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Proceeding Paper

Plantain (*Plantago lanceolata* L.) Leaf Elongation and Photosynthesis Rates Are Reduced under Waterlogging †

Samuel Wilson *, Daniel Donaghy, David Horne, Soledad Navarrete, Peter Kemp and Chris Rawlingson

School of Agriculture and Environment, Massey University, Palmerston North 4442, New Zealand; d.j.donaghy@massey.ac.nz (D.D.); d.j.horne@massey.ac.nz (D.H.); s.navarrete@massey.ac.nz (S.N.); p.kemp@massey.ac.nz (P.K.); c.n.rawlingson@massey.ac.nz (C.R.)

* Correspondence: s.s.wilson@massey.ac.nz

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Abstract: Plantain (*Plantago lanceolata* L.) has been identified by the New Zealand dairy sector as an option for reducing nitrogen losses from grazed pastures. However, there is growing concern over its poor persistence. Reports have suggested that plantain does not tolerate waterlogged soils; however, there is little scientific evidence to support those claims. Thus, the present study aimed to investigate the impact of waterlogging on plantain growth and survival. In a glasshouse, three water treatments were applied to plantain plants in pots: control (soil water below field capacity but not limited), wet (soil water marginally above field capacity), and waterlogged (water table 5 cm below the surface) for 39 days, followed by 27 days under the control watering treatment. Leaf elongation and photosynthesis were measured during the experiment. The mean leaf elongation rate of waterlogged plants was 37% lower than control plants during the stress period, but not significantly different than control plants during the recovery period. Waterlogging reduced the rate of photosynthesis in plantain leaves by 15% on average in comparison with control watering during the stress period; however, waterlogged and control plants had a similar mean photosynthesis rate during the recovery period. The results show that plantain growth and photosynthesis were significantly limited under waterlogging; however, the rapid recovery of both processes following the removal of stress suggests that important physiological functions remained intact under waterlogging, possibly due to tolerance mechanisms. These findings suggest that while waterlogging may cause limitations for plantain growth, there is no evidence to suggest that it alone could cause irreversible damage to plants and thus prevent their recovery. Rather, waterlogging stress could undermine the ability of plantain to compete with species that are tolerant of waterlogging within mixed pastures.

Keywords: narrow-leaved plantain; plant stress; stress tolerance; flooding

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1. Introduction

The loss of nitrogen (N) from grazed pastoral systems poses a significant threat to freshwater quality and has the potential to contribute to agricultural greenhouse gas emissions [1]. Plantain (*Plantago lanceolata* L.) has been identified by the New Zealand dairy sector as a low-cost option for reducing N losses from high-quality perennial ryegrass (*Lolium perenne* L.)-based pastures. When incorporated in dairy pastures, plantain (PL) can reduce N loading in urine patches [2] and suppress soil nitrification [3], leading to a reduction in N losses from the farm system [2].

However, there is growing industry concern over its poor persistence. One study found that PL contents greater than 30% on a dry matter basis in a PL–grass mixture were only achievable within the first two years following drilling [4]. This poses a problem, as at least 30% PL is required in a cow's diet to enable meaningful reductions in urine N concentration and excretion from cows [5]. Some anecdotal reports have suggested that PL does not cope well in wet or waterlogged soil conditions, which occur annually in many dairying regions of New Zealand. However, the literature detailing the effects of waterlogging on PL, in an agricultural setting, are scarce. Mook and Haeck [6] compared the demography of eight PL populations across several grassland habitats. Winter PL mortality was most affected by a high soil moisture content. Some glasshouse experiments have shown that PL possesses important waterlogging tolerance features that may allow PL to persist in periodically waterlogged soils. Grimoldi and Insausti [7] 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), which could allow for the continuation of vital plant functions while under waterlogging stress.

Given the importance the industry is placing on PL for reducing N losses from dairy farm systems, it is important that we consider both the effect of waterlogging stress on PL growth and the implications for its survival within waterlogged pastures.

2. Materials and 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 30 plastic pots with a volume of 8.96 L, which 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. PL cv. *Agritonic* seeds were planted in five locations in pots on 4 March 2022. On April 14, seedlings were thinned, leaving five remaining plants per pot.

Beginning 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) and 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 5 cm below the soil surface in the pot. The soil VWC of waterlogged pots was 37% on average during the stress period. Soil VWC was significantly different between water treatments ($p < 0.05$) throughout the treatment period and not different between treatments during the recovery period.

The following measurements were carried out on four of the total ten replicates (12 pots) throughout the experiment. Leaf elongation rate (LER) was measured in PL by selecting the two youngest growing leaves on two plants per pot. The length of leaves was measured as the distance from the base of the petiole to the tip of the leaf. The LER was determined as the average increase in the length of the two leaves divided by the number of days between the measurements. Photosynthesis was measured with a LICOR-6800 plant photosynthesis system. During the treatment period, one young growing leaf of one PL plant per replicate was scanned twice per week, and during the recovery period, once per week. Statistical analysis of data was carried out using the PROC mixed procedure in SAS (version 9.4. 2020) with water treatment as the fixed effect. Data was also analysed for repeated measures with date and treatment×date as fixed effects. Significance was declared at $p < 0.05$.

