Screening for waterlogging tolerance in strawberry clover and other perennial legumes

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Abstract
Soil waterlogging and salinity have negative impacts on the growth and productivity of perennial pasture systems. Commonly used perennial legume species such as white clover (*Trifolium repens*) and lucerne (*Medicago sativa*) are relatively salt and waterlogging sensitive, thus not suitable for areas prone to these conditions. Alternative species such as strawberry clover (*Trifolium fragiferum*) which are known to have high tolerance to waterlogging and salinity can be used to maintain productivity and persistence of legumes within affected pastures. Palestine is the major commercial strawberry clover cultivar currently used in Tasmania. Some Tasmanian breeding lines showed great potential of being commercialised. The aim of this experiment was to compare the waterlogging tolerance of these breeding lines with Palestine as well as Talish clover (*Trifolium tumens*), white clover (*Trifolium repens*), red clover (*Trifolium pratense*), Caucasian clover (*Trifolium ambiguum*) and a sensitive barley (*Hordeum vulgare*) variety. The experiment was conducted outside and the duration of the experiment from germination to harvest was 75 days with the waterlogging treatment imposed at day 54. The level of tolerance was assessed by observing the chlorophyll content, chlorophyll fluorescence and wet/dry weights of plants weekly. Three of the Tasmanian Institute of Agricultures advanced selections were observed to have the potential to have a higher tolerance to waterlogging than cv. Palestine.

Keywords
Waterlogging, strawberry clover, tolerance, establishment.

Introduction
Environmental conditions within pasture systems are constantly changing. Salinity and soil waterlogging are becoming increasingly large problems on farms across southern Australia. In 2001 it was estimated that 7.5 million hectares of land in Australia were considered at risk from rising salinity, this is expected to increase to 17 million by 2050 (ABS 2001). High salinity levels are often coupled with waterlogging in the winter months, when the level of water in the soil rises above the level utilised by the plant (Barrett-Lennard 2003). In areas that are prone to waterlogging and salinity there is a shortage of suitable perennial pasture legume species. Neither of the most commonly used perennial legumes white clover nor lucerne are suitable in these areas. Lucerne is known to have a low tolerance to waterlogging and white clover is considered moderately salt sensitive (Rogers et al. 2005). A viable alternative is strawberry clover which has a high tolerance to waterlogging and high-moderate tolerance to salinity (Rogers et al. 2005). Currently there is only one commercial cultivar of strawberry clover widely used in Australia cv. Palestine which was released in 1938 (Nichols et al. 2012). Previous research has indicated that there is a high variation in salinity and waterlogging tolerance within wild populations of strawberry clover (Rumbaugh et al. 1993). This suggests that there is potential to develop a new cultivar with improved tolerance levels to cv. Palestine. Furthermore, Nichols et al. (2012) suggested that any further breeding work done on strawberry clover should be focused on those two traits.

The Tasmanian Institute of Agriculture (TIA) has developed several advanced selections of strawberry clover from wild germplasm collected in Azerbaijan by Eric Hall while on plant collecting trip run by the Australian temperate pasture Genetic Resource Centre (Hughes et al. 2008). The aim of this experiment was to determine if any of these advanced selections are more tolerant to waterlogging than cv. Palestine, with the eventual aim of developing a new cultivar with improved tolerance.

Methods

Experimental design

The experiment was established in Launceston, Tasmania (41.48'S, 147.13'E), and conducted outside under protective bird netting. Six accessions of strawberry clover were included in the trial alongside cv. Palestine and four other legume species; red clover cv. Rubitas, white clover cv. Storm, Talish clover cv. Permatas and Caucasian clover cv. Kuratas. A barley cv. Franklin was also included to act as an indicator species as it is known to have poor tolerance to waterlogging.

The experiment was conducted in four stainless steel containers (2.05 m x 1.05 m x 0.44 m) filled with a clay loam soil. Each container was divided into twelve sections, the twelve cultivars/selections were assigned to a section using a randomised block design. 120 seeds of each variety were left to germinate for twelve days on moist filter paper in plastic containers, the next day twelve seedlings from each of the allocated cultivar/selection were pricked out per section grouped in pairs. The plants were left to establish under normal growing conditions for twenty days. Then the water in the containers was raised to 0.22 m (half the depth of the container), and the weaker plant from each pair was removed. After 45 days, three plants were randomly selected from each section, their chlorophyll content was measured using a SPAD-502 Plus meter taking three readings from each plant from the largest expanded trifoliate leaf. The chlorophyll fluorescence was also measured using a OS-30p Chlorophyll fluorometer, again taking three readings per plant from the largest expanded trifoliate leaf. After these initial readings were taken two of the containers were allocated as controls with the water level remaining at 0.22 m, the water in the remaining two containers was raised to soil saturation (the point when water was beginning to pool on the surface). The chlorophyll content and chlorophyll fluorescence were measured once per week for the duration of the experiment. After 75 days three plants were randomly selected from each section and harvested to obtain their dry and wet weights. The plants were harvested just above the soil surface at the tap root, stolons and adventitious roots were included.

