Industrial hemp response to waterlogging stress: Influence of duration and growth stage on physiological performance and yield

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Abstract

Background and Aims:

Industrial hemp is widely regarded as sensitive to waterlogging, however, supporting evidence limited. This study aimed to evaluate the effects of waterlogging duration and timing on the physiology, growth, and development of hemp under controlled conditions.

Methods:

Two glasshouse experiments were conducted using industrial hemp (*Cannabis sativa* L.) cv. Ferimon 12. The first examined five waterlogging durations: 0 (control), 3, 6, 12, and 24 days, imposed at the eight true leaf pair stage. The second examined 3- and 6-day waterlogging events imposed at six true leaf pair and flower initiation stages.

Results:

Waterlogging beyond 6 days caused severe damage, and plants died after 12 days of continuous waterlogging. Plants subjected to 3–6 days of waterlogging survived but showed reduced photosynthetic activity, biomass, and seed yield, particularly after 6 days. Sensitivity varied with the growth stage. Plants were more vulnerable during the six true leaf pair stage than at flower initiation stage.

Conclusion:

Hemp is highly sensitive to waterlogging, especially during early vegetative development, with even short-term saturation causing measurable physiological and growth decline. These findings characterise the physiological limits of hemp under controlled conditions and highlight the importance of avoiding prolonged rootzone saturation during early growth.

Introduction

Industrial hemp (*Cannabis sativa* L.) is one of the world's oldest cultivated crops and has been widely adopted due to its versatility. It is used in textiles, bioplastics, construction materials, nutritional products, and medicinal purposes, with cannabidiol rich varieties valued for their therapeutic properties. The growing demand for hemp-derived products, together with the crop's broad adaptability and the easing of cultivation restrictions in many regions, has renewed interest in understanding how environmental conditions and agronomic management practices influence hemp growth and productivity.

Hemp requires adequate moisture during early growth, especially within the first 6 weeks to ensure successful establishment (Adesina et al. 2020). However, excessive water supply can hinder growth and yield and, in severe cases, lead to crop failure.

In a previous study, Kumar et al. (2025) showed that excess water causing waterlogging during the vegetative and early flowering stages reduced photosynthesis by 20–30% and limited the growth of industrial hemp in field conditions. This field study established the impact of contrasting water regimes on crop performance. However, a precise evaluation of waterlogging effects requires controlled conditions; therefore, the present experiment was conducted in a pot-based controlled system. Medicinal cannabis is also commonly grown in commercial and research settings using pot- or container-based systems. Cannabis and hemp are taxonomically the same species, *Cannabis sativa* L., differing mainly in cannabinoid profile and end use applications. Owing to their similar growth physiology and cultivation practices, including containerised production systems, the present study has direct relevance to both medicinal cannabis and industrial hemp industries.

Waterlogging, resulting from over irrigation, poor drainage, high water tables, low soil conductivity, perched water tables, or seepage, causes prolonged rootzone saturation, creating anaerobic conditions that restrict root respiration and disrupt plant growth, nutrient uptake, and hormonal balance (Mehmood et al. 2025).

Under anaerobic conditions, plant roots shift from aerobic respiration to less efficient fermentation pathways, resulting in lower energy production (Zhang et al. 2025a) and the accumulation of toxic metabolites that damage root cells (Zhang et al. 2025a). Root hypoxia or anoxia also elevates abscisic acid (ABA) levels in leaves, resulting in stomatal closure, which restricts water and nutrient uptake, reduces photosynthetic rates, and ultimately suppresses growth and causes wilting (Manghwar et al. 2024; Umathe et al. 2025). Waterlogging further decreases chlorophyll content and chlorophyll fluorescence, indicating impaired photosynthetic performance (Aslam et al. 2023; Yang et al. 2023).