3. Results

3.1. Effect of Waterlogging on Leaf Elongation Rate

The LER of PL leaves in all treatments varied throughout the stress and recovery periods, ranging from 3 mm per day^{-1} to 19 mm per day^{-1} (Figure 1). On average, the LER of the waterlogged plants was 37% lower than that of the control plants during the stress period ($p < 0.01$). However, during the recovery period, the mean LER of the waterlogged plants was not significantly different than that of the control plants. There was no significant difference between the mean LER of PL plants under the control and the wet treatments during the stress or recovery periods. The date of measurement had a significant effect on the LER of PL plants during the stress and recovery periods, regardless of treatment ($p < 0.01$), with a noticeably higher LER occurring prior to days 8 and 35 of the stress period. There was no significant effect of an interaction between treatment and date on LER during the experiment.

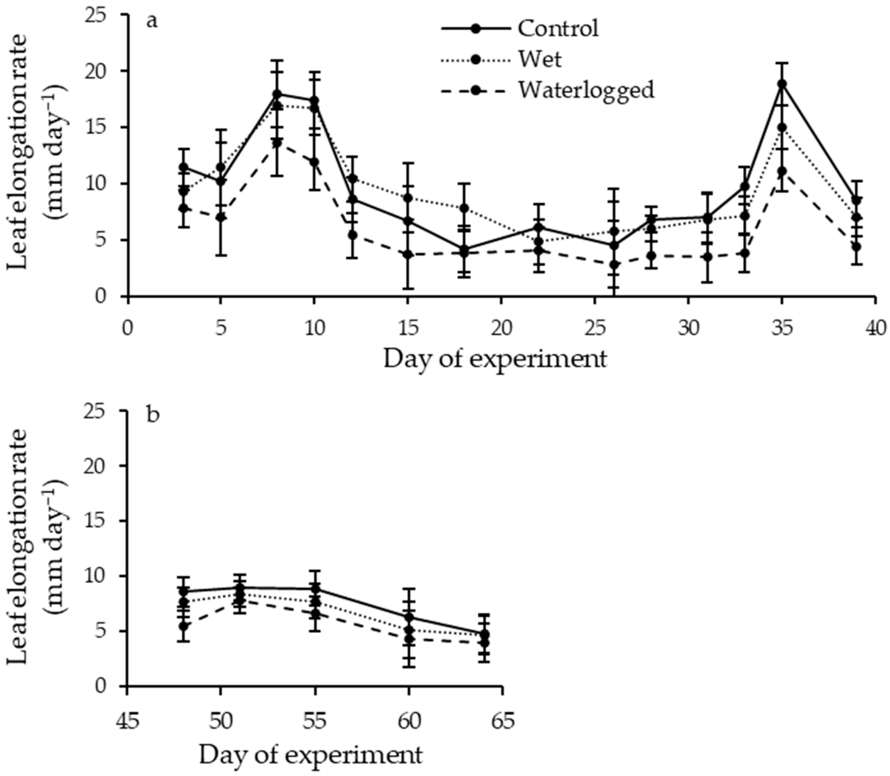

Figure 1. Leaf elongation rate of young plantain leaves under control (solid line), wet (dotted line), or waterlogged (dashed line) soil conditions during 39 days of stress treatment (**a**) and 27 days of recovery at control soil moisture (**b**). Error bars show standard error of the mean.

3.2. Effect of Waterlogging on Photosynthesis Rate

Waterlogging reduced the rate of photosynthesis in PL leaves by 15% on average in comparison with control watering during the stress period; however, waterlogged and control plants had a similar mean photosynthesis rate during the recovery period (Figure 2). The photosynthesis rate of PL plants in the control and wet treatments were similar throughout the experiment. There was no significant effect of time nor an interaction between treatment and time on the photosynthesis rate for PL leaves during the stress or recovery periods.

