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by Ann Lawrie

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Foreword

Giant Parramatta grass (GPG) (*Sporobolus fertilis*) is an aggressive perennial tussocky grass that is a declared noxious weed. It invades native pastures and reduces animal production. Its potential distribution is estimated at 23.7 million hectares in Australia.

The naturally occurring fungus, *Nigrospora oryzae*, causes a crown rot in GPG which has now been shown to reduce tussock size dramatically in the field.

Postal survey responses in this project revealed that land managers had noticed the disease was causing a decline in giant Parramatta grass and had, therefore, reduced or ceased applying herbicide with noticeable financial and environmental benefits.

Once all safety requirements are met, spraying GPG with *Nigrospora oryzae* has the potential to be commercially viable for effective control of GPG, which affects large parts of the north and mid north coast of New South Wales.

Cultivation of the disease in the laboratory is relatively simple, suggesting commercial propagation of the disease for a mycoherbicide will also be attainable.

This project was funded in Phase 1 of the National Weeds and Productivity Research Program, which was managed by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from 2008 to 2010. The Rural Industries Research and Development Corporation (RIRDC) is publishing the final reports of these projects.

Phase 2 of the Program, which is funded to 30 June 2012 by the Australian Government, is being managed by RIRDC with the goal of reducing the impact of invasive weeds on farm and forestry productivity as well as on biodiversity. RIRDC has implemented 55 projects that both extends on the research undertaken in Phase 1 and moves into new areas. These reports will be published in the second half of 2012.

This report is an addition to RIRDC's diverse range of over 2300 research publications which can be viewed and freely downloaded from our website www.rirdc.gov.au. Information on the Weeds Program is available online at www.rirdc.gov.au/weeds

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Craig Burns
Managing Director
Rural Industries Research and Development Corporation

Nana Glen property manager Gary Dew showing diseased giant Parramatta grass plants to Campbell (Tocal District Agronomist) and David Officer, Research Agronomist, NSW Industry & Investment, Grafton.

Acknowledgments

I am most grateful for the expert and generous support of David Officer and Sue Hayward of New South Wales Industry & Investment. Without them this project would have been impossible. Their local knowledge and their willingness to participate in the surveys, contact property owners, and collect and send plant materials for testing were invaluable.

Editor's note: Some information in this publication's Foreword and Executive Summary is sourced from information from David Officer in the NSW DPI Agriculture Today magazine ref: http://www.dpi.nsw.gov.au/archive/agriculture-today-stories/august-2010/giant-parramatta-grass-bio-control.

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Executive Summary

What the report is about

Giant Parramatta grass (GPG) (*Sporobolus fertilis*) is an aggressive perennial tussocky grass that is a declared noxious weed. It invades native pastures and reduces animal production. Its potential distribution is estimated at 23.7 million hectares in Australia.

The naturally occurring fungus, *Nigrospora oryzae*, causes a crown rot in GPG which has now been shown to reduce tussock size dramatically in the field.

Where are the relevant industries located in Australia?

GPG invades native pastures and reduces animal production. Its potential distribution is estimated at 23.7 million hectares in Australia

Aims/objectives

To evaluate the potential of *Nigrospora oryzae* for biological control of weedy *Sporobolus* species in Australia by:

- A postal survey and local surveying of impacts in the field
- Estimates of carriage and impacts in posted plants in a glasshouse
- Collection and testing of other provenances of plants and strains of fungi
- Identification of any resistance in plants and the greatest virulence in fungal strains
- Host specificity testing
- Development of an inoculation method for fungus
- Field inoculation and monitoring of impacts.

Surveys

Two surveys were performed to assess the degree of infection and its impact on giant Parramatta grass (*Sporobolus fertilis*) in New South Wales in 2009–2010. The first was a postal survey dispatched in October 2009 to land managers in the region around Grafton, where disease symptoms had first been noticed in the field. The second was made up of a series of three field surveys on properties with disease symptoms in November–December 2009 and late April 2010.

In October 2009, 2020 questionnaires were mailed to land managers in the New South Wales region of Grafton, where disease and decline in giant Parramatta grass (*Sporobolus fertilis*) had been reported. In all, 104 replies were received (a 5.4 per cent response rate) from land managers, all of whom had giant Parramatta grass (GPG) infestation covering a total area of 200 471 hectares. The estimated production loss was $124 500 a year, and direct control costs were $945 a year. Twenty-seven land managers reported diseased plants; 11 had observed a decline; and 13 reported GPG resistance to flupropanate. The survey and analysis are now complete, and a draft paper has been written. Dr David Officer, Research Agronomist with New South Wales Industry & Investment in Grafton, held a local meeting in May 2010 and issued a press release on 5 August 2010.

Disease was assessed in terms of GPG density, vigour (diameter, height, inflorescences) and infection at three properties that reported disease within two-and-a-half hours' travel from Grafton on three occasions—in November (density) and in early December 2009 and late April 2010 (vigour). The density of GPG had declined relative to David Officer's 2006 data. Between December 2009 and late April 2010, 32.0–47.6 per cent of GPG plants showed disease symptoms; density showed no change, but diameter had declined by up to 83 per cent, also suggesting a reduction in vigour. The symptoms are associated with a decline in GPG, as reported anecdotally.

Collection

Seeds or live plants of *Sporobolus* and other weedy grasses, pasture grasses, native grasses and crop plants have been obtained from New South Wales Industry & Investment and from the Victorian Department of Primary Industries. Samples of about 100 infected plants have been collected in order to isolate new strains of the fungus, and four species of weedy *Sporobolus* have been inoculated; scoring is expected to take about three to four months since the species develop slowly in pot trials. In November 2009 samples of characteristically orange young leaves were collected from 86 plants with symptoms of disease. All produced fungi—15 per cent *Nigrospora oryzae*, 45–70 per cent *Fusarium* sp. (a common grass pathogen) and 40–90 per cent *Alternaria alternata* (a common secondary pathogen). Isolates of *N. oryzae* showed 100 per cent mortality in seedling assays; isolates of *Fusarium* sp. and *A. alternata* did not. Thus only *N. oryzae* appears pathogenic and virulent.

Location of N. oryzae in plants and infections by other fungi

Diseased potted plants in the glasshouse at RMIT University in Melbourne were sampled and tested for where the fungus was located in the plant by surface-sterilising and plating out different parts of the plant on plates of V8 agar. The fungus was isolated from the culms, crown and roots but not the seeds. This suggests that the fungus is systemic—as is the case with *N. oryzae* infection in *Arundo donax*—but that seeds do not carry it. Five genera were isolated from 60 per cent of the seeds: *Penicillium, Cladosporium, Rhizopus, Aureobasidium* and *Chaetomium*. Seeds germinated even in the presence of these genera, so none is pathogenic.

Variation in pathogenicity and virulence according to provenance and strain

N. oryzae isolates from the material collected in the field in December 2009 were uniformly lethal on GPG seedlings in a Petri dish assay, and testing with other fungi isolated showed no lethality. Testing for other provenances of GPG has been delayed by seed dormancy (a common problem in weeds).

Centrifugal phylogenetic testing of isolates

Seeds of endemic *Sporobolus* species and other genera in the same tribe have been received and testing has begun, although this too has been delayed by dormancy problems. Seeds and live plant material of other pasture grasses and major crops in the area have been received; some have been grown and four have been inoculated to test susceptibility in a pot trial. Final results will not be available for at least three months.

Inoculation in field sites and monitoring of effects

A preliminary inspection of pasture sites at properties at Taree, New South Wales, showed infestation by GPG but no infection. Twenty property managers sent samples of GPG to RMIT for isolation of possible pathogens, and four of these produced *N. oryzae*. Suitable uninfected field sites at Taree have now been identified and the property managers' consent has been obtained for an inoculation trial. Once the centrifugal phylogenetic testing is complete, a field inoculation trial will take place. It is expected that infection will not be noted visually for four to six months, so will go beyond the time frame of the current funding. Plants will therefore be monitored by local New South Wales Industry & Investment personnel; the degree to which this will be possible depends on obtaining funding from Meat and Livestock Australia or another funding body.

Results/key findings

- The control process can take a couple of years, which allows the remaining pasture species to recover and fill in the gaps left by the dying GPG.
- Laboratory tests have shown a 70 per cent reduction in healthy GPG leaves seven months after plants were inoculated with a solution of fungal spores.
- Disease symptoms are most apparent seven to 10 days after rain in spring and summer.
- Diseased plants produce orange leaves in their new growth, which then die.
- Individual diseased tillers can be broken off at their base where the normally white crown tissue has turned black and died.

- Recent observations have shown that this disease does not affect giant rats tail grass, which is the same genus as GPG, but is known to be genetically different, which suggests the disease is quite host-specific.

Postal survey responses revealed that land managers had noticed the disease was causing a decline in giant Parramatta grass and had therefore reduced or ceased applying herbicide. This had saved almost $10,000 at Nana Glen in the preceding year – chemicals, labour, fuel and associated withholding period costs – and had reduced associated environmental costs.

Respondents also noted the need for cooperative efforts, including on roadsides, and expressed a desire to control GPG and other weeds, particularly Bahia grass. The respondents were keen to know the outcomes of the research and to participate in a local biocontrol to control GPG without constantly using mechanical or chemical means.

Surveys to assess infection and impact on giant Parramatta grass

Two surveys were performed to assess the degree of infection and its impact on giant Parramatta grass (*Sporobolus fertilis*) in New South Wales in 2009–2010. The first was a postal survey dispatched in October 2009 to land managers in the region around Grafton, where disease symptoms had first been noticed in the field. The second was made up of a series of three field surveys on properties with disease symptoms in November–December 2009 and late April 2010.

The postal survey

The postal survey used a questionnaire that was designed on the basis of similar survey studies of pasture grass weeds (McLaren et al. 2006). The questionnaire consisted of 12 questions, each relating to a specific problem and designed to elicit information about land managed, GPG infestations, management costs, herbicide use, and diseased plants and their impact.

Respondents were asked to do the following:

- provide information about the area of land they manage and the distribution of GPG infestation on their land
- estimate the proportion of GPG-infested land they manage and categorise infections as 'Dense—monoculture or close to monoculture—very few native/other species present' (more than 20 per cent); 'Medium—roughly equal proportions of GPG and native/pasture/crop species present' (5–20 per cent); 'Low or light infestations—native/pasture/crop species in much greater abundance than the target weed species (1–5 per cent); or 'Rare—single or very few plants of a target weed species are present' (less than 1%)
- classify what proportions of these infestations occurred on pastureland and native vegetation (which included native grass pastures)
- estimate the costs of control—including direct costs of materials, labour and lost production
- describe their chemical control—including what herbicides they used for GPG and their history of use
- say whether they had noticed GPG that had not died after application of a specific tussock herbicide (flupropanate or 2,2-DPA) and provide their opinion on the reason for the lack of effect (weed resistance, incorrect dosage or poor application). (Note that flupropanate and 2,2-PDA resistance has been reported from this area [Ramasamy et al. 2008].)
- give their opinion on a biological control program in Australia and who should pay for such a program—government, industry or landholders
- provide open-ended comments.

