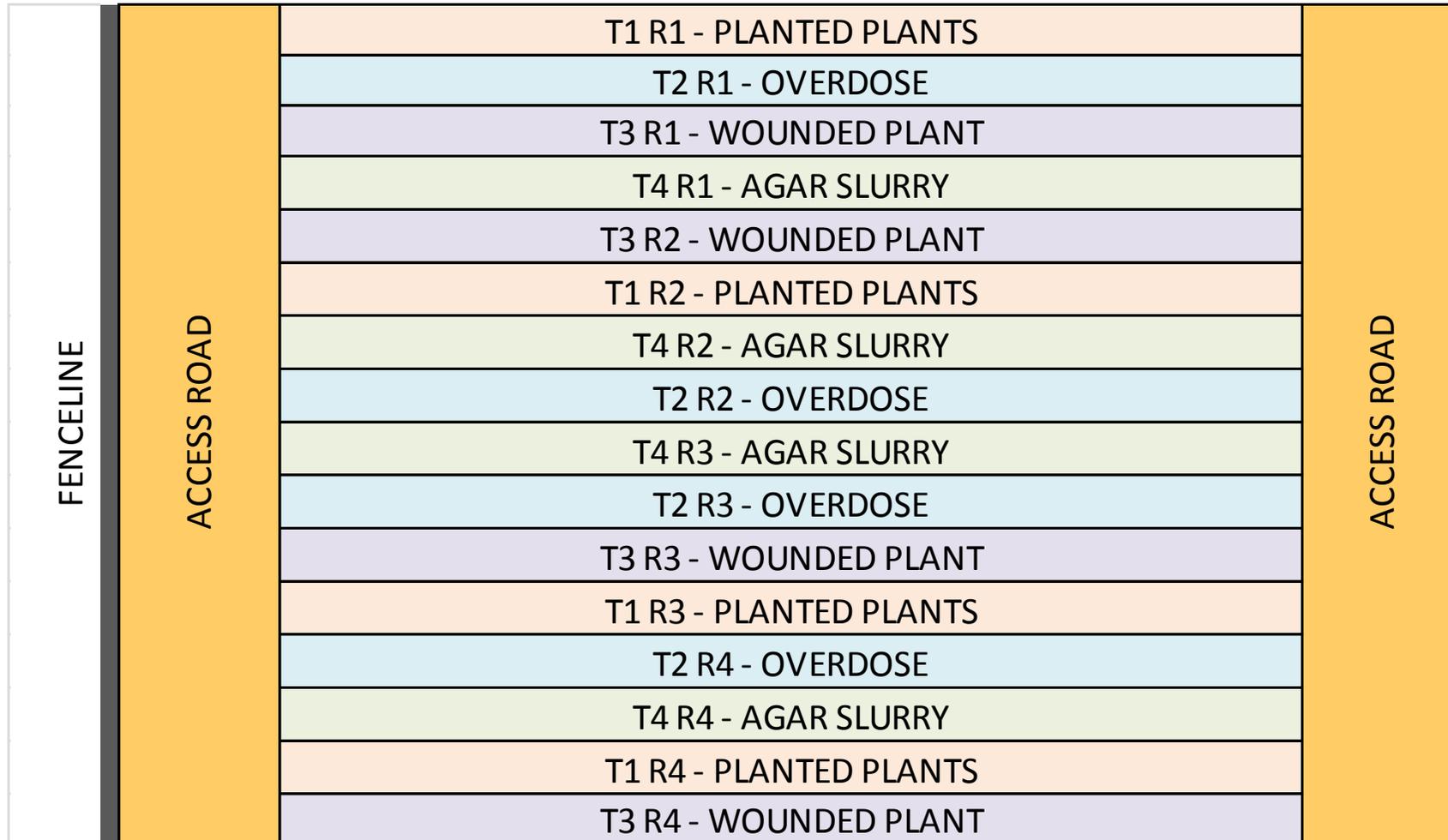


9.2 Appendix B – Trial layout “Gargett site”





9.4 Appendix D – Trial layout Clairview Site



9.5 Appendix E- Isolation work Mirani Site

Sample number	Treatment Type	Plant #	General observations	General root health	Evidence of nematodes	Results (NG = no growth; T = Trichoderma; UD = undetermined; Fus = Fusarium sp.; Rhizoc = Rhizoctonia; R = Rhizopus; Pen = Penicillium) All of 15 pieces of crown
1	Slash - R4	MS4A	Good – 1 plant dead. No actively dying plants. Living plants healthy.	Good	No	10 NG; 1T; 4 UD
2	Slash – R4	MS4B	Good – multiple dead and healthy plants. Some rot/decay on inner plants	Good	No	4 NG; 1 Fus (peach coloured, short conidiophore, monophialide); 2 T; 1 Rhizoc; 2 UD; 3 Pen
3	Slash – R4	MS4C	Good – plants small but appear healthy	Good	No	6 NG; 1 T; 8 UD
4	Slash R4	MS4D	Good - plants small, healthy, some dead plants	Good	No	9 NG; 1 T; 1 R; 2 UD; 3 UD (Brown/orange, no spores, very strong, frequent hyphal swellings)
5	Slash R1	MS1A	Good – generally small plants; some outer rot – internals look good. 1 plant with signs of internal rot	Fair – some small necrotic regions	Very low level – picture taken	9 NG; 1 T; 1 R; 4 UD (red – Cladosporium-like spores – strongly raised red and white hyphal growth); 1 UD (black – Fluffy black mycelial growth with helminthosporium-like spores).
6	Slash R1	MS1B	Good some dead plants. Live plants appear healthy, finer root hairs than other samples so far.	Good	No	14 NG; 1 T
7	Slash R1	MS1C	Good- Some dead plants, live plants appear healthy	Good	No	15 NG
8	Slash R1	MS1D	Good – Some dead plants. Live plants healthy.	Strong roots. New growth present	?	8 NG, 5 T. 2 UD
9	Slash R2	MS2A	Plants small but appear healthy	Good. Lots of fine root hairs.	No	7 NG; 1 T; 2 UD (white – very white and fluffy; fast growing), 5 UD (Red – as per #5)
10	Slash R2	MS2B	Fair, some dead plants. Live plants healthy	Fair, some small necrotic areas.	No	13 NG; 2 R
11	Slash R2	MS2C	Good. Plants small but healthy	Fair; small root mass	No	9 UG, 5 UD (white – flat radiating outward, but not very fluffy, no spores), 1 Pen
12	Slash R2	MS2D	Fair. 2 dead plants. Healthy plants good.	Fair – small mass	No	4 NG, 4 T, 2 UD; 5 UD (red – as per #5)
13	Burn R1	MB1A	Good. Small plants. Good root mass	Good	No	9 NG, 5 Pen, 1 R
14	Burn R1	MB1B	Good. Small plants. Healthy	Good	No	4 NG, 1 T, 1 R, 1 Pen, 5 Fus
15	Burn R1	MB1C	Good. Some dead plants. Small and healthy live plants.	Fair, some	No	13 NG, 1 UD (red), 1 UD

