Growth and development of industrial hemp (*Cannabis sativa L.*) in response to irrigation treatments

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Abstract
Opportunity exists for Australian growers to develop a commercial cannabis/hemp industry to meet global demand for seed, fibre and pharmaceutical products. Development of a commercial scale hemp industry requires improved knowledge of hemp crop water relations to maximise yield, understand irrigation requirements in new production regions, and for scheduling of harvest. Currently little is known of the hemp plant-water relations. This study sought to evaluate the growth, development and physiology of industrial hemp under varying irrigation treatments. A preliminary greenhouse experiment was conducted to monitor response of industrial hemp cultivar Ferimon 12 to different irrigation treatments (2, 4, 6, 8 and 10 day intervals). We found significantly taller plants for 2 day compared with the 8 and 10 day irrigation intervals. Significantly higher above ground dry biomass was observed for plants irrigated with 6, 8 and 10 day intervals compared to the 2 and 4 days interval at 42 DAS. Significantly higher PSII photochemical capacity (Fv/Fm) and leaf chlorophyll content (SPAD value) was observed for plants irrigated with 6 and 10 day intervals compared with the 2 day intervals. There for 6 day irrigation interval is probably the best frequency of irrigation. Results from the preliminary experiment will be used to inform the design of future research which will be used to develop irrigation recommendations and refine the hemp crop simulation model to further support farmer decision making regarding water management.

Key Words
Industrial hemp, irrigation interval, crop growth, crop physiology, APSIM-hemp, irrigation management

Introduction
Industrial hemp (*Cannabis sativa L.*) is becoming an important multipurpose crop globally. There are numerous uses of fiber extracted from hemp stems such as textile and paper manufacturing. Hemp seeds have an important role in food, pharmaceutical and cosmetic production. Additionally, hemp shows great versatility for wide range of growing conditions and potentially high resource use efficiency (Amaducci and Scordia et al. 2015; Fike 2016). These features together with low maintenance requirement may encourage farmers to increase hemp cultivation in Australia. Demand for hemp products also has risen given changes in legislation that permit the sale and consumption of low-THC (tetrahydrocannabinol) hemp seed foods in Australia from 2018 (Rachel 2018).

Although numerous research studies on hemp agronomy were conducted in Europe, Canada and North America (Aubin et al. 2016; Cosentino and Riggi et al. 2013), few published studies have been conducted in Australia (Lisson and Mendham 2000). Much of this existing northern hemisphere knowledge is potentially transferable to the local environment; however, there is a necessity for studies to identify management practices best suited to the Australian production system. For example, studying responses of water use efficiency of hemp varieties in Australian agricultural system.

A crop simulation model (APSIM-hemp) has been developed within the Agricultural Production System sIMulator (APSIM) to simulate the growth, development and yield of fiber hemp in response to management, genotypic, soil and climatic inputs (Lisson et al., 2000a; Lisson et al., 2000b; Lisson et al., 2000c; Lisson et al., 2000d: Van der Werf, 1991). However, the model requires further improvements specifically regarding irrigation management of seed-based hemp cultivation. This study aims to fill these knowledge gaps by conducting series of experiments regarding irrigation management on seed-based hemp.

This paper presents some of the physiological and agronomical response of industrial hemp cultivar Ferimon 12 to different irrigation treatments from a preliminary greenhouse experiment undertaken in Tasmania.

Methods
Cultural conditions and plant material

The preliminary experiment was conducted in the March /July 2017 in controlled conditions at the University of Tasmania's Sandy Bay campus, Australia. The greenhouse temperature was maintained at around 25 ^{0}C and day length was maintained 13 hours 40 min to maximize the vegetative growth and prevent early flowering. Commercially grown monoecious industrial hemp cultivar (Ferimon 12) was grown in 30 L bags filled with potting mix which consisted of composted pine bark, coco peat and coarse sand (18:1:1 ratio). Dolomite 4.7 kg/m^3, Osmocote 10.0 kg/m^3 and Micromax 1.1 kg/m^3 were added to the potting mix to satisfy plant nutritional demands for the duration of the experiment. Seeds were over sown to a depth of 3 cm and the plant population were hand thinned to one plant per pot. Plants were irrigated using an overhead sprinkler irrigation system between sowing until the 5-6 true leaf pair stage. There were five different irrigation interval treatments; 2(W1), 4(W2), 6(W3), 8(W4) and 10(W5) day with eight replicates in a Randomized Complete Block Design. Irrigation treatments were started at 5-6 true leaf pair stage (20 days after seed sowing (DAS)) of the plant and were hand watered until drained upper limit was reached at each irrigation event. Drained upper limit was determined by gravimetrically.