In October 2009, 2020 survey packages were mailed to land managers either via the local body responsible for weed control or to individuals directly (see Figure 1). The package contained a questionnaire and a prepaid envelope for returning the completed survey form to RMIT University in Melbourne. Five hundred survey forms were sent to the Clarence Valley Weeds Authority, South Grafton; 500 to 'Far North Coast Weeds', Lismore; 200 to Nambucca Shire Council, Macksville; 300 to Shoalhaven City Council, Nowra; 250 to Coffs Harbour City Council; 150 to Hunter Council's Environment Division, Thornton; and 100 to the Illawarra District Weeds Authority, Kiama. This was because the properties in the Grafton region had earlier been reported to have diseased GPG plants but a decline in GPG infestation rates after the winter rains. An additional 20 surveys were mailed to 'Mid North Coast NSW Farmers, Nana Glen', to land managers who had reported GPG infestations on their properties.

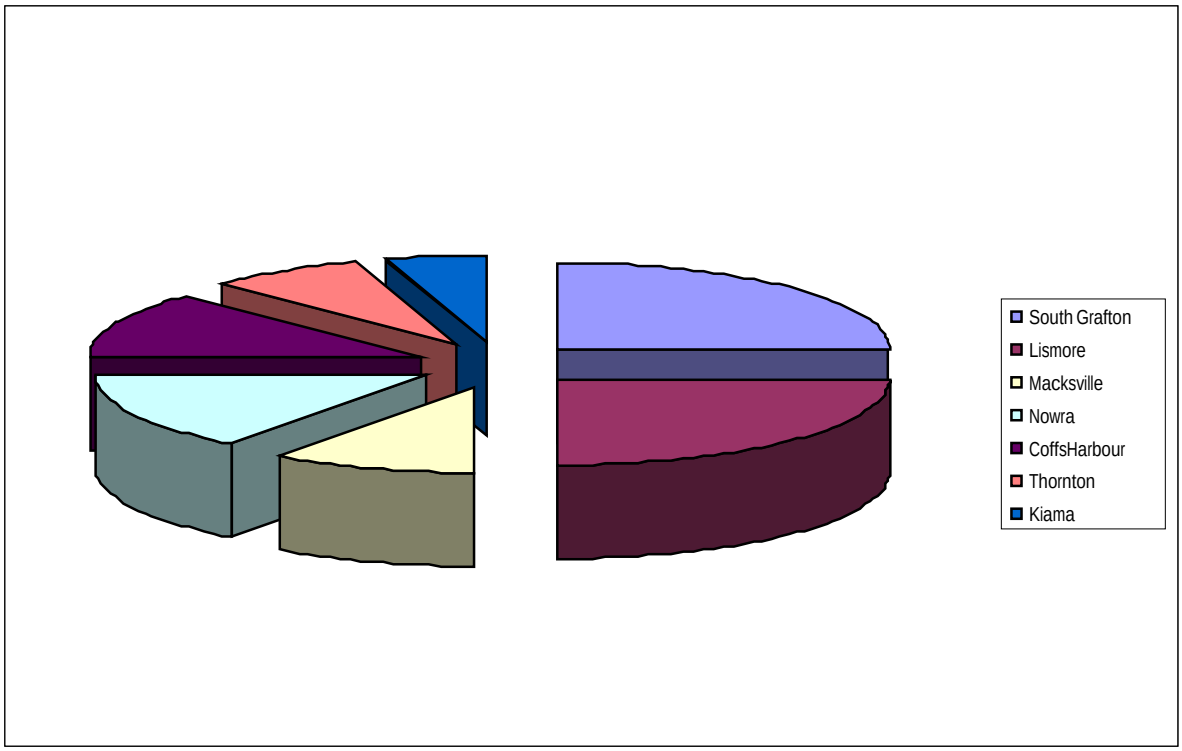


Figure 1 Distribution of postal survey area

Survey results

Responses

In all, 104 replies were received; 75 survey packs were returned as 'Receiver unknown' since property managers had either moved or sold the property, and the rest were not returned. This gave a response rate of 5.4 per cent—about normal for this type of survey. The 104 respondents managed a total of 200 471 hectares, with 94 per cent of that area classified 'native' because of one return for 174 245 hectares managed by NSW Parks & Wildlife. (This was not the main target of the survey, so it is ignored in most of the following analysis.) This left a total of 26 225 hectares, 42 per cent of which was pasture managed mainly for cattle grazing (see Table 1).

Table 1 Types of areas managed by respondents

	Area (ha)		
Respondents	**Pasture**	**Native**	**Total**
All respondents	11 088	189 383	200 470
Respondents excluding NSW Parks & Wildlife	11 088	15 138	26 225
Percentage excluding NSW Parks & Wildlife	42	58	100

Infestation

Most land managers reported infestations of GPG, amounting to 9778 hectares (see Table 2). Respondents estimated that pasture was 66 per cent infested, native vegetation 92 per cent infested, and 'other uses' 100 per cent infested. The density of infestation was most commonly reported as 'low' for all types of land use (see Figure 2), and pasture contrasted with native vegetation in having large areas free of infestation—reported by respondents to be a result of their unremitting efforts.

Table 2 GPG infestation on non–national park land, by category

Land category	% infestation	Infestation by category (ha)					
		Dense	Medium	Low	Rare	None	Total
Pasture	66	226	1194	2129	1494	2558	7601
Native vegetation	92	153	437	1168	219	170	2146
Other	100	0	8	10	13	0	31
Total		379	1639	3307	1726	2728	9778

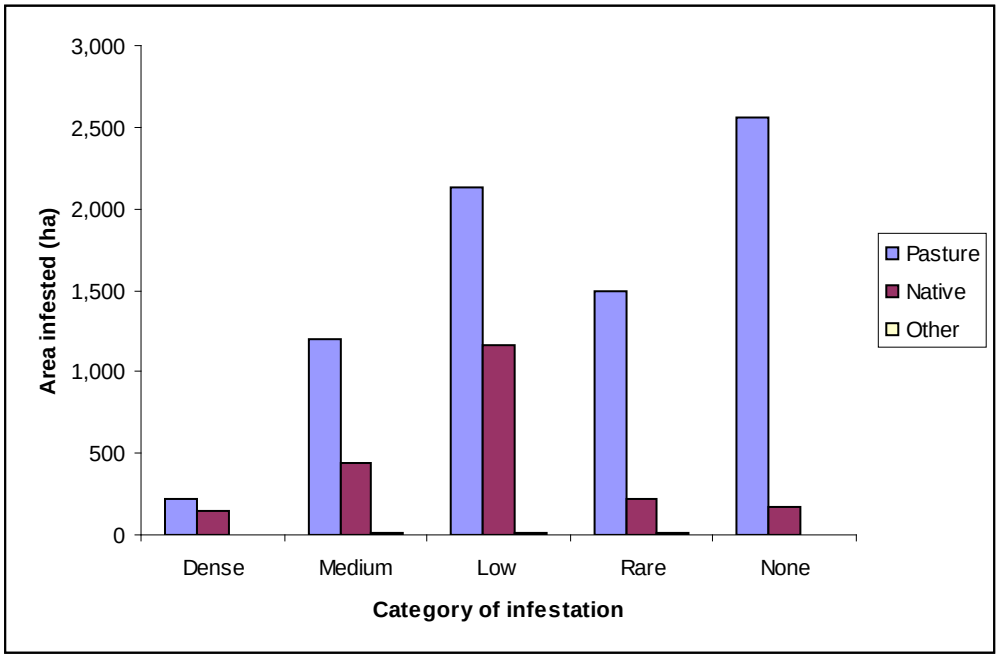


Figure 2 Area and intensity of GPG infestation on non–national park land

Cost

The total cost of GPG control was estimated at $205 960 a year—about half of that for materials (including herbicides) and half for labour, at 748 days per year (see Table 3). Open-ended comments suggested that wick wiping with glyphosate was successful, provided it was done regularly, and that bahia grass was a problem after GPG control with herbicide. The importance of constant (monthly) attention and preventing seeding was mentioned many times. Several respondents who had noted disease in GPG said they had not sprayed the GPG with herbicide since then because it was declining by itself.

Table 3 Costs of GPG control

Land type	Materials ($/yr)	Labour ($/yr)	Time (days)	Other ($/yr)	Direct cost ($/yr)	Lost production ($/yr)	Total ($/yr)
Pasture	42 660	44 900	411	12 190	99 750		
Native	3 920	14 170	337	2 620	20 710		
Total	46 580	59 070	748	6 810	112 460	93 500	205 960

Herbicides

Most respondents had used some form of herbicide many times (some over 100 times) in their efforts to control GPG in the preceding 10 years; some herbicide use dated back 40 years. The most widely used herbicides were flupropanate and glyphosate, some property managers using both (see Figure 3). In these circumstances it seems inevitable that resistance would eventually occur.

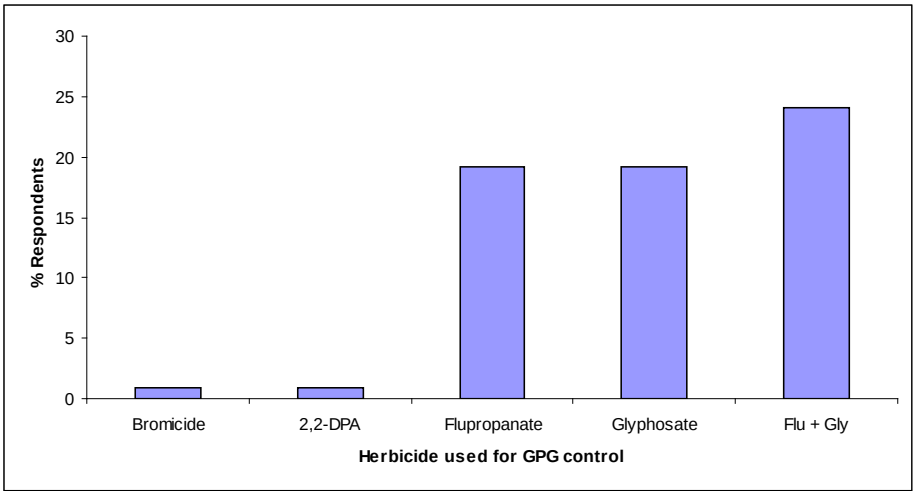


Figure 3 Herbicides used for GPG control

Herbicide resistance

Twenty-two land managers reported that GPG plants had not died after being sprayed with herbicide—15 with flupropanate and seven with glyphosate (see Table 4). In five cases this was ascribed to operational failures (poor application or incorrect dosage), but in 13 cases the lack of success was ascribed to herbicide resistance (eight to flupropanate and five to glyphosate). Flupropanate resistance had previously been verified from only one of these properties, at Nana Glen, and reports of glyphosate resistance were new. All need to be verified experimentally by stringent testing.

