Plantain (*Plantago lanceolata* L.) growth is limited under waterlogging

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Key words: Plantain; perennial ryegrass; waterlogging; abiotic stress; stress tolerance; pasture; persistence.

Abstract

Plantain (*Plantago lanceolata* L.; PL) is becoming an increasingly important component of New Zealand dairy pastures because of its ability to reduce nitrogen losses to the environment. However, widespread suggestions of its poor persistence, have led to industry concern over its suitability for dairy systems. Anecdotal evidence suggests that PL does not cope well in waterlogged soil, yet there is little scientific literature relevant to this issue. Thus, the aim of this study was to investigate the impact of waterlogging stress on PL growth and survival. In a glasshouse, the performance of PL under waterlogging was evaluated against perennial ryegrass (*Lolium perenne* L.; PRG), the predominant plant species in New Zealand dairy pastures. Three watering regimes were applied to PL and PRG plants in plastic pots: control (optimal watering), wet (soil at field capacity), and waterlogged (water table 5cm below the soil surface) for 39 days, before 27 days of recovery under control watering. Plant dry mass (DM) harvests were made on days 22, 39 and 66. The leaf DM of PL was reduced by the waterlogging treatment by 37% and 38% respectively during the treatment and recovery periods, in comparison with the control. In contrast, the leaf DM of PRG plants was only reduced by 18% and 3% by the waterlogging treatment during the treatment and recovery periods respectively, and so PRG produced 28% and 45% more leaf DM under waterlogging than PL, during the treatment and recovery periods, respectively. The root DM of PRG was 33% higher under waterlogging in comparison with the control at day 39, while PL root DM was not significantly affected by water treatment. Whilst PL growth appears to be sensitive to waterlogging stress, the survival of PL under waterlogged conditions suggests that it possesses some waterlogging tolerance strategies. PRG exhibited an enhanced ability for coping under, and recovering from waterlogging stress, which may have been associated with the maintenance of root growth under waterlogging. These findings suggest that PL growth may be somewhat limited in periodically waterlogged pastures, and the enhanced ability of PRG to cope with waterlogging stress, could provide it with a competitive advantage over PL under such conditions, should they coexist in a mixed sward.

Introduction

Plantain is becoming an increasingly important component of New Zealand dairy pastures because of its ability to reduce harmful environmental impacts. Plantain has secondary metabolites that, when included in a cow's diet, can increase urine volume (Minnée et al., 2020; Navarrete et al., 2020), reduce urine nitrogen concentration (Box et al., 2017; Totty et al., 2013), and suppress soil nitrification (Gardiner et al., 2020; Judson et al., 2019). These attributes, along with a high nutritive value, have made it a suitable, low-cost tool for reducing nitrate (NO_3^-) and nitrous oxide (N_2O) losses from highly productive, PRG (*Lolium perenne* L.) based, dairy systems (Doole et al., 2021). However there is growing anecdotal and scientific evidence that PL does not persist well under conventional pasture management strategies (Ayala et al., 2011a). Plantain is generally considered a short-lived forage species (Kuiper & Bos, 1992). Data from commercial farms in New Zealand suggests that high PL contents (>30% on a biomass basis) are attainable in the first two years following drilling, but cannot be maintained (Dodd et al., 2019). Competition from other pasture species (Cranston et al., 2015), livestock treading damage, compaction (Stewart, 1996) and over-grazing (Ayala et al., 2011b; Cranston et al., 2015) are potential contributing factors to declines in PL density in mixed pastures (Bryant et al., 2019). There is also anecdotal evidence that PL does not cope well in wet or waterlogged soils. An increased frequency and intensity of rainfall events, as well as fine-textured soil types, combine to create these conditions which typically occur from autumn to spring in many of the dairying regions of New Zealand. Literature detailing the effects of waterlogging on PL, in an agricultural field setting, are scarce. Mook et al. (1989) compared the demography of eight PL populations, across several grassland habitats. Winter PL mortality was most affected by a high soil moisture content. This effect was also found in PL in a fen habitat, where young plants in particular, exhibited a high winter mortality, even in mild winters (Van Groenendael, 1985). Glasshouse experiments have shown that PL possesses important waterlogging tolerance features. Grimoldi et al. (2005) found that PL possesses the ability to respond to flooding conditions by increasing root porosity through the generation of lysigenous aerenchyma (intercellular spaces for air transport). Pasture species vary in their tolerance of waterlogging and thus waterlogging has the potential to influence the botanical composition of a pasture (Bolton & McKenzie, 1946; Grieve et al., 1986). Perennial ryegrass can tolerate extended periods of high soil moisture (McFarlane et al., 2003; Ordóñez Vásquez, 2020), potentially allowing it to out-compete less tolerant species, such as PL, during wetter seasons. This may be an important dynamic involved in the loss of PL density that occurs in ryegrass-based pastures. Given the importance the industry is placing on PL for reducing nitrogen losses from dairying systems, it is therefore important that we consider the responses of PL and its most important companion species, PRG, to waterlogging stress. The current study investigated the effect of waterlogging on the regrowth and survival of PL and PRG in a glasshouse.