Results and Discussion

Conditions of waterlogging can cause plants to become stressed, which decreases productivity and can negatively affect plant health. A change in a plant chlorophyll content is a good indicator of a change in the stress levels in the plant, a decrease in chlorophyll content indicates a high level of stress (Pavlović et al. 2014). It appeared that the waterlogging treatment quickly caused the plants to become stressed. As was expected there was no significant ($P>0.05$) difference in the chlorophyll contents between plants prior to the waterlogging treatment being applied. However, one week after the treatment had been applied cv. Palestine already had a markedly lower chlorophyll content in plants under the waterlogged treatment in comparison to the control (Figure 1b). None of the other strawberry clover selections saw this rapid decrease in chlorophyll content, although most decreased over time (Figure 1a). Two weeks after the waterlogging treatment was applied Talish clover, Caucasian clover and barley we all measuring markedly lower chlorophyll contents under waterlogged conditions compared with the control treatments. These three species are known to have low tolerance to waterlogging so it was expected that these plants would perform poorly (Hall and Hurst 2012b; 2012a; Zhou 2012).

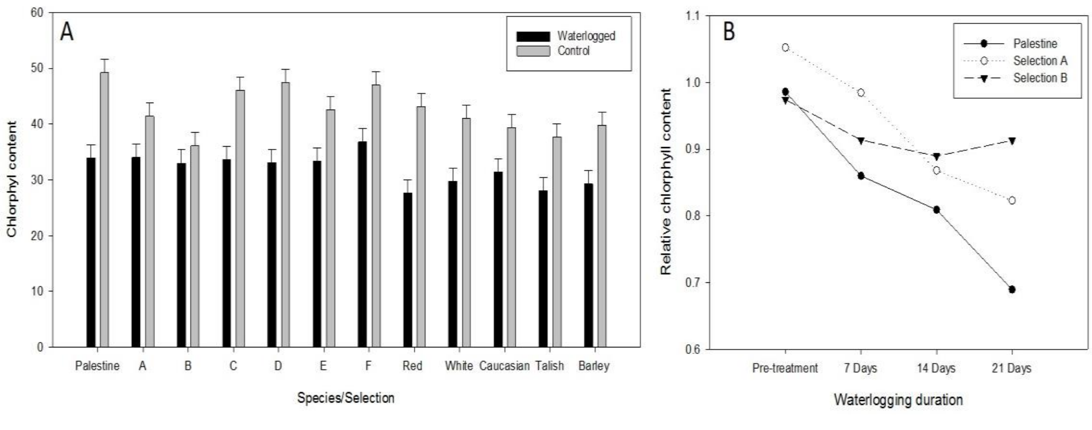

Figure 1. Chlorophyll content 21 days after waterlogging treatment imposed. Letters A-F represent strawberry clover selections. Red, white, Caucasian and Talish are all clovers (A) relative chlorophyll content of waterlogged strawberry clover accessions vs control plants over the experimental period (B).

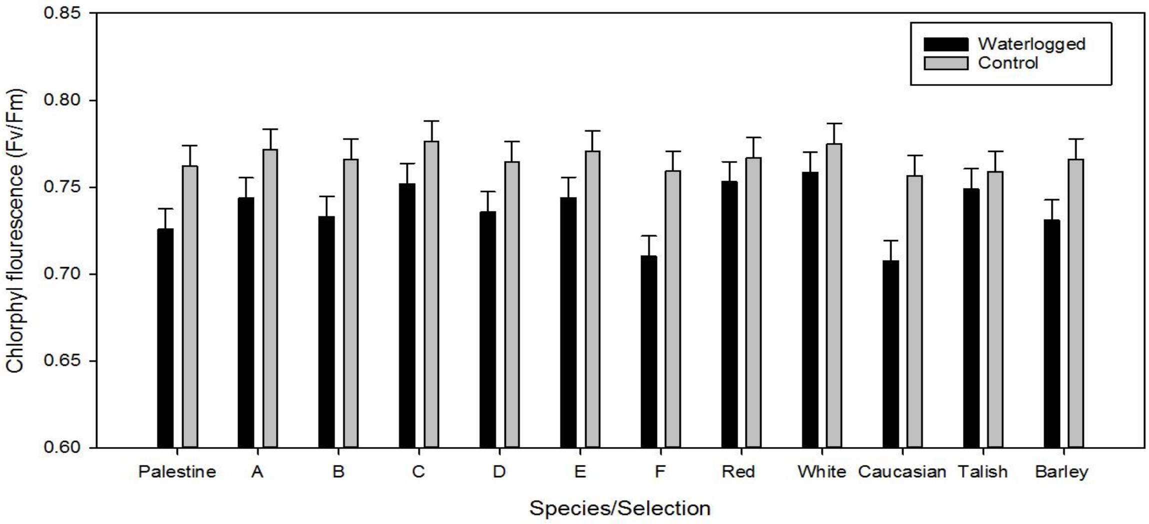

Figure 2. Chlorophyll fluorescence 21 days after waterlogging treatment imposed. Letters A-F represent strawberry clover selections. Red, white, Caucasian and Talish are all clovers.