Table 4 Herbicide control failures and reasons given

		Reason suspected				
Herbicide	**No. not dead after spraying**	**Poor application**	**Incorrect dosage**	**Resistance**	**Don't know**	**Total**
Flupropanate	15	2	1	8	5	31
Glyphosate	7	1	1	5	2	16
Total	22	3	2	13	7	47

Disease

Twenty-seven respondents (26 per cent) reported diseased GPG plants; of these respondents, 13 (48 per cent) had observed a decline in GPG infestation. In contrast, 77 respondents who had not observed disease reported only a 14 per cent decline (see Figure 4). The questionnaire was not specific enough to distinguish between a decline in the frequency of GPG and the size of GPG plants on properties. Several respondents commented that they had stopped spraying herbicides for GPG control because disease had resulted in a decline in GPG.

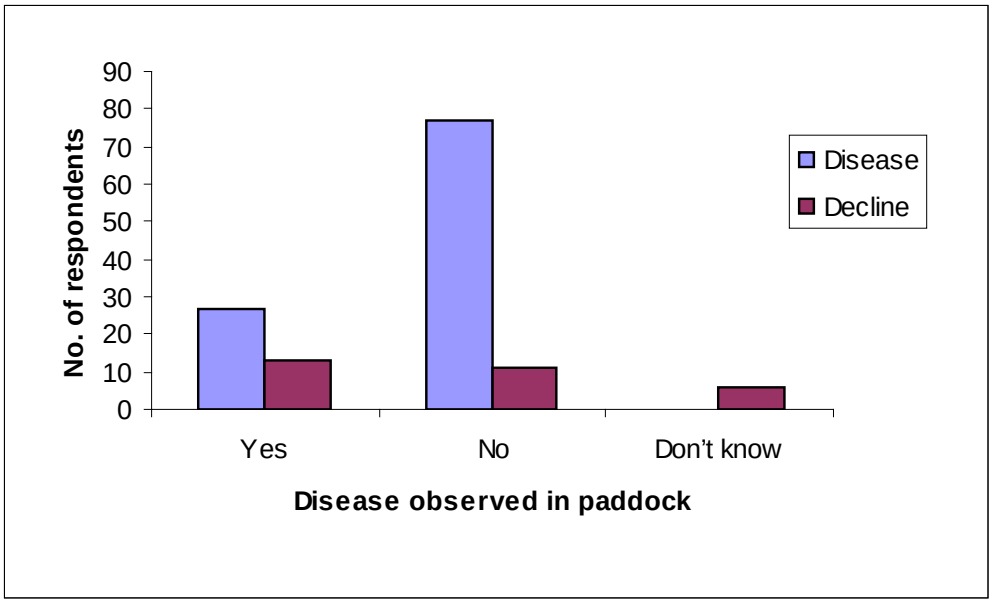


Figure 4 Disease and decline of GPG

Properties reporting diseased GPG were mapped: this revealed three clusters, around Grafton, Nana Glen and Bowraville (see Figure 5).

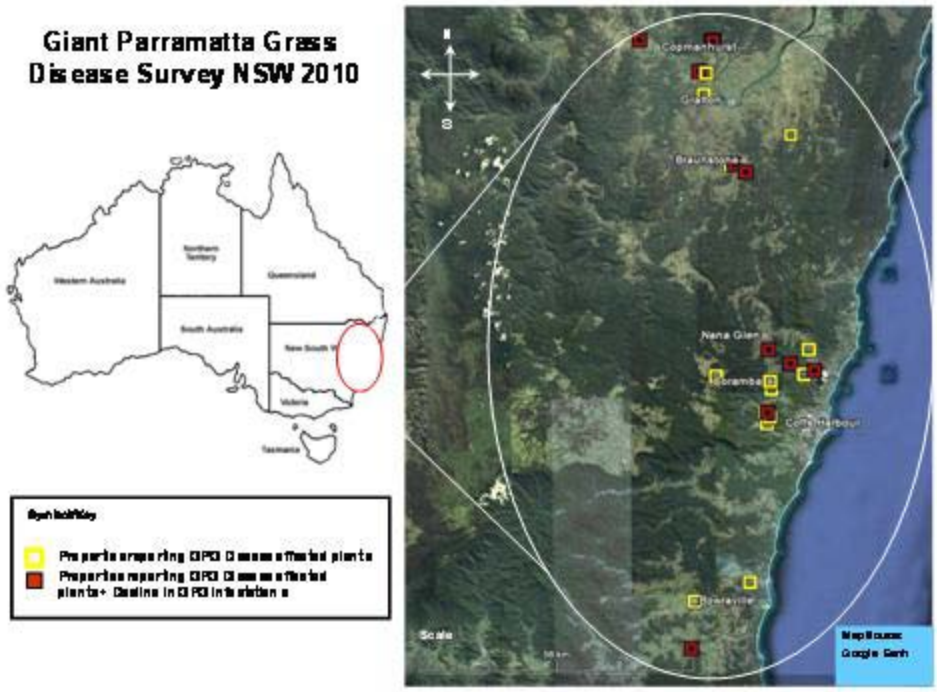


Figure 5 Properties with GPG disease and decline in 2009–10

Funding

Sixty-seven per cent of the land managers believed that government should fund research into biocontrol of GPG, although 31 per cent of them believed that industry and landholders should contribute (see Figure 6).

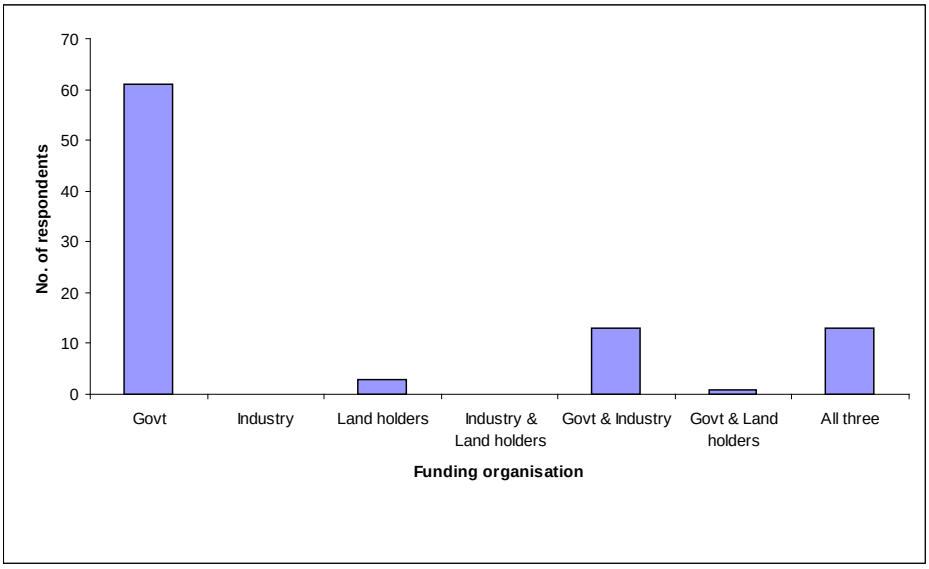


Figure 6 Respondents' views on funding for biocontrol of GPG

Summary

The postal survey showed that land managers in the Grafton area had major GPG infestations and incurred significant costs associated with its management, that herbicide resistance was suspected, and that some property managers had noticed a decline in GPG associated with the diagnostic disease symptoms.

Local property managers' interest in this project has been very high, and David Officer (of New South Wales Industry & Investment) organised a meeting in May 2010 in order to present the results of the mail survey and updates on the research. A press release about the research appeared in *Agriculture Today* on 5 August 2010 (http://www.dpi.nsw.gov.au/aboutus/news/agriculture-today/august-2010/giant-parramatta-grass-bio-control) and a fact sheet is being prepared for distribution to property managers in the area.

The field surveys

The aim of the field surveys was to assess the incidence and impact of disease on sites reported to have fungus-infected GPG using two survey methods. The first method involved the use of small quadrats (0.25 square metres) and followed up on previous data David Officer acquired using the same method on three properties from 2005 to 2006; this estimated GPG and disease frequency. The second method used four large (250 square metre) permanent quadrats as monitoring sites on two properties with diseased GPG and estimated GPG plant size by diameter and height. The aim was to find out whether the decline caused by disease, as reported in the questionnaire and anecdotally, was in GPG density or plant size or both.

The small-quadrat survey

As noted, the first field survey, in November 2009, used small non-permanent quadrats on properties from which David Officer had collected data during a separate trial of ways of controlling GPG in 2004 to 2006. Part of the aim was to find out if the incidence of GPG and disease symptoms had changed since then.

Materials and methods

The field sites at Tabulam (28°53'13.91"S, 152°34'6.79"E), Nana Glen (30°8'10.32"S, 153°0'17.47"E) and South Gate (30°9'45.2"S, 153°00'56.1"E) were chosen on the basis of the prevalence of GPG disease in the region and the availability of a history of GPG since 2005. The weather before the assessment was wet for that time of year, with October and November rainfall in 2009 at 160 and 97 millimetres respectively. Three people threw quadrats (each 0.5 x 0.5 metres square) at random over 1.5 hectares on each site. The number of diseased and healthy GPG plants was counted for each quadrat. The typical orange foliage exhibited by the diseased GPG plants was used as an indicator of disease (see Figure 7).

Figure 7 GPG without (left) and with (right) diagnostic orange leaf symptoms

Results

GPG frequency varied from 33 per cent at Nana Glen and Southgate to 45 per cent at Tabulam (see Table 5). GPG with disease symptoms varied from 25 per cent at Southgate to 45 per cent at Tabulam (see Figure 8). Greater severity of disease was observed near creeks and on gully slopes, where higher humidity and soil moisture are to be expected.

