Pasture dieback on the North Coast of New South Wales. 1. Initial diagnostics to identify the causal agent

SP Boschma[A], A Daly[B], PS Gillespie[C], O Wildman[B], M Shrivasatava[B], N Jennings[D],
JM Geary[D] and SJ Baker[A]

[A]NSW Department of Primary Industries, 4 Marsden Park Rd, Calala NSW 2340:
suzanne.boschma@dpi.nsw.gov.au
[B]NSW Department of Primary Industries, Woodbridge Road, Menangle NSW 2568
[C]NSW Department of Primary Industries, 1447 Forest Road Orange NSW 2800
[D]North Coast Local Land Services, 79 Conway Street Lismore NSW 2480

Abstract: *Pasture dieback is a complex condition that results in poor growth and premature death of summer growing grasses. The condition has become widespread since 2015 and was identified on the North Coast of NSW in March 2020. In NSW, symptomatic and non-symptomatic plants, soils and insects were collected and analysed by the NSW Department of Primary Industries diagnostic laboratories. A range of fungal organisms were isolated including* Gaeumannomyces, Fusarium, Curvularia, Rhizoctonia *and* Bipolaris *spp. A number of species of nematodes were detected, though only a few were plant parasitic species. Samples were tested for viruses and several possible* Luteovirus-*like particles were observed. Insects identified included pasture mealybug (*Heliococcus summervillei*) which is associated with the condition in Queensland and mealybug ladybird (*Cryptolaemus montrouzieri*) which is a predator of mealybug.*

Key words: Plant Health Diagnostic Service, Biosecurity Collections Unit, fungi, virus, insect.

Introduction

Pasture dieback (PD) is a condition affecting large areas of sown and native summer growing grass pasture in Queensland (Buck 2017, 2021; AgForce 2019) and more recently on the North Coast of NSW. Dieback affected grasses are unthrifty and die prematurely. The condition was reported in buffel grass (*Cenchrus ciliaris*) pastures in Central Queensland in the 1990s (Graham and Conway 2000; Makiela 2008). However, there has been significant spread throughout eastern Queensland since 2015, with many grass species now affected. The area affected by PD in Queensland is difficult to determine, but estimates range from a conservative 200,000 ha to 4.4 million ha (AgForce 2019).

Buffel grass dieback in Central Queensland has been investigated several times but no specific cause, neither abiotic or biotic, has been established (Graham and Conway 2000; Makiela 2008; Makiela and Harrower 2008). Since 2017, in response to the current outbreak, Meat and Livestock Australia (MLA) in collaboration with multiple organisations has been conducting research to identify potential causal agent(s) (e.g. MLA 2018). Two insects are under investigation for their role in the condition: pasture mealybug (*Heliococcus summervillei;* Brookes 1978) (QUT 2018) and white ground pearl (*Margarodes australis*; Jakubuski 1965) (Thomson 2019; Thomson *et al.* 2021). Work is also being conducted to understand the role pathogens and the environment may be contributing to undermining the health of pastures leaving them more vulnerable to attack by these insect pests.

Pasture dieback was confirmed in the Tweed Valley on the North Coast of NSW in March 2020. To identify agent(s) that may have a role in the condition in NSW, we collected and analysed symptomatic and non-symptomatic plants, soil and insects from across the region. In this paper we summarise the findings of our initial analyses. Details of the expression of pasture dieback in the field and agronomic activities underway are provided in Boschma *et al. (2021).*

Methods

We collected samples of symptomatic and non-symptomatic plants from PD affected and neighbouring areas. Plant and soil samples were placed in plastic bags and kept cool. Insects found in affected areas were collected and placed in sealed vessels. All samples were inspected by NSW Department of Primary Industries' Plant Health Diagnostic Service and Biosecurity Collections Unit teams using a range of diagnostic techniques to identify microorganisms, nematodes and insects including light and electron microscopy, general and selective agar culture and DNA sequencing of taxonomically informative gene regions.