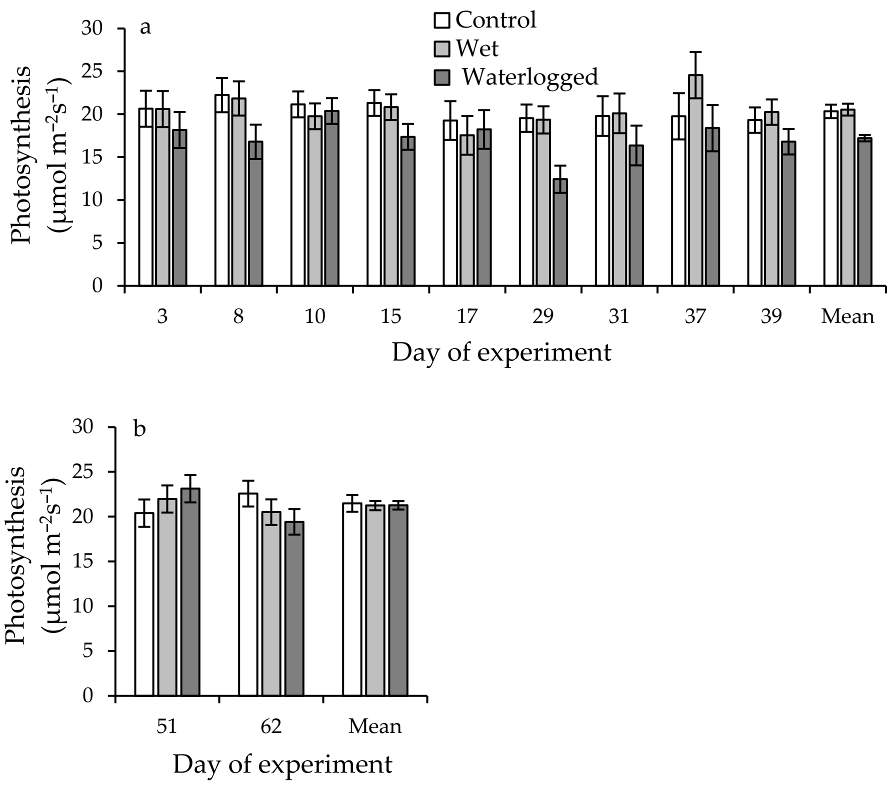

Figure 2. Photosynthesis rate of young plantain leaves under control (white bars), wet (light grey bars), or waterlogged (dark grey bars) soil conditions during 39 days of stress treatment (**a**) and 27 days of recovery at control soil moisture (**b**). Error bars show standard error of the mean.

4. Discussion

LER is highly sensitive to changes in plant water status and has been identified as an early indicator of plant sensitivity to waterlogging stress [8]. The reduction in the LER of PL leaves under waterlogging stress was similar to that of *Paspalum* grass (−40%), subjected to flooding for 28 days [9], and indicates that PL is sensitive to waterlogging [8]. However, the waterlogging stress did not cause a total cessation of growth in PL leaves, suggesting that PL may possess waterlogging tolerance features. In the pasture grasses cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* S.), the restoration of oxygen supply to waterlogged tissues through morphological changes was a major waterlogging tolerance mechanism which allowed for the continuation of above-ground growth [10]. In the current study, waterlogged PL plants may have developed additional adventitious roots close to the oxygenated soil surface or drawn oxygen from above the soil surface through lysigenous aerenchyma cells [7,10]. Additionally, the similarity in the LER of control and waterlogged PL plants during the recovery period suggests that plants recovered quickly from waterlogging, possibly due to the maintenance of important growth functions while under stress.

While the waterlogging stress did not lead to the death of PL plants in this experiment, a longer stress period, or the combination of stresses that normally occur in a grazed pasture, could have dire consequences for PL survival in the field. The reduction in the LER of a PL plant would limit its ability to generate leaf area and, thus, to capture light for use in photosynthesis [11]. This could be particularly critical for PL, as it has been suggested that PL has a limited capacity for energy storage in its crown and roots [12] and thus may be reliant on energy produced in photosynthesis for leaf re-growth following defoliation. Therefore, the decrease in the LER of PL under waterlogging could lead to further reductions in light interception, energy production, and growth following defoliation. This effect might be exacerbated if it were to co-exist in a grazed pasture with perennial ryegrass, which is productive and therefore competitive, in waterlogged soil [13,14].

Waterlogging stress may also result in the production of reactive oxygen species (ROS) that can lead to photosynthetic machinery destruction [15]. In lucerne (*Medicago sativa* L.) seedlings subjected to 10 days of waterlogging, a reduction in net photosynthesis and photochemical efficiency occurred concurrently with an increase in cell lipid peroxidation, suggesting that ROS had caused damage in the chloroplasts [15]. While the rate of photosynthesis was reduced in PL leaves under waterlogging stress, the rapid recovery of the photosynthesis rate following the removal of the stress could suggest that there was no permanent damage to photosynthetic apparatuses. This could be due to an adaptive process, such as in increase in antioxidant activity, which has been shown to be a potential waterlogging tolerance mechanism in perennial ryegrass [14]. The reduction in photosynthesis was likely related to something less permanent, such as a reduction in stomatal conductance [13].

The results show that PL growth and photosynthesis were limited under waterlogging; however, the rapid recovery of both processes following the removal of stress suggests that important physiological functions remained intact under waterlogging, possibly due to tolerance mechanisms. These findings suggest that while waterlogging may cause limitations for PL growth, there is no evidence to suggest that it alone could cause irreversible damage to plants and thus prevent their recovery. Rather, waterlogging stress could undermine the ability of PL to compete with waterlogging tolerant species within mixed pastures.

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