Table 5 Frequency of GPG and diseased GPG at field sites using small quadrats, November 2009

Field site	Total quadrats	% of GPG	% of diseased GPG
South Gate	1461	33.0	32.2
Nana Glen	1450	33.2	48.8
Tabulam	720	44.9	47.6

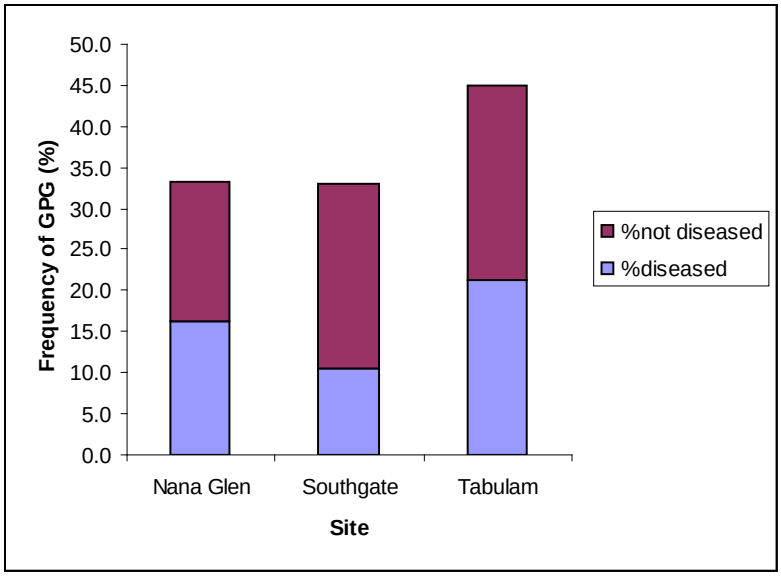
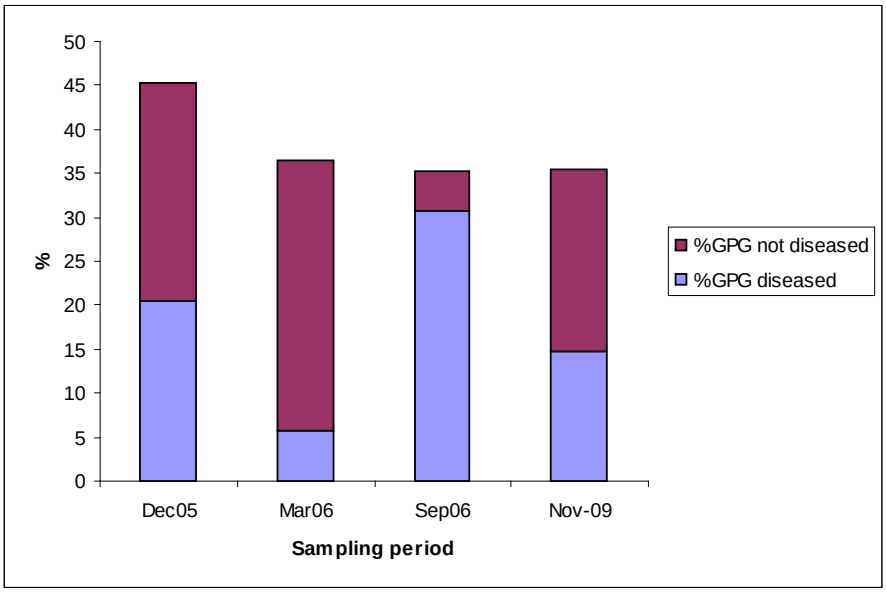


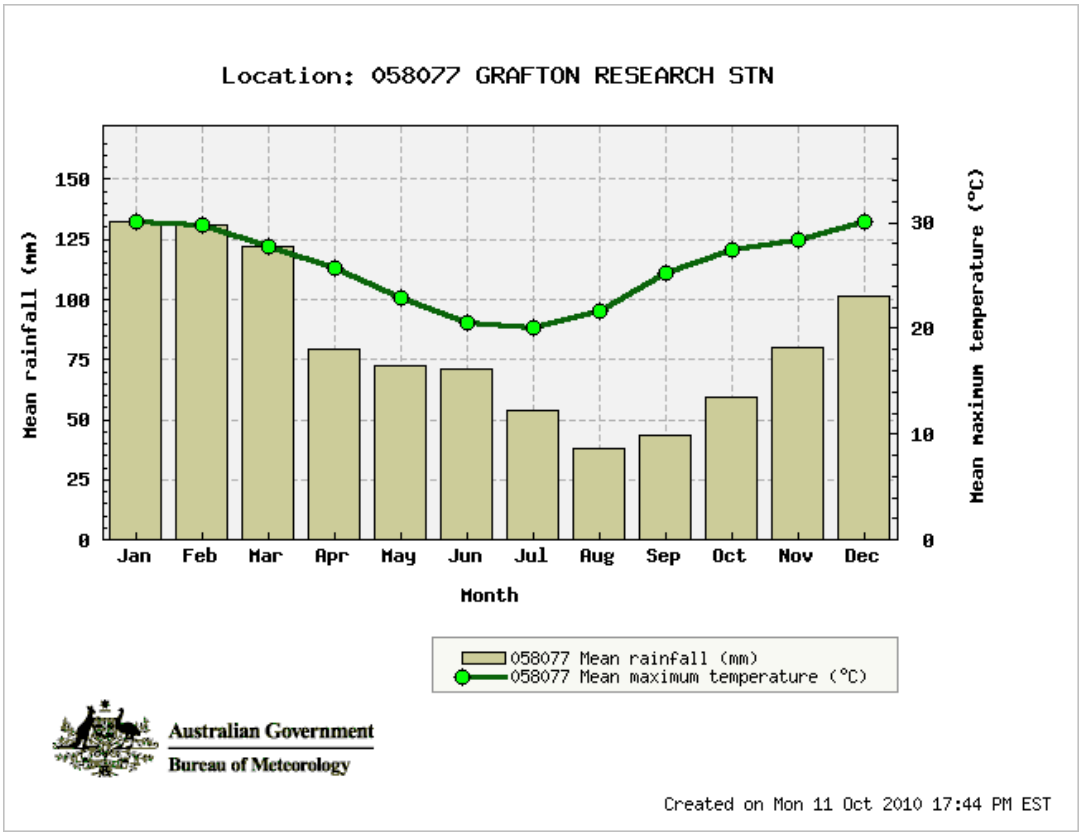
Figure 8 Frequency of GPG with and without disease symptoms at field sites using small quadrats, November 2009

At Nana Glen the frequency of GPG had declined by 10 per cent in comparison with the 2005–2006 data (see Figure 9) and the incidence of disease was comparable: just less than half the GPG plants showed disease symptoms, although disease varied seasonally, from 15 per cent in March 2006 to 87 per cent in September 2006. This suggests that disease led to a decline in plant frequency (and hence density) and that disease persisted from year to year. The largest quantity of symptomatic plants in September coincides with the lowest seasonal rainfall, and the smaller quantity in March coincides with the end of the summer-season rainfall (see Figure 10). This is consistent with disease symptoms being maximal because the GPG plants are stressed by lack of water and minimal when the plants are not so stressed and able to produce new growth towards the end of the period of greater water availability. This is the opposite of the pattern that would be expected with a foliage disease—for example, a leaf spot—and consistent with a crown rot.



Note: 2005 and 2006 data from Dr David Ireland, New South Wales Industry & Investment.

Figure 9 Variation in frequency of GPG and disease symptoms, Nana Glen, 2005 to 2009



Source: Bureau of Meteorology.

Figure 10 Weather patterns, Grafton Research Station, 1917 to 2010

The large-site survey

As noted, the second type of survey involved using large permanent rectangular quadrats at four sites on two properties identified from the questionnaire responses and anecdotally as having disease symptoms associated with the decline of GPG. The aim was to monitor the effects of disease on the size of individual plants over five months, from early December 2009 until late April 2010.

Materials and methods

Three field sites were chosen at Nana Glen and one at Southgate, and field surveys were conducted in December 2009 and late April 2010. There had been 257 millimetres of rain during October–November 2009, just before sampling in December, and disease symptoms were evident on both visits. Sampling was delayed until the end of November because the start of the wet season was late, and disease symptoms were easily observable two weeks later (David Officer, New South Wales Industry & Investment, pers. comm.).

At each site a quadrat 50 metres long by 5 metres wide was marked out with tape and its position recorded by GPS. Within each quadrat the position of each plant was marked by GPS, and measures of its vigour (diameter, height and inflorescences) were recorded. In addition, samples of orange leaves were pulled out from the crown of 86 plants, sealed in clean plastic bags and taken back to RMIT, where they were stored at 4°C for pathogen isolation as soon as possible after return.

Results

In early December 2009 the proportion of plants with disease symptoms ranged from 31 to 70 per cent and the proportion flowering ranged from 12 to 71 per cent (see Figure 11). There was no consistent difference in flowering between plants with disease symptoms and plants without symptoms in December 2009, so this was not assessed in April 2010.