The impact of waterlogging on crop performance varies with species sensitivity, the duration of flooding and the growth stage at which it occurs (Githui et al. 2022). Kumar et al. (2025) reported that a short duration of waterlogging at the vegetative stage of industrial hemp in a field trial reduced photosynthetic traits; however, these traits recovered by the end of the growth stage. In cotton, for example, waterlogging during flowering substantially reduces morphological traits, including plant height, leaf area, and stem diameter and diminishes fibre quality by reducing fibre length, strength, and uniformity (Beegum et al. 2023; Najeeb et al. 2015). Although short periods of waterlogging may allow partial recovery, prolonged exposure consistently leads to significant reductions in biomass accumulation, growth, and final yield (Ashraf et al. 2012; Ghobadi & Ghobadi 2010; Najeeb et al. 2015; Wang et al. 2017).

Although the effects of waterlogging have been extensively examined in crops such as cotton, wheat and barley (Beegum et al. 2023; Celedonio et al. 2016; Masoni et al. 2016; Pampana et al. 2016; Wang et al. 2017), very few published studies have investigated how hemp responds to this stress (Kumar et al. 2025). Anecdotal reports also indicate that hemp may be particularly sensitive to waterlogging; however, the influence of waterlogging duration and the growth stage at which it occurs on hemp survival, photosynthesis, growth, phenology, and yield remains unclear.

This study addresses this gap by evaluating the effects of waterlogging imposed at different growth stages and for varying durations on hemp grown under controlled glasshouse conditions.

Materials and methods

Two glasshouse experiments were conducted to evaluate the effects of waterlogging on hemp growth and physiological responses. The first experiment assessed plant tolerance to different durations of waterlogging, while the second experiment was further refined based on initial findings to examine plant responses to moderate waterlogging imposed at different growth stages.

Both experiments used a single genotype grown in a uniform substrate to ensure consistency and minimise genetic and edaphic variability. The use of a substrate also allowed precise control of waterlogging duration and timing by reducing physical and chemical heterogeneity typical of field soils. This design enabled clear attribution of plant responses to the imposed treatments and allowed physiological responses to rootzone hypoxia to be isolated from confounding soil effects. This controlled approach aligns with the study's objective of establishing a foundational understanding of the physiological and morphological responses of hemp to waterlogging.

Location and growth conditions of the experiments

The experiments were conducted in a glasshouse at the Horticulture Research Centre at the University of Tasmania, Hobart, Australia. The first experiment was conducted from February to June 2019 and the second experiment from August to December 2019. Daylength was maintained at 13 h 40 min to delay flowering (Lisson et al. 2000), using natural daylight supplemented, when required, with two 400 W mercury vapour lamps providing approximately 40–80 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR). Supplemental lighting was used solely for photoperiod extension and supplied low intensity light, typical of glasshouse photoperiod control, with the duration adjusted seasonally.

Daily maximum and minimum temperatures were recorded using a Tiny Tag data logger. Glasshouse temperatures were generally maintained, through ventilation, within an approximate average maximum of 25°C and an average minimum of 15°C, which falls within the optimal range for hemp germination and growth. However, a few higher temperature events (up to ~ 31°C) were recorded due to occasional environmental fluctuations; these instances were infrequent.

Cultural methods

Hemp seeds (cv. Ferimon 12, monoecious) were sown in 15 L woven polypropylene bags (24 cm height) filled with a substrate consisting of a 1:1:1 mix of peat moss, perlite, and vermiculite. A bulk density of 0.13 g cm^{-3} was assumed for unit conversions, consistent with values reported for similar substrates. The substrate pH was adjusted to 6.0-6.5 by incorporating dolomite at 36.2 g kg^{-1}, providing Ca and Mg while buffering acidity.

Basal nutrients were supplied by adding a controlled-release fertiliser (Osmocote, 7.7 g kg^{-1}, N:P:K ratio 15:4:9 plus micronutrients) and a micronutrient blend (Micromax, 8.5 g kg^{-1}, Fe 6% w/w, Mn 2.5% w/w, Zn 1% w/w, Cu 0.5% w/w, B 0.1% w/w, Mo 0.05% w/w), applied according to manufacturer recommendations to meet nutritional requirements.