Methods

The experiment was conducted in a glasshouse, under ambient light, at Massey University's plant growth unit in Palmerston North, between March and August 2021. The trial consisted of 60 plastic pots with a volume of 8.96L. The pots were filled with dried soil in a 2:1 mix of Manawatu silt loam and common builder's sand. Soil fertility was non-limiting in this experiment. The mean soil bulk density of the pots at the commencement of the experiment was 1.46 g/cm^3. Plantain cv. *Agritonic* and perennial ryegrass cv. *Maxsyn* seeds were planted in five locations in pots on March 4, 2022. On April 14, seedlings were thinned, to leave five remaining plants per pot. Plants in all pots were cut to 5cm above the soil surface by hand on May 5, May 26, and June 7 before the commencement of the treatment period on June 8. The water treatments were imposed for 39 days, before a 27-day recovery period. Water treatments were defined by soil volumetric water content (VWC), calculated by dividing the volume of water in the pot (measured by weight) by the volume of the pot. Field capacity was determined to be 31% soil VWC. Pots in the control and wet treatments were topped up to 23% and 31.5% soil VWC respectively every two days throughout the treatment period. Pots in the waterlogged treatment were placed into large tubs, where the water level was maintained at 5cm below the soil surface in the pot. Holes in the bottom of the pots facilitated the waterlogging of soil within the pots. The soil VWC of waterlogged pots was on average 37%. Soil VWC was significantly different between treatments ($P<0.05$) throughout the water treatment period and not different between treatments during the recovery period. One mid-stress leaf DM cut was made on day 22 of the treatment period, and two destructive harvests of leaf DM and root DM were made on day 39 (end of treatment period) and day 66 (end of recovery period). The cumulative leaf DM, up to day 39, was calculated by adding leaf DM from the day 22 and day 39 harvests together. Unless otherwise stated, the leaf DM comparisons for the water treatment period are based on the cumulative DM produced up to day 39. Only control and waterlogged pots were sample for root material. The root to shoot DM ratio (R:S) was calculated by dividing total shoot DM (leaf + stem) by root DM. Statistical analysis of data was carried out using the PROC mixed procedure in SAS (SAS.Institute, 2020).

Results and Discussion

Waterlogging significantly reduced leaf DM production, regardless of species, at all harvests, in comparison with the control (Table 1). Plants in the wet treatment produced a similar amount of leaf DM to plants in the control treatment, suggesting that the wet treatment did not place any significant stress on plants. The leaf DM accumulation of PL was reduced by the waterlogging treatment by 37% and 38% respectively during the treatment and recovery periods, in comparison with the control. This result indicates that PL regrowth is particularly sensitive to waterlogging stress and demonstrates the risk that waterlogging poses for PL production on farm (Ordóñez Vásquez, 2020). However, it is important to note that no PL plants died under waterlogged conditions during the experiment (data not shown), suggesting that PL possesses some capacity for surviving under waterlogging stress (Grimoldi et al., 2005). Additionally, PL produced a similar amount of leaf DM to PRG under waterlogging during the first phase (day 1-22) of the treatment period, suggesting that the short-term waterlogging tolerance of these species is similar. However, the leaf DM of PRG plants was only reduced by 18% and 3% by waterlogging during the treatment and recovery periods respectively, in comparison with the control. This finding agrees with previous work showing PRG has an enhanced ability to cope with and recover from waterlogging stress, in comparison with other pasture species (Di Bella et al., 2022). The improved waterlogging tolerance of PRG may be related to its ability to sustain normal water soluble carbohydrate concentration in the shoot (Di Bella et al., 2022; Liu & Jiang, 2015) or an enhanced ability for reducing lipid peroxidation through the maintenance of antioxidant activity under stress (Liu & Jiang, 2015). Perennial ryegrass has also been shown to exhibit compensatory growth following the cessation of an abiotic stress (Korte & Chu, 1983; Nicholas et al., 2004), which could partly explain its rapid recovery once the waterlogging stress had been removed. Perennial ryegrass plants produced 28% and 45% more leaf DM respectively under waterlogging than PL plants, during the treatment and recovery periods respectively. This improved capacity for withstanding, and recovering from waterlogging stress, would likely provide PRG with a competitive advantage over PL if they were to co-exist in a pasture under these conditions (Ordóñez Vásquez, 2020).