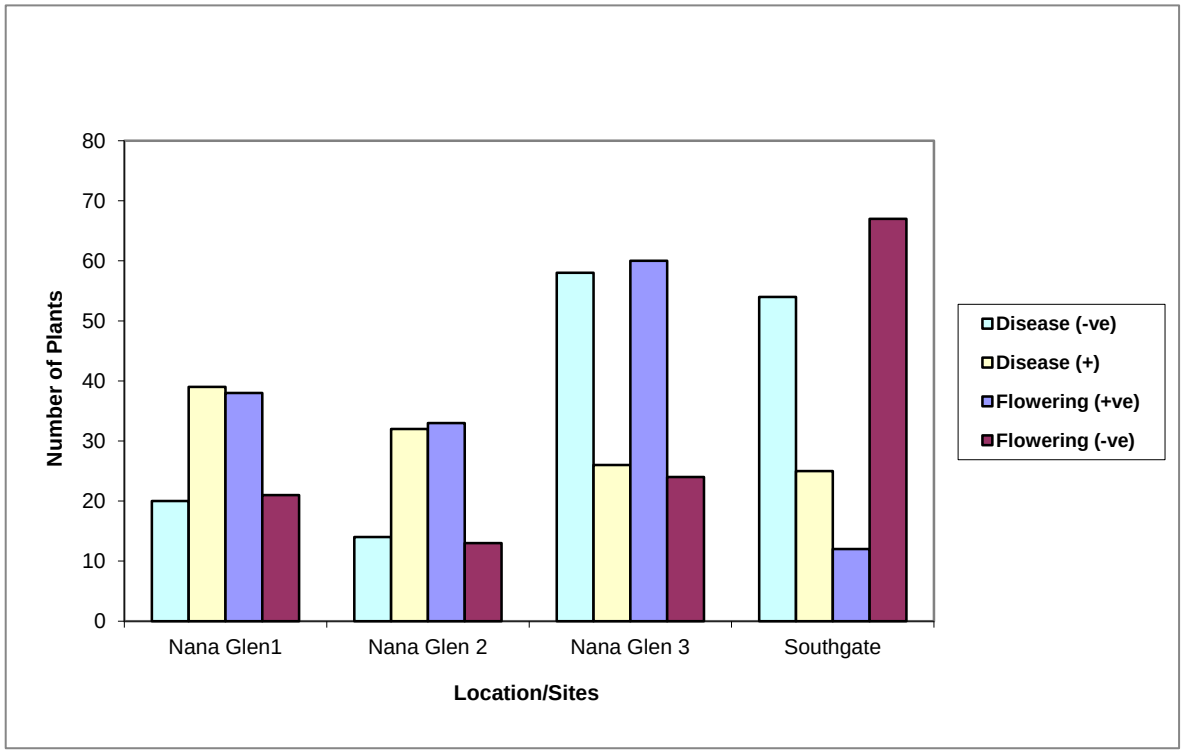
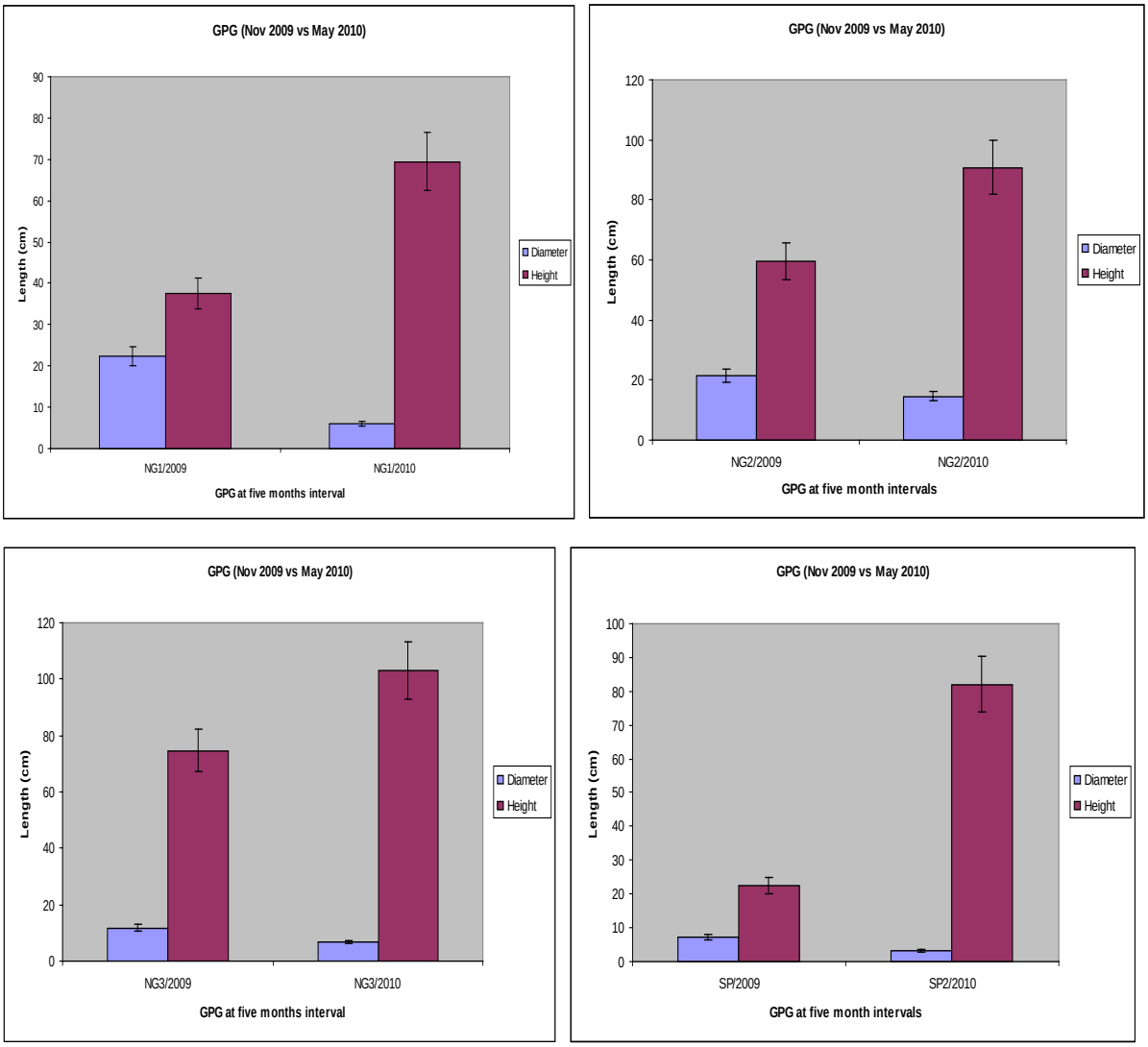


Figure 11 Disease (as judged by symptoms) and flowering, Nana Glen and Southgate, early December 2009

The same sites were sampled again in late April 2010 (almost five months later, after the end of the wet season). Between early December 2009 and late April 2010, the mean diameter of GPG plants at monitoring sites in diseased paddocks declined significantly at both Nana Glen and Southgate, while the height increased significantly (see Figure 12). The decrease in diameter reflected smaller leaf length while the increase in height was due to flowering (in season). This suggested that the 'decline' reported by land managers was also a consequence of a reduction in plant vigour.



Note: NG1 = Nana Glen site 1; NG2 = Nana Glen site 2; NG3 = Nana Glen site 3; SP = Southgate site.

Figure 12 Height and diameter of GPG plants at five-month intervals, large permanent quadrats, November 2009 and May 2010

Final conclusions from field surveys

The presence of disease symptoms in GPG in the field was associated with a 10 per cent reduction in the frequency of GPG in the small-quadrat survey and a halving of tussock diameter in five months in the large-site survey, suggesting that disease symptoms are indeed associated with a decline, as reported in the questionnaire responses. The decline land managers reported was therefore in both plant size and frequency as individual plants decrease in size and eventually die out.

Isolation of *Nigrospora oryzae* and other micro-organisms from symptomatic GPG plants

Isolation of micro-organisms from potted plants at RMIT University in 2009

The aim of this research was to locate *Nigrospora oryzae* in symptomatic plants in pots in a glasshouse at RMIT University in Melbourne.

Materials and methods

Asymptomatic potted giant Parramatta grass plants grown from seed from properties near Grafton in New South Wales had previously been grown in a glasshouse at RMIT and had been infected with *N. oryzae*, and displayed typical orange leaf symptoms. *N. oryzae* had been isolated from the bases of the symptomatic shoots.

In the case of vegetative parts, the GPG plants were sampled in duplicate and tested for where the fungus was in the plant by surface-sterilising with 1 per cent sodium hypochlorite and plating out on plates of V8 juice agar different vegetative parts of the plants:

- the crown—the base of the symptomatic shoot
- the culm—a section of stem below infected leaves
- the leaf—symptomatic leaf and leaf sheath.

In the case of seeds, four replicates of 20 seeds per replicate plate were subjected to one of three treatments to determine if seed carriage was surface or systemic:

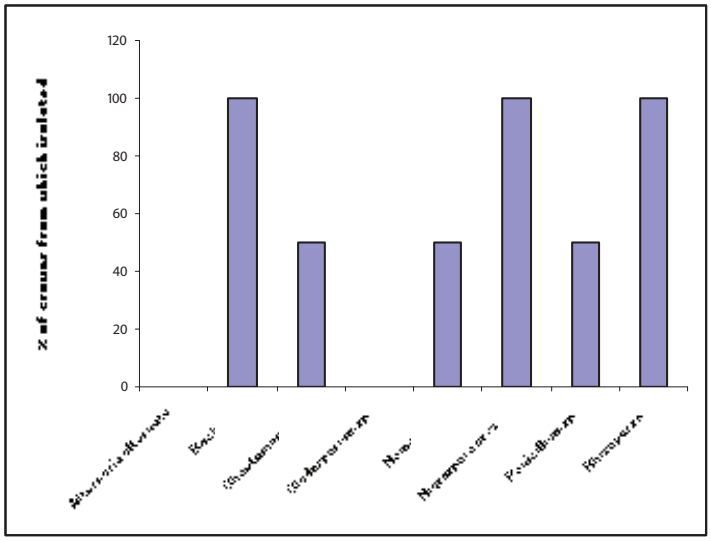
- treatment 1—surface-sterilised with 1 per cent sodium hypochlorite for two minutes and rinsed three times with sterile water
- treatment 2—rinsed three times with sterile water
- treatment 3—unwashed.

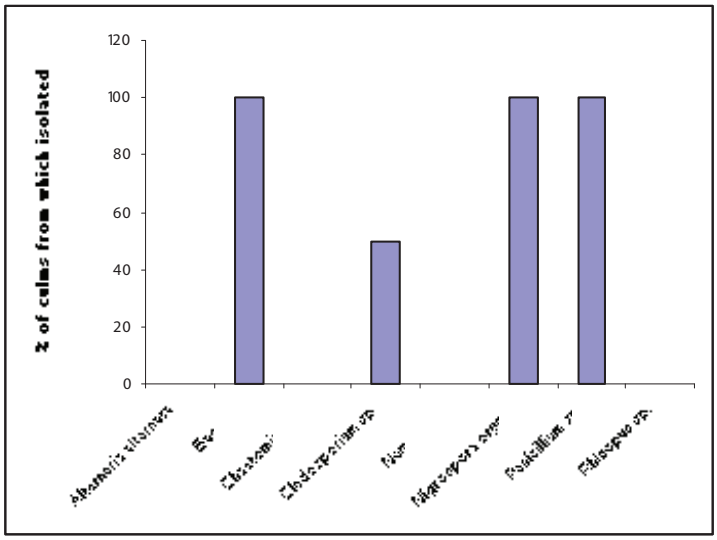
Seeds were plated onto freshly prepared V8 agar—200 millilitres of V8 juice, 2 grams of calcium carbonate, 20 grams of agar, pH 6.0–6.5—and incubated at 25°C in a 12-hour photoperiod using fluorescent tubes for seven days. Micro-organisms growing from the seeds were examined microscopically and identified using standard texts.

Results

The fungus was isolated from all three vegetative parts—crown, culm and leaf (see Figure 13). This suggests that the fungus is systemic rather than existing on the surface of the plants, as is the case with *N. oryzae* infection in *Arundo donax*, a large weedy grass in a tribe close to GPG and for which *N. oryzae* has been suggested as a biological control. The implication of this is that infected plants transmit the disease to all shoots and so all shoots and new leaves should become infected, leading to the decline observed near Grafton.

Seed germination averaged about 40 per cent, there being no differences between the treatments (see Figure 14). Bacteria and *Penicillium* sp. were isolated from all treatments, suggesting that the surface-sterilisation was insufficient or did not penetrate sufficiently inside the glumes. Other fungi, including *N. oryzae*, were isolated only from seeds that had not been surface-sterilised, suggesting that the seed carried them externally rather than internally. The seed thus appeared not to be infected systemically with *N. oryzae* (as is the case with many other systemic micro-organisms) and as a result infected seeds could not be used as inocula for biocontrol.