Four seeds were sown per pot at ~ 3 cm depth and thinned to one plant per pot after emergence.

Treatments

Experiment 1

Waterlogging treatments began at 43 Days After Sowing (DAS) (eight true leaf pair stage). Before treatment, each pot received approximately 1.27 L of water per day via overhead sprinklers (19 L h^{-1}). After treatment commenced, control plants continued to receive irrigation for 8 min per day, applied in three intervals.

A randomised complete block design was used with six replicates and five waterlogging durations: 0 (control), 3, 6, 12, and 24 days. Pots were immersed in 50 L containers, with water maintained 2.5 cm above the surface of the substrate. Following each waterlogging period, pots were drained for 24 h without irrigation, after which normal irrigation resumed.

Experiment 2

Similar to Experiment 1, before treatment each pot received 1.27 L of water per day via overhead sprinklers (19 L h^{-1}). After treatment commenced, control plants continued to receive irrigation for 8 min per day, applied in three intervals.

A randomised complete block design was used with five replicates and three waterlogging durations: 0 (control), 3, and 6 days. Waterlogging was applied at two growth stages: six true leaf pair (35 DAS, GS 1012) and flower initiation (49 DAS, GS 2301). Following each waterlogging period, pots were drained for 24 h without irrigation, after which normal irrigation resumed.

The treatments therefore included: 0-day waterlogging (**control**), 3-day waterlogging at six true leaf pair (**3 VE**), 6-day waterlogging at six true leaf pair (**6 VE**), 3-day waterlogging at flower initiation (**3 FI**), and 6-day waterlogging at flower initiation (**6 FI**).

Measurements

Volumetric water content of the substrate was recorded weekly at depths of 4.2, 12.5, and 20.8 cm using an Acclima TDR-315 sensor. Values were averaged across depth and summed to obtain total substrate water content.

From 4 DAS (after seedling emergence), plant development was monitored to calculate thermal time to flower initiation (first visible pistils), end of flowering (95% male flowers withered), and seed maturity (90% hard seeds), based on daily maximum and minimum temperatures and a base temperature of 5.7°C (Cosentino et al. 2013; Van der werf et al. 1995). Growth stages followed Mediavilla et al. (1998).

Chlorophyll content was measured at 11:00 am on the two youngest fully expanded leaves using a SPAD meter (Apogee Instruments, Logan UT, USA). Chlorophyll fluorescence (F_v/F_m) was measured after 30 min dark adaptation using a phase amplitude modulated (PAM) fluorometer (OS-30 chlorophyll fluorometer, Opti Sciences, Hudson NH, USA) on two youngest fully expanded leaves to assess PSII efficiency.

Net CO_2 assimilation rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs) were measured weekly using a Li-Cor 6400XT infrared gas analyser (IRGA). Before measurement, plants were exposed to full sunlight for 1 h near midday. Measurements were taken on two youngest fully expanded leaves. The IRGA chamber-maintained leaf temperature at 20 ^{o}C, CO_2 concentration at 400 µmol mol^{-1}, CO_2 flow rate at 400 µmol s^{-1} and PAR at 1500 µmol m^{-2} s^{-1}. Photosynthetic water use efficiency (PWUE) was calculated as Pn/Tr.

Plant height and stem diameter were measured weekly and at harvest.

Plants were harvested at the end of the seed maturity; dead and senesced leaves were excluded. In experiment 1, control and 3-day treatments were harvested at 142 DAS, and the 6-day treatment at 138 DAS. In experiment 2, control and 3 VE treatments were harvested at 146 DAS. Waterlogged plants matured earlier: 6 VE plants were harvested at 139 DAS (with two plants dying before maturity), and 6 FI plants at 133 DAS.

At each harvest, fresh weight and leaf area were recorded. Bark proportion was determined from stem sections. Stem, leaf, and reproductive tissues were separated and oven dried at 60°C for 48 h to determine dry yield. Seeds were separated, cleaned and weighed, and total seed yield (g m^{-2}) and thousand seed weight (TSW) were calculated for each treatment.