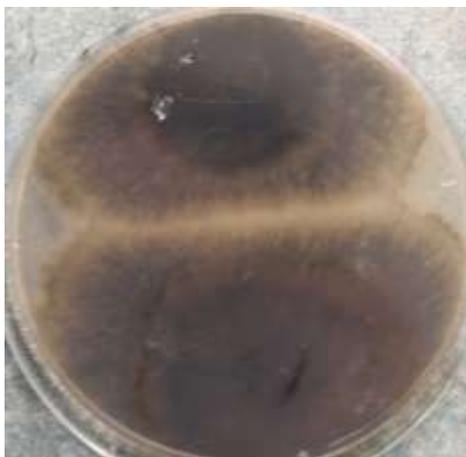
Sample number	Treatment Type	Plant #	General observations	General root health	Evidence of nematodes	Results (NG = no growth; T = Trichoderma; UD = undetermined; Fus = Fusarium sp.; Rhizoc = Rhizoctonia; R = Rhizopus; Pen = Penicillium) All of 15 pieces of crown
				dieback		
16	Burn R1	MB1D	Good. One dead plant. Small and healthy.	Good	No	8 NG, 1 R, 2 UD, 4 Fus (light purple – contaminated)
17	Slash R3	MS3A	Good, healthy large plants.	Good some minor necrotic regions	No	NG 13, 1 Pen, 1 UD
18	Slash R3	MS3B	Fair, some dead plants, healthy plants good.	Fair some necrosis	No	NG 12, 2 Pen, 1 T
19	Slash R3	MS3C	Good, some dead plants, healthy plants good, small and large plants. Some Nigrospora observed on dead plant material (old leaves).	Good	No	14 NG, 1 Fus (Peach – slightly darker than #2, short conidiophores, mono-phialide)
20	Slash R3	MS3D	Good, Strong healthy plants, 2 dead plants.	Good	No	13 NG, 1 R, 1 UD (red – as per #5)
21	Burn R2	MB2A	Fair, some dead plants. Healthy plants good.	Good, some small necrotic areas	No	14 NG, 1 Fus (as per #19)
22	Burn R2	MB2B	Fair, small plants healthy. Some dead plants with regrowth	Good	No	5 NG, 6 UD (red) 3 Pen
23	Burn R2	MB2C	Good, plants small, but appear healthy	Good	No	14 NG, 1 UD
24	Burn R2	MB2D	Good. One dead plant. Healthy ones good	Good	No	14 NG, 1 UD
25	Burn R3	MB3A	Good. Some dead plants. Live plants healthy.	Good	No	8 NG, 1 UD (red) 2 Pen, 3 UD
26	Burn R3	MB3B	Fair, some dead plants, live plants healthy, some crown rot (mechanical damage?)	Good	No	3 NG, 8 T, 2 R, 2 UD
27	Burn R3	MB3C	Good, some dead plants. Live plants healthy.	Fair – Some necrotic regions.	No	6 NG, 4 T, 1 Pen, 4 UD, 2 R.
28	Burn R3	MB3D	Good, some dead plants. Live plants small, healthy.	Good strong growth	No	10 NG, 1 R, 2 Pen, 1 T, 1 UD
29	Burn R4	MB4A	Fair, some dead plants. Small plants, healthy.	Good	No	5 NG, 1 Pen, 1 R, 2 T, 1 UD (Red), 4 UD
30	Burn R4	MB4B	Fair, some dead plants. Live plants small and healthy.	Good	No	10 NG, 2 Pen, 3 UD, 1 Ud (red)
31	Burn R4	MB4C	Good. Some dead plants, many small, healthy plants.	Fair, small root mass fine roots.	No	NG 7, 5 Pen, 1 Trich, 2 Fus (Purple, short conidiophores, mono-phialide).
32	Burn R4	MB4D	Good. Some dead. Live plants healthy.	Good	No	9 NG, 2 T, 1 Pen, 2 Fus, 1 UD
33	Nigro R1	MN1A	Good. 3 plants. Healthy	Good	No	3 NG, 2 R, 1 Pen, 9 Fus (as per