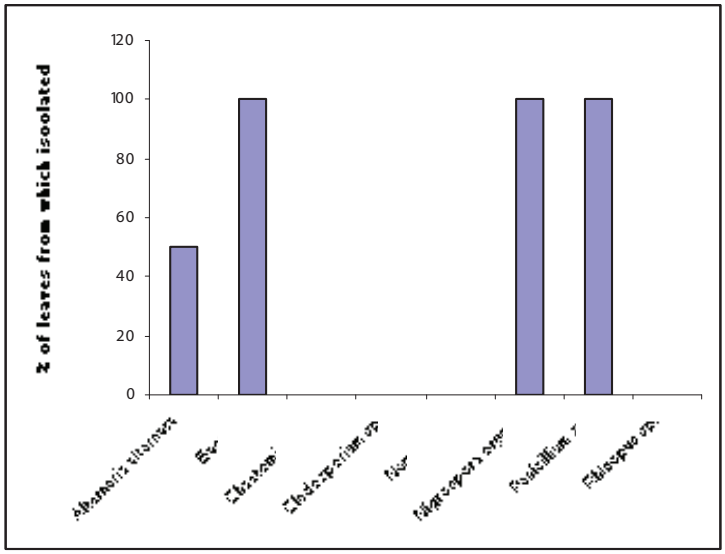


Figure 13 Micro-organisms isolated from crown, culm and leaf of GPG plants in pots in a glasshouse, RMIT, 2009

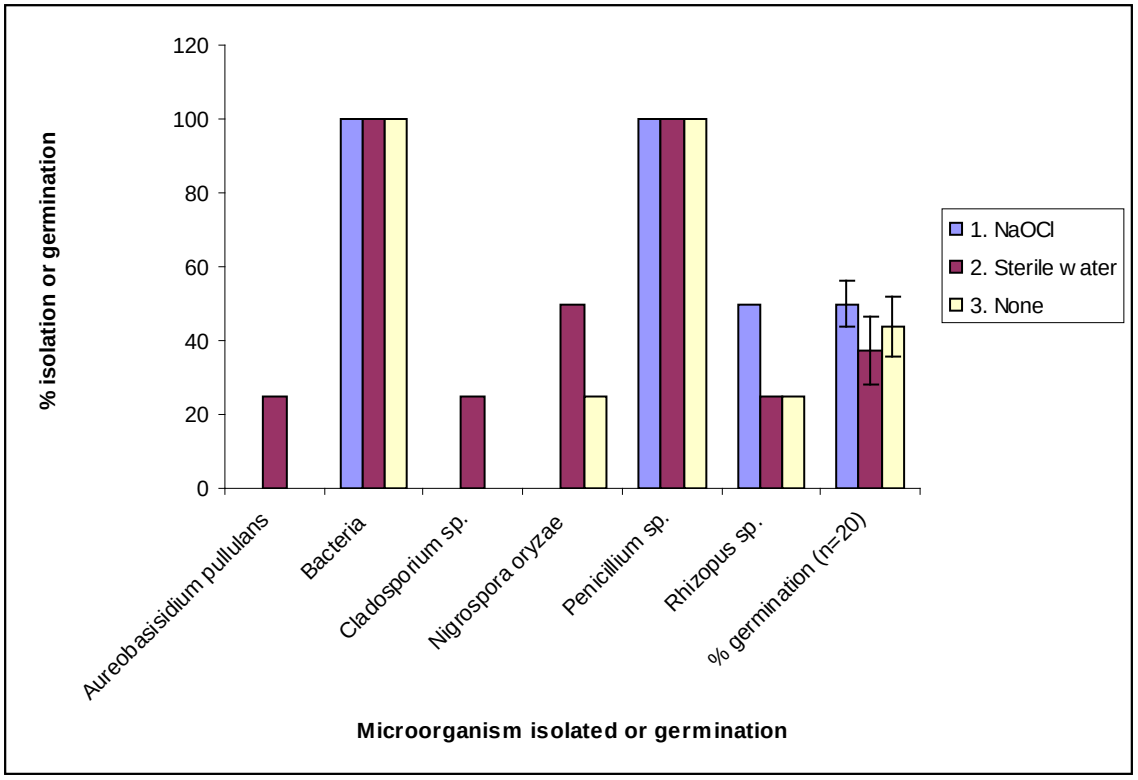


Figure 14 Micro-organisms isolated from and germination of seeds of glasshouse-grown GPG plants in pots after three treatments, RMIT, 2009

Isolation of micro-organisms from symptomatic plants in the field in December 2009

The aim of the research was to see if the field symptoms—the distinctive orange leaves—were exclusively associated with any potential pathogen, especially *N. oryzae*.

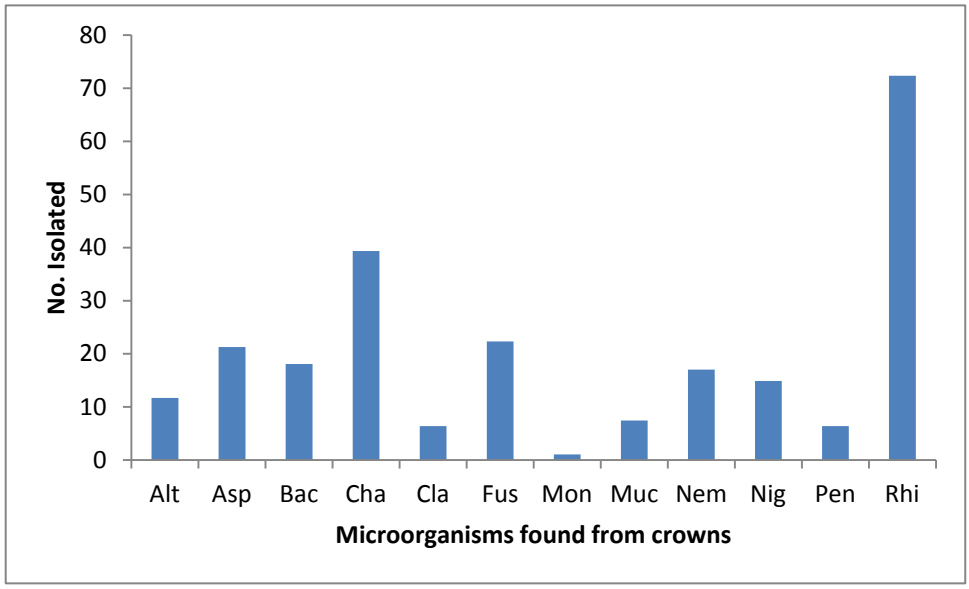
Materials and methods

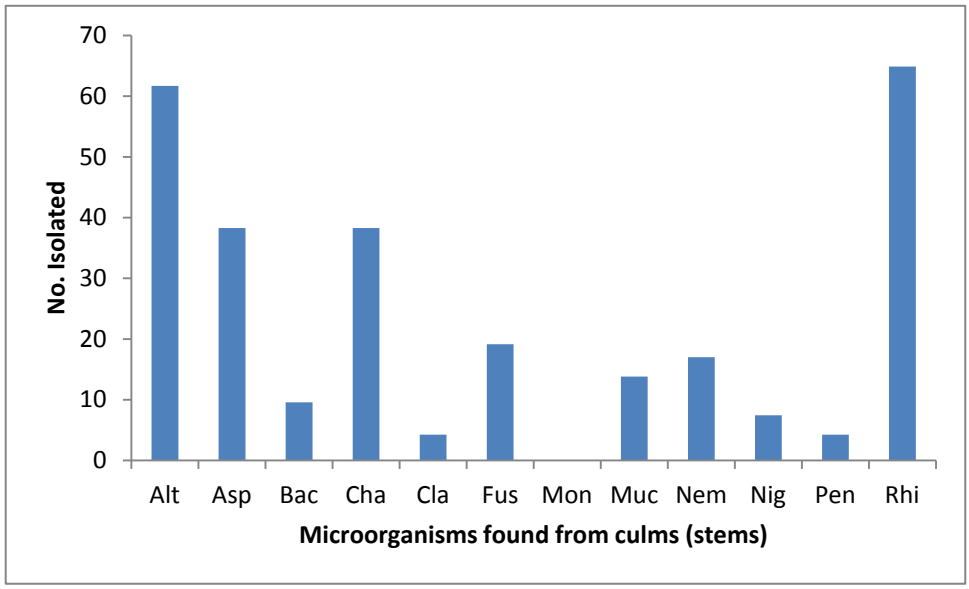
Diseased GPG samples were collected systematically from 86 shoots of symptomatic plants pulled out of the ground from all four sites for isolation of causative micro-organisms and stored at 4ºC until treated as soon as possible on their arrival at RMIT in Melbourne. Samples (rhizome/roots, stem and leaf) were washed in tap water to remove soil and other plant debris, dipped in 70 per cent ethanol for one minute, surface-sterilised with 1 per cent sodium hypochlorite for two minutes, and rinsed three times with sterile distilled water. The surface-sterilised samples were left on sterile tissues for five minutes in the laminar flow cabinet to remove the moisture and were then plated onto freshly prepared V8 medium—200 millilitres V8 juice, 2 grams of calcium carbonate, 20 grams of agar, pH 6.0–6.5, autoclaved at 121ºC for 20 minutes, and poured into 90–millimetre diameter plastic Petri dishes—and incubated at 25°C with a 12-hour photoperiod using fluorescent tubes placed 0.5 metres above the plates (27 µmoles m^{-2} s^{-1}) for up to one month or until growth was evident. Fungi were identified by standard morphological methods.

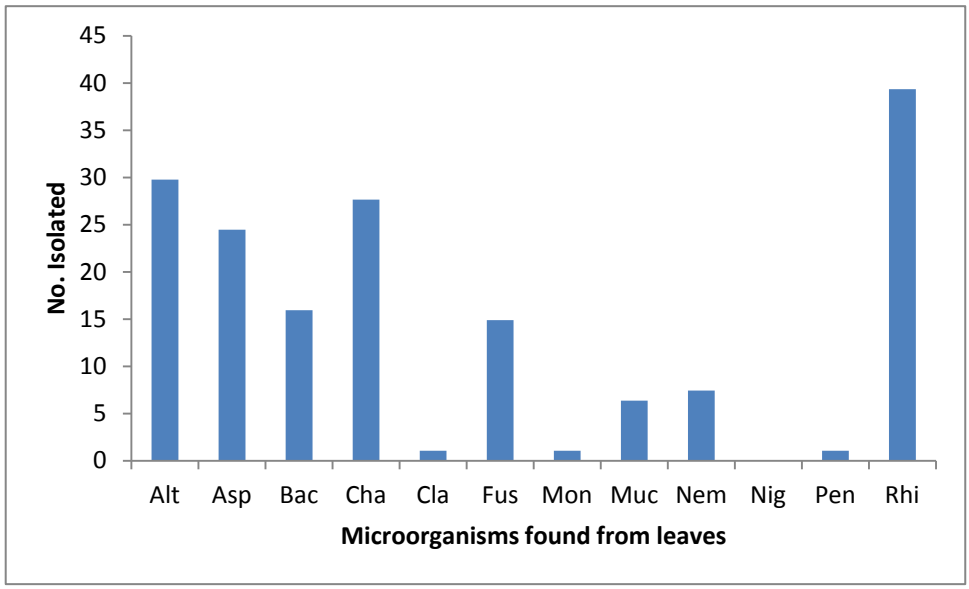
Results

Micro-organisms were found more frequently on the isolation plates from crown and culm samples than from leaves (see Figure 15). Twelve types of micro-organism were found on the isolation plates. Of these, 10 were fungi, one was one or more types of bacteria and the other was non–plant pathogenic nematodes (no stylets). The most frequent fungus was *Rhizopus* sp., which is unlikely to be the primary cause of the diagnostic orange leaves in the field because it causes only post-harvest fruit and vegetable rot. Four other fungi—species of *Aspergillus, Penicillium, Chaetomium* and *Cladosporium*—are common soil fungi that either are associated only with seed disease or are not

pathogenic. Of the potential pathogens, *Monilia* sp. was isolated from relatively few specimens and typically causes leaf spots, so is unlikely to be the cause of the symptoms. *N. oryzae* and *Fusarium* sp. are potential primary pathogens, but *A. alternata* is normally a secondary pathogen that accompanies later stages of a primary infection, although all were found in all sites (see Figure 16). *N. oryzae* is recorded as causing crown rot and dieback in *Arundo donax*, a reasonably close relative of *S. fertilis* in the grass family, and so is a potential cause of the symptoms observed in the field, but it was isolated from only 7 to 15 per cent of crowns and culms and was not isolated from the leaves. This does not, however, exclude it as a cause of the symptoms: the leaf symptoms are likely to be the result of crown rot because they lack lesions and are uniformly chlorotic, as is typical of a crown rot. *N. oryzae* was never isolated alone from any plant but was frequently isolated without any other pathogenic fungus—for example, with *Chaetomium* and *Rhizopus* sp.