Data analysis

Proc Mixed in SAS version 9.4 was used for data analysis. Repeated measurements collected over time were presented graphically with standard error (SE) bars to illustrate treatment trends and variability. Data from destructive measurements were analysed using analysis of variance (ANOVA) to evaluate the effects of waterlogging on plant responses.

In Experiment 1, which included only waterlogging duration treatments (0-control, 3, 6, 12, and 24 days), treatment effects were assessed using one way ANOVA.

In Experiment 2, waterlogged treatments were analysed using two-way ANOVA, with waterlogging duration (3 and 6 days) and growth stage (vegetative and flowering initiation) as fixed factors; the non-

waterlogged control was excluded from the interaction analysis.

Statistical significance was determined using Type III F tests at $P \leq 0.05$. When interactions were significant, treatment means were separated using Tukey's HSD test. For variables with non-significant interactions, main effects were interpreted and means compared using Tukey's HSD test.

Results

Experiment 1

Volumetric water content of the substrate

Total volumetric water content in the control (0-day) treatment remained stable throughout the experiment. Waterlogging increased substrate water content by approximately 0.0417 L L^{-1} in the 3-day treatment, 0.0625 L L^{-1} in the 6- and 12-day treatments, and 0.083 L L^{-1} in the 24-day treatment. Plants subjected to 12- and 24 days of waterlogging died by 66 DAS (23 days after waterlogging). After waterlogging ceased, water content declined across all treatments. Differences in substrate water content among treatments were minimal both before and after the waterlogging events. (Data not shown).

Leaf chlorophyll, CO_2 exchange, stomatal conductance and PWUE

Each waterlogging duration followed a similar overall trend for relative chlorophyll content, Pn, Tr, Gs, and photosynthetic water-use efficiency (PWUE) across the crop cycle (Fig. 1a-c; Tr and Gs data not shown). In the control, these variables increased to a plateau near flowering and then gradually declined after about 108 DAS, consistently remaining higher than in waterlogged plants. The 3-day treatment showed an initial decline but subsequently recovered, following a similar, though lower, trajectory to the control (Fig. 1a-c).

Plants exposed to the 6 days of waterlogging showed a more prolonged decline in the above variables, with recovery beginning around 74 DAS. By the end of the cycle, their values approached those of the control and 3-day treatments (Fig. 1a-b). PWUE in 6-day waterlogged plants, however, remained lower than both the control and 3-day plants until the end of the crop cycle (Fig. 1c). Plants subjected to 12- and 24-day of waterlogging showed a decline in physiological parameters from the outset and died at 23 days after waterlogging began (Fig. 1a-c).

[Insert Fig. 1 here]

Crop Phenology

Waterlogging duration did not affect the calendar and thermal time required for flower initiation (data not shown). The average thermal time for flower initiation was 662.2 ^{o}Cd. However, the duration from flower

initiation to the end of flowering was significantly shorter ($P < 0.05$) in the 6-day waterlogging treatment (343 ^{o}Cd) compared with the control and 3-day treatments (393 ^{o}Cd). All plants in the 12- and 24-day treatments died shortly after flowering, whereas plants in the other treatments continued to grow. The thermal time from the end of flowering to seed maturity was consistent (~ 763 ^{o}Cd) across the control, 3-day, and 6-day treatments, with no significant differences.

Plant height and stem diameter

In the control, plant height and stem diameter increased steadily until flowering (~ 66 DAS) and then plateaued. The 3-day waterlogging treatment caused a temporary slowdown of growth, with full recovery by ~ 94 DAS. In contrast, plants waterlogged for 6 days experienced a prolonged growth setback and only partial recovery, resulting in reduced final height and diameter compared with both the control and 3-day treatments. Plants subjected to 12- and 24-day waterlogging showed irreversible growth suppression and died by 66 DAS (Fig. 2).