Results and discussion

We collected and assessed about 80 plant and insect samples from 30 sites. The symptomatic plants were predominantly *Paspalum* spp. (e.g. broadleaf paspalum and common paspalum), plus kikuyu (*Pennisetum clandestinum*) and Rhodes grass (*Chloris gayana*). A range of fungal organisms that can be pathogenic to plants were isolated. They included isolates from genera: *Gaeumannomyces, Rhizoctonia, Fusarium, Cladosporium, Alternaria, Curvularia, Bipolaris* and *Stemphyllium*. *Pythium* was occasionally detected also. However, no microorganism was isolated from all symptomatic samples or locations (Table 1). Additionally, we detected several possible Luteovirus-like particles via electron microscopy. Plant parasitic nematodes were detected at several sites, but only in low numbers not expected to be harmful (Table 1). The nematodes we identified were similar to those from a study conducted in Southern Queensland (Young *et al.* 2019).

We identified a range of insects, including pasture mealybug which was found at all sites. This same species is associated with the condition and under investigation in Queensland (QUT 2018). Mealybug ladybird (*Cryptolaemus montrouzieri*), native ants (Solenopsis sp.) and mites were also detected. The larva of mealybug ladybird, which is sometimes called mealybug destroyer is a known predator of mealybug (QUT 2018) while ants can aid mealybugs (QUT 2018). We did not detect ground pearl at any PD sites.

Pasture mealybug is not a new pest in Australia. It was first identified in the Cooroy district, Queensland in 1926 (Summerville 1928) then in Atherton, Queensland in 1938 (Brookes 1978). It was identified again in 2018 (Schutze *et al.* 2019) and was found at many, but not all, pasture dieback sites in Queensland. This mealybug was responsible for extensive damage to grass species in New Caledonia (Brinon *et al.* 2004). We believe that pasture mealybug is associated with the condition in NSW as it has been found at all sites; those sampled and described above, and sites we have visited subsequently. It is unclear whether the insect is a sole agent, vectoring a pathogen (e.g. virus or non-culturable microorganism) or the final component in a complex interaction of multiple agents and factors. It is important to note that morphological and molecular identification of specimens of *H. summervillei* from Queensland and NSW are the same species, but they show some variation to the specimens described by Brookes (1978). This may mean that the mealybug associated in pasture dieback is a different species. However, the differences are small and ecologically this mealybug fits the same niche and habits as that of its twenty plus northern European congeners.

The inconsistency in symptom expression of PD (M. Thomson, UQ, pers. comm.) and presence/absence of different pests and pathogens across sites in Queensland suggests that multiple conditions may be operating even though they are commonly referred to as 'pasture dieback'. We think that defining, accurately naming and understanding each agent needs to be a priority so that effective strategies can be developed for each. This will allow targeted research to be conducted addressing specific agents and be a better outcome for industry.

R&D priorities for NSW

Pasture mealybug is associated with PD on the North Coast NSW. However, it is not understood whether it is acting alone, vectoring a plant pathogen, or if it is the final pest that results in the collapse of a pasture that has been compromised by other agents and environmental factors. Current activities being conducted in Queensland would benefit by being extended to NSW. The southward spread of PD into different climates and poses a risk to different farming and grazing systems to most of Queensland.

There is a need to model the potential distribution of pasture mealybug using models such as CLIMEX. This analysis could predict the potential impact of this mealybug on a range of industries that may be threatened. In Queensland, PD is predominantly distributed throughout areas with 600+ mm annual average rainfall (S. Buck, QDAF, pers. comm). Based on rainfall alone, this means that large areas of northern NSW and along the NSW coast may be at risk of this condition. The interaction with temperature also needs to be evaluated.

In NSW, work is needed to understand the effect of the condition and pasture mealybug on sown and native summer and winter growing annual and perennial grass pasture and forage species as well as crops. As PD moves south and west in NSW, more species, farming systems and enterprises are at risk of becoming affected. Some native grass species have been identified as susceptible to the condition in Queensland. There are potentially many more susceptible native species. New species and systems will be exposed if PD moves into new agroecological zones.

Conclusions

Pasture dieback is having a devasting impact in pasture productivity and therefore livestock production on the North Coast of NSW. Work is continuing to identify the components of this complex condition in Queensland and NSW.

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Table 1. Microorganisms found in association with symptomatic pasture dieback samples.