Note: Alt = *Alternaria alternata*; Asp = *Aspergillus* sp.; Bac = bacteria; Cha = *Chaetomium* sp.; Cla = *Cladosporium*; Fus = *Fusarium* sp.; Mon = *Monilia*; Muc = *Mucor* sp.; Nem = nematodes: Nig = *Nigrospora oryzae*; Pen = *Penicillium* sp.; Rhi = *Rhizopus* sp. (probably *R. oryzae*).

Figure 15 Fungi isolated from symptomatic plants collected in early December 2009

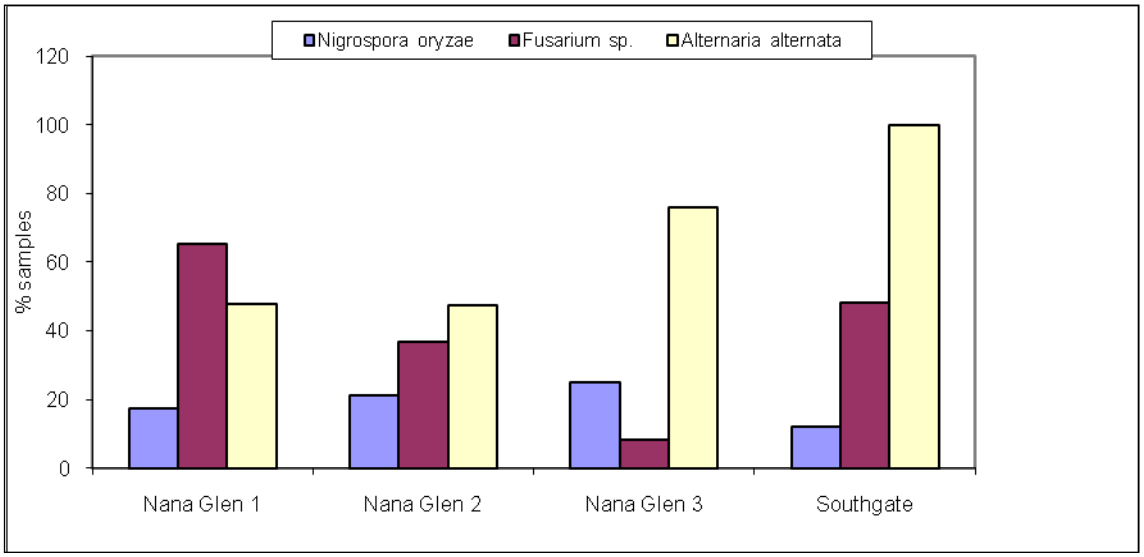


Figure 16 Major likely pathogens isolated from symptomatic plants collected in the field in early December 2009

Isolation of micro-organisms from field-collected seeds of GPG, 2009 and 2010

The aim of the research was to determine if seeds of plants infected in the field carried *N. oryzae* or other pathogens and to compare these plants with glasshouse-grown symptomatic plants infected with *N. oryzae*.

Materials and methods

GPG seeds were collected from three properties near Grafton, at Nana Glen, Southgate and Tabulam, in late 2009 and early 2010. Seeds from Grafton and Sconbiens had been similarly collected in 2006. Seeds were also collected anew from previously infected glasshouse-grown GPG plants from near Grafton for comparison with field samples and results from the 2009 tests. The seeds were stored at 4°C for up to five months, until May 2010, to overcome dormancy. They received the treatments used previously for the 2009 trials with glasshouse-grown plants—sodium hypochlorite, sterile water, or no treatment.

Results

Germination varied greatly, from 0 to 95 per cent, with no germination in the 2009–2010 field-collected seed (due to dormancy) and high germination in both the 2006 field-collected seed and the 2010 glasshouse-collected seed (see Table 6). Only seven species of micro-organism, all fungi, were isolated. *N. oryzae* was not isolated from seed from any source. Most of the fungi isolated were common soil saprophytes (such as *Aspergillus* and *Penicillium*) and only *Fusarium oxysporum* was a potential primary pathogen. There was no relationship between fungi isolated and germination. As with the previous seed testing, *N. oryzae* was not carried by seeds internally, and in this case it was not carried externally.

Table 6 Micro-organisms isolated and germination from field- and glasshouse-collected seed, May 2010

Seed source	Time of collection	Treatment	Germination (%)	Micro-organisms isolated
Nana Glen	Nov 2009	1. NaOCl	0	None
		2. Sterile water	0	*Alternaria alternata* *Aspergillus fumigatus*
		3. None	0	*Alternaria alternata*
Southgate	May 2010	1. NaOCl	0	*Alternaria alternata* *Aspergillus fumigatus* *Fusarium oxysporum* *Penicillium* sp.
		2. Sterile water	0	*Alternaria alternata* *Fusarium oxysporum* *Penicillium* sp.
		3. None	0	*Alternaria alternata* *Fusarium oxysporum* *Penicillium* sp.
Tabulam	Nov 2009	1. NaOCl	0	None
		2. Sterile water	0	*Alternaria alternata* *Aspergillus fumigatus* *Penicillium* sp.
		3. None	0	*Alternaria alternata* *Penicillium* sp.
Sconbiens	2006	1. NaOCl	66	None
		2. Sterile water	78	*Aspergillus fumigatus*
		3. None	75	*Aspergillus fumigatus*
Grafton	2006	1. NaOCl	88	*Aureobasidium pullulans* *Penicillium* sp.
		2. Sterile water	90	*Penicillium* sp.
		3. None	90	*Aureobasidium pullulans* *Penicillium* sp.
Nana Glen[a]	May 2010	1. NaOCl	23	*Aspergillus fumigatus*
		2. Sterile water	14	*Aspergillus fumigatus* *Aureobasidium pullulans*
		3. None	20	*Alternaria alternata* *Aspergillus fumigatus* *Penicillium* sp.
RMIT[a]	May 2010	1. NaOCl	95	*Alternaria alternata* *Aspergillus* sp. *Scedospoium* sp.
		2. Sterile water	79	*Alternaria alternata* *Aspergillus* sp. *Scedospoium* sp.
		3. None	79	*Alternaria alternata* *Aspergillus* sp. *Scedospoium* sp.

a. Seeds glasshouse grown; the remainder field collected.

Variation in pathogenicity and virulence of *Nigrospora oryzae* isolates

The aim of the research was to determine whether all cultures isolated from field-collected giant Parramatta grass plants were pathogenic and virulent.

Materials and methods

Because of time constraints, a seedling assay was used instead of a potted plant assay. All cultures of *Nigrospora oryzae* isolated from field-collected materials were cultured on V8 agar and tested against GPG seedlings grown from 2006 seeds in a Petri dish assay, as described by Ramasamy et al. (2008). It had been intended to test isolates against different provenances of GPG but dormancy in seeds collected in 2009 and 2010 made this impossible: as Table 6 shows, none germinated.

Results

All isolates were pathogenic, virulent and uniformly lethal. All might belong to the same genotype that is virulent on GPG, but no genetic testing was done to determine this. It should, however, be done in order to find out whether a mixture of isolates is needed for a biocontrol preparation. If proven genetically different by RAPD (randomly amplified DNA polymorphism) testing, isolates should be assayed against mature potted plants as for the original isolate.

Centrifugal phylogenetic testing of isolates

The aim of the research was to determine whether other species were affected by *Nigrospora oryzae* and so assess its impact if inoculated as a biocontrol organism on site.

Materials and methods

A list of 45 species (58 cultivars) for testing was drawn up in consultation with David Officer (New South Wales Industry & Investment) and local Grafton land managers (see Table 7). Seeds, cuttings or whole plants of these species were acquired from the Grafton area and sent to RMIT for testing. Seeds were germinated where possible within the time frame and plants placed in pots to grow. Potted plants of four *Sporobolus* species have been inoculated with *N. oryzae* using 1 millilitre of 10^6 conidia/millilitres, as described by Ramasamy et al. (2008).

Results

Eight *Sporobolus* species were identified for testing; of these, four have been obtained as seed and been raised. Testing with *N. oryzae* is in progress in pots in the glasshouse at RMIT: the symptoms take three to six months to develop, so it is not possible to report the results here.

Four other important weedy grasses (*Nassella* spp.) were identified and seeds from all have been obtained. Ten pasture grasses important in the region were identified; seed has been obtained for seven of these. Thirteen native grasses (also important in pasture) were identified; seed has been obtained for four of these, all of which have been germinated and planted in pots. Ten important crop species (22 cultivars) were identified and, of these, seed of the cereals has been obtained, germinated and planted in pots. Live shoot cuttings of four of the five sugar cane cultivars were obtained and have been planted in pots in a glasshouse at RMIT. Once these cultivars reach a suitable stage they will be crown-inoculated and left for up to six months to test *N. oryzae*'s ability to cause disease. Uninoculated control plants have been included. It would be desirable to house these uninoculated plants in a different glasshouse, without infected GPG plants, but space restrictions preclude this. Funding for this project has now ceased, so monitoring and inoculation will be done by the recipient of the grant, who will try to obtain further funding for research assistance to continue this vital step before field inoculation.