[Insert Fig. 2 here]

Specific leaf area (SLA), dry biomass, bark yield and seed yield

At harvest, SLA declined significantly as waterlogging duration increased from 0 to 6 days ($P < 0.05$). Similarly, leaf, stem, and total aboveground dry weights (TADW) decreased with longer waterlogging, with the 6-day treatment producing significantly lower values than both the control and 3-day treatments ($P < 0.05$) (Table 1).

Table 1
Mean SLA, dry biomass production, bark yield and seed yield at harvest in Experiment 1

Waterlogging duration (days)	SLA (m^2 kg^{-1})	DW leaves (g $plant^{-1}$)	DW stems (g $plant^{-1}$)	TADW (g $plant^{-1}$)	Bark yield (g $plant^{-1}$)	Seed yield (g $plant^{-1}$)
0 (control)	15.0^{a}	21.1^{a}	40.1^{a}	77.0^{a}	15.0^{a}	8.2^{a}
3	13.7^{b}	16.3^{a}	34.4^{a}	62.4^{a}	11.0^{a}	6.9^{a}
6	8.6^{c}	10.0^{b}	16.8^{b}	34.7^{b}	5.0^{b}	4.3^{b}

Mean value of data. Within a column, data means followed by the different letters are significantly different at the $P < 0.05$ level. SLA, specific leaf area; DW, dry weight; TADW, total above ground dry weight.

Waterlogging had no significant effect on bark proportion at seed maturity (data not shown). However, both bark and seed yield declined with waterlogging duration, with the lowest yields recorded in the 6-day treatment ($P < 0.05$) (Table 1).

[Insert Table 1 here]

Experiment 2

Volumetric water content of the substrate

Water content in the control treatment (0-day) remained relatively consistent throughout the experiment. In the waterlogged treatments, substrate water content increased by ~ 0.083 L L^{-1} following waterlogging and then declined after the waterlogging conditions were removed. Similar to Experiment 1, differences in water content among treatments were minimal both before and after the waterlogging events. (Data not shown).

Leaf chlorophyll, CO2 exchange, stomatal conductance and PWUE

Each treatment showed a similar overall trend for relative chlorophyll content, F_v/F_m, Pn, Tr and Gs across the crop cycle (Fig. 3a-c; Tr and Gs data not shown). Following the initial decline during waterlogging, plants in the 3-day treatments (3 VE and 3 FI) recovered and closely followed the control trajectory, although at slightly lower values.

In contrast, plants exposed to 6 days of waterlogging (6 VE and 6 FI) showed a more prolonged reduction in relative chlorophyll content, chlorophyll fluorescence, Pn, Tr and Gs, before gradually recovering to values comparable to the control (Fig. 3a-c).

PWUE in plants waterlogged at flower initiation (3 FI and 6 FI) was comparable to the control throughout the experiment. The 3 VE plants showed slightly lower PWUE until 105 DAS, while 6 VE plants exhibited a marked reduction immediately after waterlogging, followed by gradual recovery after 40 DAS and convergence with the control by 105 DAS (Fig. 3d).

[Insert Fig. 3 here]

Crop Phenology

Flower initiation occurred 7 days earlier ($P < 0.05$) in 6 VE plants, which required 517 ^{o}Cd of thermal time compared with the other treatments. The 6 FI plants progressed more rapidly ($P < 0.05$) through both subsequent developmental phases, requiring ~ 335°Cd from flower initiation to the end of flowering and ~ 847°Cd from the end of flowering to seed maturity. In comparison, all other treatments required approximately ~ 416°Cd and ~ 854°Cd, respectively, for these two phases. (Data not shown).

Specific leaf area (SLA), dry biomass, plant height, stem diameter, bark yield and seed yield

An overview of the statistical analysis indicated that waterlogging duration and growth stage significantly influenced most measured traits. Interaction effects between duration and growth stage

were limited, with significance detected only for SLA (Table 2). Detailed responses of individual traits are described in the following sections.