Grass species affected	Microorganisms identified	Taxon	Method of Identification	Microorganisms not detected as a result of specific assays[1]
Digitaria eriantha	*Gaeumannomyces*, *Rhizoctonia*-like, *Fusarium*, *Alternaria*, *Absidia* and *Penicillium* spp.	Fungus	Light microscopy, agar culture, DNA sequencing	*Xylella*, *Phytophthora*, bacteria spp.
	Pythium sp.	Oomycete (water mould)	Selective agar culture	
Cenchrus ciliaris	*Gaeumannomyces* sp. and *Fusarium incarnatum/equiseti*	Fungus	Light microscopy, agar culture, DNA sequencing	N/A
Pennisetum clandestinum	*Gaeumannomyces*, *Curvularia*, *Bipolaris*, *Fusarium* and *Penicillium* spp.	Fungus	Light microscopy, agar culture	*Phytophthora*, *Pythium*, *Verrucalvus* spp. (water moulds)
Paspalum sp., *Chloris gayana*	*Fusarium* and *Epicoccum* spp.	Fungus	Light microscopy, agar culture	*Phytophthora* spp.
C. gayana	*Pythium* sp.	Protist	Selective agar culture	
P. clandestinum	*Gaeumannomyces* sp.	Fungus	Light microscopy	N/A
Paspalum sp.	*Gaeumannomyces*-like, *Rhizoctonia*-like, *Stemphyllium*, *Cladosporium* and *Epicoccum* spp.		Light microscopy	N/A
Unknown	Slime mould	Protist	Light microscopy	N/A
P. notatum	*Gaeumannomyces* sp.	Fungus	Light microscopy	WSMV, BYDV, CYDV
	Pratylenchus zeae	Nematode	Light microscopy, DNA sequencing	N/A
Paspalum sp.	*Gaeumannomyces*, *Cladosporium* and various *Dematiaceous* spp. (e.g. *Curvularia*) and *Fusarium chlamydosporum/equiseti*	Fungus	Light microscopy, agar culture, DNA sequencing	WSMV, BYDV, CYDV
	Ditylenchus sp.	Nematode	Light microscopy, DNA sequencing	
Cynodon dactylon	*Gaeumannomyces*-like, various *Dematiaceous* spp. (e.g. *Curvularia*)	Fungus	Light microscopy	WSMV, BYDV, CYDV
	Potyvirus-like	Virus	Electron microscopy	
Paspalum sp.	Various *Dematiaceous* spp. (e.g. *Alternaria*, *Dreschlera*-like)	Fungus	Light microscopy	N/A
	Pratylenchus zeae	Nematode	Light microscopy, DNA sequencing	
Paspalum sp	*Helicotylenchus dihysteria*	Nematode	Light microscopy, DNA sequencing	N/A
Paspalum sp.	*Fusarium* sp.	Fungus	Agar culture, DNA sequencing	N/A

Table 1. Microorganisms found in association with symptomatic pasture dieback samples.(continued)

C. gayana	N/A	N/A	N/A	Virus particles, WSMV, BYDV, CYDV
Paspalum sp.	*Fusarium solani*-like sp.	Fungus	Agar culture	N/A
Setaria sphacelata	*Bipolaris setariae*	Fungus	Agar culture, DNA sequencing	Virus particles, WSMV, BYDV, CYDV
Paspalum sp.	Possible *Luteovirus*-like particles	Virus	Electron microscopy	WSMV, BYDV, CYDV
Paspalum sp.	Possible *Luteovirus*-like particles	Virus	Electron microscopy	WSMV, BYDV, CYDV
Paspalum sp.	Possible *Luteovirus*-like particles	Virus	Electron microscopy	WSMV, BYDV, CYDV
Unknown	*Xiphinema* sp.	Nematode	Light microscopy, DNA sequencing	Virus particles
P. clandestinum	*Fusarium oxysporum*-like, *Fusarium*, *Dreschlera* and *Trichoderma* spp.	Fungus	Agar culture	*Phytophthora* spp.
	Pythium sp.	Oomycete (water mould)	Selective agar culture	
Paspalum sp.	N/A	N/A	N/A	Virus particles

[1] WSMV: wheat streak mosaic virus; BYDV: barley yellow dwarf virus; CYDV: cereal yellow dwarf virus.