Table 7 Species and materials obtained for phylogenetic testing

Classification	Botanical name	Common name	Cultivar	Seeds obtained	Live plants obtained	Testing in progress
Sporobolus spp.	*Sporobolus africanus*	Paramatta grass				
	Sporobolus creber			Yes		Yes
	Sporobolus fertilis	Paramatta grass		Yes		Yes
	Sporobolus indicus	Paramatta grass		Yes		Yes
	Sporobolus jacquemontii	American rat's tail grass				
	Sporobolus natalensis	Giant rat's tail grass				
	Sporobolus pyramidalis	Giant rat's tail grass				
	Sporobolus virginicus	Carpet grass		Yes		Yes
Nassella spp.	*Nassella hyalina*	Cane needle grass		Yes		
	Nassella neesiana	Chilean needle grass		Yes		
	Nassella tenuissima	Mexican feather grass		Yes		
	Nassella trichotoma	Serrated tussock		Yes		
Pasture grasses	*Aristida behriana*	Brush wiregrass		Yes		
	Aristida ramosa	Purple wiregrass		Yes		
	Achnatherum caudatum	Broad kernel espartillo				
	Chloris truncata	Windmill grass		Yes		
	Cynodon dactylon	Couch grass		Yes		
	Dicanthium sericeum	Rhodes grass	Katambara	Yes		
	Festuca arundinacea syn. *elatior*	Tall fescue				
	Lolium perenne	Perennial rye grass		Yes		
	Pennisetum clandestinum	Kikuyu		Yes		
	Phalaris aquatica	Toowomba canary grass				
	Phragmites australis	Common reed		Yes		
Native grasses	*Austrodanthonia eriantha*	Hill wallaby-grass				
	Austrodanthonia setacea	Bristly wallaby-grass				
	Austrostipa blackii	Crested speargrass				
	Austrostipa breviglumis	Bamboo speargrass				
	Austrostipa curticoma	None				
	Austrostipa elegantissima	Feather speargrass				
	Austrostipa gibbosa	None				
	Austrostipa mollis	Soft speargrass				
	Austrostipa stuposa	Tasmanian speargrass				
	Bothriocloa macra	Red grass		Yes—planted		
	Cymbopogon refractus	Barbwire grass		Yes—planted		
	Macrolaena stipoides	Weeping grass		Yes—planted		
	Themeda triandra	Kangaroo grass		Yes—planted		
Crops	*Avena sativa*	Oats		Yes—planted		
	Erimo	Azuki bean				
	Glycine max	Soybean	Cowrie			
	Glycine max	Soybean	Surf			
	Glycine max	Soybean	Zeus			
	Glycine max	Soybean	Poseidon			
	Hordeum vulgare	Two-rowed barley	Franklin	Yes—planted		
	Hordeum vulgare	Barley	Kaputar	Yes—planted		

Classification	Botanical name	Common name	Cultivar	Seeds obtained	Live plants obtained	Testing in progress
	Hordeum vulgare	Barley	Keel	Yes—planted		
	Oryza sativa	Rice	Jarrah	Yes—planted		
	Oryza sativa	Rice	Japonica	Yes—planted		
	Saccharum officinarum	Sugar cane	BIV 83 - 3120			
	Saccharum officinarum	Sugar cane	Q - 203		Yes—planted	
	Saccharum officinarum	Sugar cane	Q - 155		Yes—planted	
	Saccharum officinarum	Sugar cane	Empire		Yes—planted	
	Saccharum officinarum	Sugar cane	Q-232		Yes—planted	
	Secale cereale	Rye		Yes—planted		
	Sorghum bicolor	Sweet sorghum		Yes—planted		
	Triticale	Triticale—wheat	Speedee	Yes—planted		
	Triticale	Triticale—wheat	Dural	Yes—planted		
	Triticum aestivum	Wheat		Yes—planted		
	Zea mays	Sweetcorn		Yes—planted		

Seed sources: rice—Bede Clarke (District Agronomist, Casino), Gary Wooley (Dungarubba); native seed—Ian Chivers (Native Seeds Pty Ltd, Cheltenham, Vic 3192); *Sporobolus creber*—Leahwyn Seed, NSW Seed Bank, Botanic Gardens Trust, Sydney; Pasture grasses and crops—David Officer (Research Agronomist, Grafton Primary Industries Institute, New South Wales Industry & Investment, Grafton); weedy and native grasses—Dr David McLaren (Principal Research Scientist—Weeds, BioSciences Research, Department of Primary Industries, Victoria).

Inoculation in the field and monitoring of effects

The aim of the research was to find a pasture field site without giant Parramatta grass disease but within the same climatic zone and that could be a trial inoculation site if the results of phylogenetic testing suggest that *Nigrospora oryzae* has significant effects on GPG and other weedy *Sporobolus* species.

Materials and methods

As noted, David Officer held a primary producers session in response to demand by those who responded to the questionnaire. He reported on the results of both the postal and the field surveys. Following that session, he explored primary producers' interest in hosting a possible trial site for inoculation with *N. oryzae*. Samples of GPG were collected from 20 properties in the Grafton area and sent to RMIT for isolation of any pathogenic fungi, to check for pre-existing infection (see Table 8).

Results

Fungi and occasionally bacteria and nematodes were isolated from GPG from most properties, but the majority contained only species of *Aspergillus, Chaetomium, Penicillium* and *Rhizopus* that are not thought to be pathogenic and have been isolated from pots and field sites elsewhere. *N. oryzae* was isolated from four of the 20 locations, although none showed characteristic symptoms and might represent early stages in disease. These properties would not be suitable as field trial sites. Suitable uninfected field sites at Taree have been identified and the property managers' consent obtained. Preliminary inspection of two possible sites did not find any diseased GPG. Once phylogenetic testing is complete, GPG at the identified properties will be inoculated with a spore suspension of *N. oryzae* and the effects will be monitored. It is expected that infection will not be observable visually for four to six months, which puts it well beyond the time frame of the current funding. Plants will therefore be monitored by local Industry & Investment personnel; the degree to which this will be possible depends on obtaining future funding from Meat and Livestock Australia or another funding body.

Table 8 Micro-organism assessment of GPG samples sent by Taree–Forster district property owners in 2010

Property no.	Location	Crown	Culm	Leaf
1	Site A Nherrol Flat 633 Rod Spice	*Aspergillus nidulans* *Chaemotium* sp. *Fusarium* sp. *Rhizopus* sp.	*Aspergillus nidulans* *Fusarium* sp. *Rhizopus* sp.	Bacteria
2	Site B Murray Rd Primary Sched Vieta	Nematodes *Rhizopus* sp.	*Penicillium* sp.	None
3	Site C Rod Spice Chrissy Gollaw Railway Crossing (North) Wingham	*Penicillium* sp. *Rhizopus* sp.	*Penicillium* sp.	None
4	Site D Rod Spice John BA Bridgeworks Rd Winghale Side	Bacteria *Chaemotium* sp. *Rhizopus* sp.	None	Bacteria *Penicillium* sp.
5	Site E 57 Denuer Rd Taree South	*Chaemotium* sp. *Penicillium* sp. *Rhizopus* sp.	*Rhizopus* sp.	*Fusarium* sp. *Rhizopus* sp.
6	Palmero IS (Opp. School) Kabunai	*Penicillium* sp. *Rhizopus* sp.	*Alternaria alternata* *Fusarium* sp. *Rhizopus* sp.	*Fusarium* sp.
7	Glen Esr Rd Upper Rolland Plains—1	*Fusarium* sp. *Rhizopus* sp.	*Alternaria alternata* *Penicillium* sp.	*Alternaria alternata*
8	Glen Esr Rd Upper Rolland Plains—2	*Rhizopus* sp.	*Fusarium* sp. *Rhizopus* sp.	*Alternaria alternata* *Fusarium* sp. *Rhizopus* sp.
9	Sewage Treatment Plant Mark Tull Gloucester Shire Council	*Rhizopus* sp.	Bacteria *Fusarium* sp.	Bacteria
10	693 Sherry Lane Ulmarra	*Penicillium* sp. *Rhizopus* sp.	None	None
11	Krambach Bucketts Way Old Bar Terry Inksons Place	*Fusarium* sp. *Rhizopus* sp. *Penicillium* sp. *Rhizopus* sp.	*Fusarium* sp. *Fusarium* sp. *Penicillium* sp.	None *Penicillium* sp.
12	Coolongolook Riverlands Estate	*Penicillium* sp. *Rhizopus* sp.	*Penicillium* sp.	*Aspergillus nidulans* Bacteria
13	Tionee 49 Denva Rd South Taree	*Chaemotium* sp. *Fusarium* sp. *Rhizopus* sp.	*Chaemotium* sp. *Fusarium* sp. Nematodes	*Rhizopus* sp.
14	Tucabia Cold Stream Rd	*Aspergillus* sp. *Chaemotium* sp. *Rhizopus* sp.	*Rhizopus* sp.	*Fusarium* sp. *Rhizopus* sp.
15	Tulloch Dargavilles Rd Nabige	*Chaemotium* sp. Nematodes *Penicillium* sp. *Rhizopus* sp.	*Fusarium* sp. *Rhizopus* sp.	None
16	Yates Dargavilles Rd Nabine	*Fusarium* sp. *Rhizopus* sp.	None	*Chaemotium* sp. *Fusarium* sp.

Property no.	Location	Crown	Culm	Leaf
17	Haydans Wharf Rd (West)	*Chaemotium* sp. *Nigrospora oryzae* *Penicillium* sp. *Rhizopus* sp.	*Nigrospora oryzae* *Rhizopus* sp.	*Fusarium* sp. *Penicillium* sp.
18	Haydans Wharf Rd (East)	*Nigrospora oryzae* *Penicillium* sp. *Rhizopus* sp.	*Fusarium* sp. *Nigrospora oryzae*	*Rhizopus* sp.
19	Noakes Inlet Rd	*Chaemotium* sp. *Nigrospora oryzae* *Penicillium* sp.	*Alternaria alternata* *Nigrospora oryzae*	*Aspergillus nidulans*
20	Combas	*Nigrospora oryzae* *Penicillium* sp. *Rhizopus* sp.	*Rhizopus* sp.	*Rhizopus* sp.

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Ramasamy, S, McLaren, DA, Pritchard, G, Officer, D, Bonilla, J, Preston, C et al. 2008, '2,2-DPA resistance in giant Parramatta grass (*Sporobolus fertilis*)', in *Proceedings of 16th Australian Weeds Conference, 18–22 May*, Cairns, pp. 98–100.

Using the Fungus *Nigrospora oryzae* for the Biological Control of Giant Parramatta Grass

by Ann Lawrie

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Giant Parramatta grass (GPG) (*Sporobolus fertilis*) is an aggressive perennial tussocky grass that is a declared noxious weed. It invades native pastures and reduces animal production. Its potential distribution is estimated at 23.7 million hectares in Australia.

The naturally occurring fungus, Nigrospora oryzae, causes a crown rot in GPG which has now been shown to reduce tussock size dramatically in the field.

The herbicide used in this research is based on spores of the naturally occurring fungus isolated from giant Parramatta grass growing in the Clarence Valley of northern NSW. This project was funded in Phase 1 of the National Weeds and Productivity Research Program, which was managed by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from 2008 to 2010. The Rural Industries Research and Development Corporation (RIRDC) is now publishing the final reports of these projects.

This report is an addition to RIRDC's diverse range of over 2000 research publications which can be viewed and freely downloaded from our website www.rirdc.gov.au. Information on the Weeds Program is available online at www.rirdc.gov.au/weeds

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Cover photos: Giant Parramatta grass

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