Table 2
Two-way ANOVA interaction-test for the measured traits in Experiment 2

SLA	Waterlogging duration (D)	Growth stage (S)	D × S
	***	***	***
DW leaves	**	ns	ns
DW stems	**	*	ns
TADW	***	*	ns
Height	***	**	ns
Diameter	**	*	ns
Bark yield	***	ns	ns
Seed yield	**	*	ns

ns = not significant; * P < 0.05; ** P < 0.01; *** P < 0.001. SLA, specific leaf area; DW, dry weight; TADW, total above ground dry weight.

[Insert Table 2 here]

SLA declined most strongly in the 6 VE treatment, whereas SLA at flower initiation remained relatively stable even after prolonged waterlogging (Table 3).

Table 3
Interaction of waterlogging duration and growth stage on SLA in Experiment 2

Treatment	SLA (m^2 kg^{-1})
3 VE	14.00^{a}
6 VE	8.70^{d}
3 FI	13.15^{b}
6 FI	13.08^{c}

Means followed by different lowercase letters indicate significant differences among waterlogging duration × growth stage combinations at P ≤ 0.05 (two-way ANOVA; interaction effect significant, followed by post-hoc multiple comparison test). SLA, specific leaf area; 3 VE, 3-day waterlogging at six true leaf pair; 6 VE, 6-day waterlogging at six true leaf pair; 3 FI, 3-day waterlogging at flower initiation, 6 FI, 6-day waterlogging at flower initiation.

[Insert Table 3 here]

For all other variables, only main effects were significant (Table 4). Increasing waterlogging duration from 3 to 6 days reduced leaf dry weight, stem dry weight, TADW, plant height, stem diameter, bark yield, and seed yield, while waterlogged at flower initiation consistently showed higher stem DW, TADW, plant height, stem diameter and seed yield than those waterlogged during the vegetative stage (Table 4).

Table 4
Main effect of waterlogging duration and growth stage on measured traits in Experiment 2

	Waterlogging duration	
	3 days	**6 days**
DW leaves (g)	19.52[a]	11.22[b]
DW stems (g)	27.39[a]	13.01[b]
TADW (g)	61.86[a]	38.76[b]
Height (cm)	125.60[a]	100.40[b]
Diameter (mm)	12.74[a]	10.01[b]
Bark yield (g)	11.57[a]	5.10[b]
Seed yield (g)	6.46[a]	4.02[b]
	Growth stage	
	VE	**FI**
DW stems (g)	12.41[b]	28.00[a]
TADW (g)	44.23[b]	56.39[a]
Height (cm)	102.40[b]	123.60[a]
Diameter (mm)	9.43[b]	12.92[a]
Seed yield (g)	4.40[b]	6.08[a]

Values are means. Within each row, means followed by different lowercase letters differ significantly at $P \leq 0.05$ based on ANOVA and Tukey's HSD test. SLA, specific leaf area; DW, dry weight; TADW, total above ground dry weight. 3 VE, 3-day waterlogging at six true leaf pair; 6 VE, 6-day waterlogging at six true leaf pair; 3 FI, 3-day waterlogging at flower initiation, 6 FI, 6-day waterlogging at flower initiation.

[Insert Table 4 here]

Discussion

In these experiments, hemp, like many other crops, showed poor tolerance to prolonged waterlogging, particularly when saturation occurred early in development. Plant growth and development declined progressively as waterlogging duration increased, regardless of growth stage at which it was imposed. Plants subjected to more than 12 days of waterlogging at the eight true leaf pair stage did not survive, confirming severe sensitivity to extended rootzone saturation. In contrast, plants exposed to shorter periods of waterlogging (3–6 days) during the vegetative phase recovered, although recovery was slower after 6 days. Despite this recovery, long-term reductions in photosynthesis, growth, dry matter production, and yield were observed compared with the non-waterlogged control.