Sample number	Treatment Type	Plant #	General observations	General root health	Evidence of nematodes	Results (NG = no growth; T = Trichoderma; UD = undetermined; Fus = Fusarium sp.; Rhizoc = Rhizoctonia; R = Rhizopus; Pen = Penicillium) All of 15 pieces of crown #19)
34	Nigro R1	MN1B	Good, 1 dead plant, remainder healthy	Good	No	6 NG, 4 T, 1 R, 4 UD
35	Nigro R1	MN1C	Good, small, healthy plants.	Good large root mass	No	1 NG, 9 T, 5 R
36	Nigro R1	MN1D	Good. 2 dead plants. Live plants healthy, small	Good	No	1 NG, 14 R
37	Nigro R2	MN3A	Fair, plants show new growth. Some dead plants.	Fair, some overall rot	No	2 UD (black – as per #5), 11 T, 2 R
38	Nigro R2	MN3B	Good, dense growth. 1 dead plant.	Fair, some root lesions (photo)	No	All were Pen, T or R.
39	Nigro R2	MN3C	Fair, some dieback with new growth.	Fair, some necrosis	No	6 NG, 1 UD (red – as per #5), 1 T, 1 UD, 6 Fus (as per #19)
40	Nigro R2	MN3D	Good. 1 dead plant. Large healthy plants. Good growth.	Good, small necrotic regions	No.	12 NG, 1 T, 2 Pen.
41	Nigro R3	MN4A	Poor. Plants mostly dead. 4 very small plants (regrowth)	Fair, small necrotic regions	No	All T and R.
42	Nigro R3	MN4B	Poor, numerous dead plants. Small regrowth	Fair, some necrotic lesions	No	All T, Pen, R
44	Nigro R3	MN4C	Poor, numerous dead plants with regrowth	Good	No	All T, Pen, R
45	Nigro R3	MN4D	Poor, rot on external areas, regrowth absent on some plants	Poor. Roots slow rot/necrosis. Truncated	No	All T and R
45	Nigro R4	MN2B	Good. Strong growth, numerous plants	Good	No	2 UD (red – as per #5), 13 T, R and P
46	Nigro R4	MN2D	Good, thin plants	Fair, small root mas. Roots present are healthy	No	1 NG, 1 UD, 3 Fus (as per 19), 10 R
47	Control R1	MC1C	Good, thin plants. Healthy	Good.	No	14 NG, 1 T
48	Control	MC1D	Fair, some dead plants. Good	Good	No	NG 5, 10 R

Sample number	Treatment Type	Plant #	General observations	General root health	Evidence of nematodes	Results (NG = no growth; T = Trichoderma; UD = undetermined; Fus = Fusarium sp.; Rhizoc = Rhizoctonia; R = Rhizopus; Pen = Penicillium) All of 15 pieces of crown
	R1		regrowth on live plants.			
49	Control R2	MC2C	Fair, some dead plants with thin new growth	Good. Fine roots prevalent	No	NG 8, 7 Fus (as per 19)
50	Control R2	MC2D	Good. 1 dead plant. Strong and healthy live plants.	Good	No	10 NG, 5 R
51	Control R3	MC3C	Good. 1 dead plant, live plants healthy with good growth. 1 with internal rot.	Good	No	5 NG, 4 Pen, 1 UD (red – as per #5), 3 T, 2 UD
52	Control R3	MC3D	Fair, some dead plants. Live plants healthy. Regrowth on dead plants	Good	No	12 NG, 2 Pen, 1 T.
53	Control R4	MC4C	Fair, some dead plants. Good regrowth on dead plants.	Fair	?	11 NG, 2 Fus (as per #19), 7 UD, 1 Pen
54	Control R4	MC4D	Poor, most plants dead, limited to no regrowth on live plants	Good	No	10 NG, 2 Pen, 1 T, 2 UD (Red – as per #5)



Unidentified brown orange fungus, strong growing with frequent hyphal swellings



Unidentified black fungus with helminthosporium like spores



Red Cladosporium-like spores , strongly raised red and white hyphal growth



Purple, short conidiophores, mono-phialide