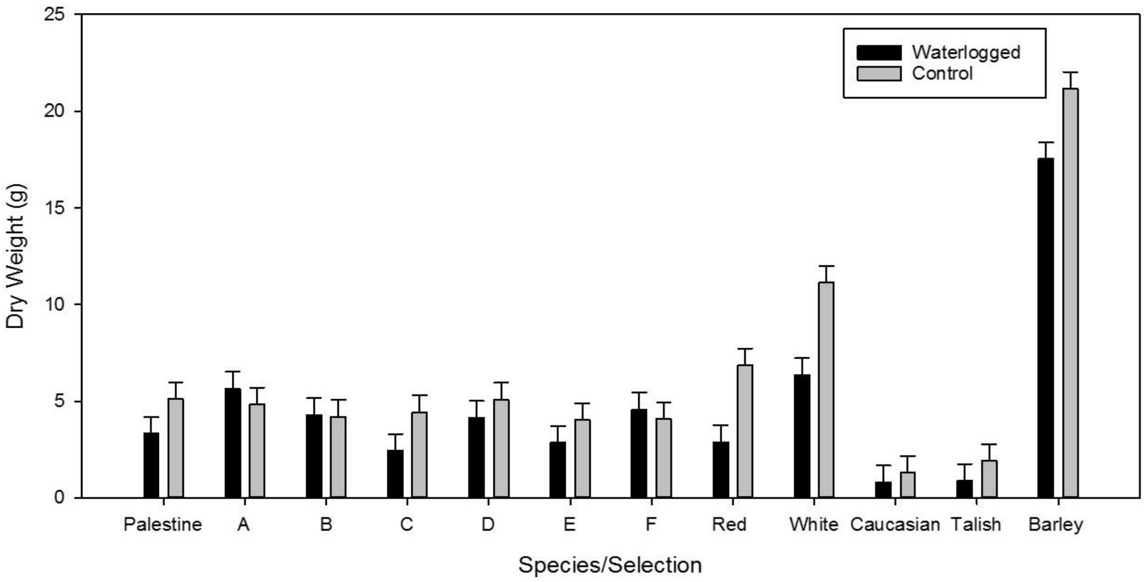

Figure 3. DM Yield of legume treatments 21 days after waterlogging treatment imposed. Letters A-F represent strawberry clover selections. Red, white, Caucasian and Talish are all clovers.

Three weeks after the application of the waterlogging 'selection B' was the only cultivar/selection not to have a significant ($P<0.05$) difference in chlorophyll content between the plants in the control and waterlogged treatments (Figure 1a). This indicates that this selection may be the most tolerant to waterlogging of the plants screened and potentially have higher tolerance to waterlogging than cv. Palestine. There are several mechanisms which are believed to be responsible for the high waterlogging tolerance of strawberry clover such as an increase in adventitious root and aerenchyma as displayed in previous experiments by Rogers and West (1993).

There were also significant ($P<0.05$) differences in chlorophyll fluorescence between waterlogged and control treatments after three weeks of waterlogging conditions (Figure 2). The largest differences were observed between plants in the waterlogged and control treatments of 'selection F and Caucasian clover'.

The dry weight yields can give an indication of available biomass and an indication of plant productivity. Of the clover species, white clover produced the largest amount of biomass under the control treatment, in contrast Talish clover and Caucasian clover produced the lowest amounts of biomass under (Figure 3). The DM yields of Talish clover and Caucasian clover are known to be low during their slow establishment (Hall and Hurst 2012a; 2012b). Under the waterlogged conditions the high productivity of white clover was matched by ‘selection A and ‘selection B’. ‘Selection A’ and ‘selection F’ recorded a marginal increase in biomass produced under waterlogged conditions while ‘selection B’ saw no change, this indicates that they might have increased tolerance to waterlogging as their productivity was not significantly decreased.

A plants ability to effectively photosynthesise is related to the amount of dry matter a plant produces (Long et al. 2006). Using Pearson’s correlation a moderate (r=0.35, P=0.01) correlation was found between Chlorophyll content and plant dry matter. These correlation were highlighted most strongly by strawberry clover selections A and B. As they saw the lowest decrease in chlorophyll content (Figure 1a) under waterlogged conditions, while presenting marginally higher dry matter yields (Figure 3). Increased levels of chlorophyll allow plants to increase their capacity to photosynthesise, producing more carbohydrates which can be transformed into biomass.

Conclusion

There was observed variation in waterlogging tolerance between the selections of strawberry clover and other screened clover species. Both white clover and strawberry clover demonstrated a relative tolerance to conditions of waterlogging, when compared with other species. Three of TIA’s advanced selections ‘selection A, B and F’ displayed the potential to have an increased tolerance to waterlogging than cv. Palestine. Further research is required on the traits and mechanisms relating to waterlogging tolerance in strawberry clover including aerenchyma in the adventitious roots and increased rates of nodulation on adventitious roots of strawberry clover under waterlogged conditions.

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