Table 1. Leaf dry mass (DM) (g/pot) of Plantain and Perennial ryegrass following 22 and 39 days under control, wet, and waterlogged soil conditions, and a further 27 days (day 66) under control soil moisture conditions. Cumulative leaf DM, up to day 39, was calculated by adding leaf DM from the day 22 and day 39 harvests together. Means with different letters within the same column are significantly different (P<0.05).

Species	**Treatment**	**Leaf DM**			
		22-day harvest	39-day harvest	Cumulative to day 39	66-day harvest
Plantain	Control	9.38b	6.28a	15.66b	9.60a
	Waterlogged	6.81c	3.09c	9.90d	6.00c
	Wet	10.75a	6.60a	17.35a	8.17b
Perennial ryegrass	Control	9.07b	6.51a	15.58b	8.96ab
	Waterlogged	7.51c	5.21b	12.72c	8.71ab
	Wet	10.11a	6.31a	16.42ab	8.02b
Standard Error		0.4174	0.3329	0.6511	0.4722
Significance	Species	0.7885	0.0022	0.1972	0.1129
	Treatment	<.0001	<.0001	<.0001	0.0021
	Species x Treatment	0.1951	<.0001	0.0049	0.0041

The root DM of PRG was 33% higher under waterlogging in comparison with the control, while PL root DM was unaffected by water treatment (Table 2). The mean R:S significantly increased under waterlogging, regardless of species, although this effect disappeared over the recovery period. The reduction in R:S was likely a combination of a reduction in leaf DM production of plants under waterlogging, but the preservation of root mass. The R:S of PRG appeared to increase under waterlogging but was similar between treatments for PL. The maintenance of root growth under waterlogging has been associated with waterlogging tolerance in PRG (Di Bella et al., 2022; McFarlane et al., 2003). While increased root growth did not appear to be a waterlogging tolerance strategy of PL, root DM may have remained constant under waterlogging, through the replacement of dead primary roots with new adventitious roots in oxygenated areas close to the soil surface, which has been observed in waterlogged wheat (Malik et al., 2001). However, this mechanism requires further investigation in PL.

Table 2. Total root dry mass (DM) (g/pot) and root to shoot DM ratio of plantain and perennial ryegrass following 39 days under control and waterlogged soil conditions and a further 27 days (day 66) under control soil moisture conditions. Means with different letters within the same column are significantly different (P<0.05).

Species	**Treatment**	**Root DM**	**Root: Shoot DM**	**Root DM**	**Root: Shoot DM**
		39-day harvest		66-day harvest	
Plantain	Control	6.97b	0.66b	7.04b	0.40c
	Waterlogged	6.85b	0.86b	5.35b	0.43c
Perennial ryegrass	Control	8.45b	0.83b	12.13a	0.70b
	Waterlogged	11.23a	1.20a	15.09a	0.94a
Standard Error		0.92	0.10	1.35	0.08
Significance	Species	0.117	0.013	<.0001	<.0001
	Treatment	0.003	0.008	0.581	0.077
	Species x Treatment	0.091	0.364	0.061	0.158

Conclusions and/or Implications

Plantain regrowth was particularly sensitive to waterlogging during this experiment. However, the survival of PL under the waterlogging stress suggests that PL possesses some tolerance strategies for coping with waterlogging. Perennial ryegrass proved to have an enhanced ability for coping under waterlogging stress, in comparison with PL. The maintenance of leaf production by PRG under the waterlogging treatment may have been associated with an increase in root growth under waterlogging. These findings suggest that PL regrowth and survival may be limited in periodically waterlogged pastures. The enhanced ability of PRG to cope with waterlogging stress could provide it with a competitive advantage over PL under such conditions, should they coexist in a mixed sward.

Acknowledgements

The authors acknowledge the T.R. Ellett Agricultural Research Trust for project funding, the Kathleen Spragg Agricultural Trust for travel funding, and Massey University Plant Growth Unit staff along with Kate Fairbairn for their technical assistance.

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