Measurement and analysis of data

Days after sowing to reach 50% emergence of plants, 50% flower initiation and 50% flowering from each treatment were recorded. The most fully expanded leaf was assessed for relative chlorophyll content using a SPAD chlorophyll meter (Apogee Instruments, Logan UT, USA). PSII function and efficiency was measured from recently fully expanded leaf using a Phase Amplitude Modulated (PAM) fluorometry (OS-30 chlorophyll fluorometer, Opti Sciences, Hudson NH, USA) under prolonged dark conditions. SPAD and fluorometry were only measured on plants under 2, 6 and 10 days irrigation interval treatments. There were two consecutive harvests. First harvest was at 50% flower initiation (42 DAS). The second harvest was at the end of flowering at 105 DAS. Stem height was measured just before initiation of harvest from the soil surface to the growing tip of the plant. Plant stems were cut at the soil surface and separated into leaves, stem, petiole and reproductive parts. Harvested plant material was placed in paper bags and oven dried to constant weight at 65 ^{0}C and weighed when dried. Data were analyzed using Proc Mixed in SAS version 9.3. Repeated measure analysis was conducted for chlorophyll content and chlorophyll fluorescence.

Results and Discussion

The significant increases of Fv/Fm (PSII photochemical capacity) (Figure 1) had been noted from 53 DAS for the 6 day and 10 day irrigation interval. SPAD values (relative chlorophyll content) were significantly larger for 6 day and 10 day interval irrigation treatments compared with the 2 day interval treatment from 42 DAS onwards (Figure 2). The reason could be the reduction of individual leaf size when irrigation interval increased and thus the increases of density of chlorophyll pigment molecules. However, reduction of both Fv/Fm (PSII photochemical capacity) and SPAD value (chlorophyll content) were expected when increase irrigation interval as in some findings water stress has caused reduction of SPAD value and Fv/Fm due to destruction of chlorophyll molecules in some crop species (Shahenshah and Isoda, 2010). Therefore, the increases of irrigation interval may not cause any damage the chlorophyll molecules in this study.

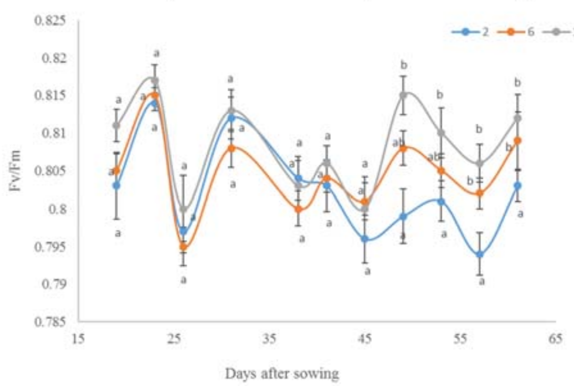

Figure 1. Weekly measurement of mean Fv/Fm of a fully expanded leaf of plant in each treatment group. Different letters above bars represent significant (p<0.05) difference between water treatments in each measurement. Bars represent the SE.

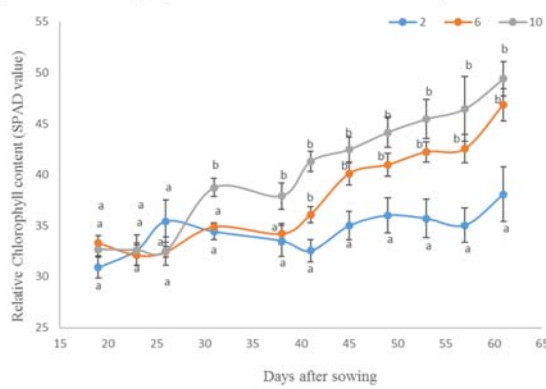

Figure 2. Weekly measurement of mean relative chlorophyll content of a fully expanded leaf of plant in each treatment group. Different letters above bars represent significant (p<0.05) difference between water treatments in each measurement. Bars represent the SE.

Table 1 illustrates the total above ground dry biomass of leaves, stem, petioles and reproductive parts of the plants at two consecutive harvests, 42 DAS and 105 DAS respectively. The 6, 8 and 10 day irrigation intervals showed significantly greater total dry biomass compared with the 2 and 4 day at 42 DAS harvest. Yet, at 105 DAS, irrigation treatment had no significant effect on total above ground dry biomass. At 105 DAS stems were significantly taller for the 2 day irrigation interval compared with the 8 or 10 day interval treatments (Figure 3). A similar trend was observed in an experiment conducted by Lisson and Mendham (1998), with the cultivar Kompolti under different irrigation regimes.

Another possibility is that the shorter frequency treatments were waterlogged early and this effect reduced later as plants and roots were larger. Also, the effect of frequent irrigation causing 'rank growth' cannot be ignored. This could actually be the reason for taller plants and lower SPAD readings on the 2 day treatment.

Therefore the 6 day interval treatment is probably the best as there was no reduction of biomass at 42 DAS compared to the other better treatments; height difference better than 8 and 10 days intervals, but no different to 4 day interval.

Table 1. Mean dry biomass per plant ± standard error from each irrigation interval treatment at 42 DAS and at 105 DAS. Different letters in the same column denote significant difference (P<0.05).

	Total dry biomass/plant (g) ± SE	
Irrigation intervals (days)	42 DAS	105 DAS
2	1.910a± 0.124	119.18a ±11.331
4	1.889a±0.143	110.69a±14.771
6	2.840b±0.267	108.34a±12.979
8	2.540b±0.169	96.89a±8.386
10	2.563b±0.343	100.16a±4.819

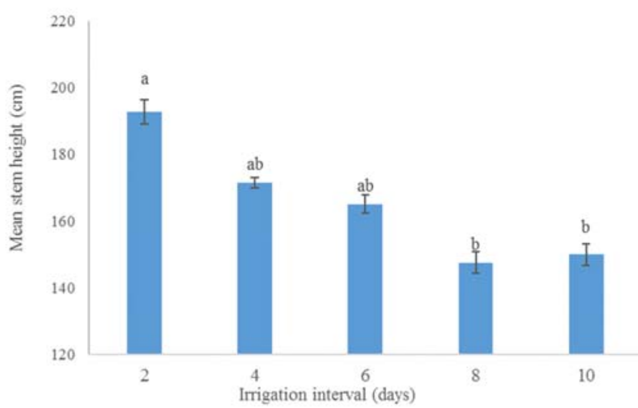

Figure 3. Mean stem height of plants at 105 DAS in each treatment group. Different letters above bars represent significant (p<0.05) difference between water treatments. Bars represent the SE.

Thermal time (day degrees above 1 ^{0}C) for 50% emergence of seedlings was 60 ^{0}Cd. Irrigation treatments had no significant effect on thermal time required for 50% flower initiation and 50% flowering. Average thermal time for 50% flower initiation and 50% flowering of across all treatments were 689 ^{0}Cd and 776 ^{0}Cd respectively. The results show that potentially the stress was not severe enough early to affect development; it is possible though that increasing the severity of water stress might accelerate early flowering (Takeno 2016).

Conclusions

Significant reduction of stem height and significant increases of Fv/Fm and SPAD values (due to decline of leaf size potentially affecting leaf expansion) were noted when irrigation interval was increased. This experiment will help to inform the necessary irrigation treatments to elucidate effects on Hemp growth under different water relations. Future greenhouse experiments will be conducted with refined irrigation treatments (e.g. 2, 5, 10 and 15 day irrigation intervals). Treatments will also be started at different growth stages such as at vegetative (5-6 true leaf pairs stage), flower initiation, 50% flowering, and at start of seed setting. Collected data from ongoing and future experiments will also be used to improve APSIM-hemp model which will support to cannabis farmers for efficient use of water for improve yield and quality of seed-based hemp.

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