The physiological impairment underlying these responses is consistent with well-established effects of hypoxia on root function. Waterlogging restricts oxygen diffusion and creates hypoxic or anoxic conditions that inhibit root respiration, reduce energy production, and impair nutrient uptake. Prolonged oxygen deprivation also causes oxidative damage to root tissues (Zhang et al., 2025a). These below-ground stresses disrupt canopy development and reduce photosynthesis and yield, as reported other crops exposed to waterlogging (Aslam et al. 2023; Manghwar et al. 2024; Umathe et al. 2025; Yang et al. 2023).

Given the limited comprehensive research on waterlogging tolerance in hemp, comparisons with other crops provide useful context. Tolerance thresholds differ widely among species; wheat and barley can survive and recover from 14–16 days of waterlogging without complete plant loss (Pais et al. 2021; Pang et al. 2022), whereas durum wheat shows yield reductions only beyond 20 days of saturation (Pampana et al. 2016). Cotton, by comparison, exhibits far lower tolerance; Zhang et al. (2025b) reported complete mortality after more than 10 days of waterlogging plant mortality in some genotypes. In the present study, hemp yield declined by more than 50% after only 6 days of waterlogging, indicating a level of sensitivity comparable to or exceeding that of cotton and considerably lower than that of most cereals.

Physiologically, waterlogging in hemp caused marked reductions in chlorophyll content, Pn, Tr, Gs, and F_v/F_m, consistent with responses reported for cotton, barley, and wheat (Ghobi et al. 2010; Masoni et al. 2016; Ploschuk et al. 2018; Celedonio et al. 2016; Jiang et al. 2022; Wang et al. 2017; Zhang et al. 2025b). Ren et al. (2016) have shown that 6 days of waterlogging at the 3-leaf stage in maize can reduce leaf area index, chlorophyll content, photosynthesis similar to those observed in hemp after 6 days. Mahmood et al. (2021) found that 7 days of waterlogging in maize reduced chlorophyll content to a degree comparable to the reduction observed here in hemp after 6 days.

Reductions in Tr and Gs were also notable. In hemp, a 15–25% decline occurred after only 3 days of waterlogging during the most sensitive developmental stage (six true leaf pair). Comparable responses have been reported in other crops; Kubota et al. (2024) observed reduced Tr and Gs in soybean after more than 3 days of waterlogging. Consistent responses were observed by Ploschuk et al. (2023), who observed declines in Photosynthetic parameters in barley and rapeseed following 14 days of flooding at late growth stages, and in field pea at both early and late developmental stages.

Reductions in photosynthetic performance resulted translated directly into losses in biomass accumulation, TADW, and yield. Although yield declines in hemp were greater than those commonly observed in cereals, the overall pattern aligns with findings across species: as waterlogging duration increases, growth and productively decline proportionally. For example, Pampana et al. (2016) showed a 19–30% reduction in yield in durum wheat after 40–60 days of waterlogging, whereas hemp in this study exhibited > 50% yield loss after only 6 days of waterlogging, further highlighting its sensitivity.

SLA in hemp declined progressively with increasing waterlogging duration. The greatest reduction (~ 40%) occurred after 6 days of waterlogging at the vegetative stage, similar to reductions reported in wheat, barley, and maize under comparable stress (Ploschuk et al. 2018; Tian et al. 2021).

Waterlogging also accelerated hemp phenology, with earlier flower initiation and faster progression through reproductive stages in severely stressed plants. These findings are consistent with Ploschuk et al. (2018), who reported that late stage waterlogging hastened maturity in rapeseed, but differ from other studies showing delayed development in wheat and cotton. For example, Wang et al. (2017) found that 6 days of waterlogging at the seedling stage did not affect cotton phenology, while Celedonio et al. (2016) reported that waterlogging delayed flowering and reduced tiller appearance in wheat cultivars. Such variation underscores species-specific and stage-specific responses to hypoxia and suggests that hemp may shift phenology as a stress-avoidance mechanism under severe oxygen limitation.

Overall, this study highlights the pronounced susceptibility of hemp to waterlogging stress and demonstrates that both the duration and developmental timing of saturation strongly influence physiological function, growth, and yield.

The use of a single genotype and a uniform substrate was an intentional design that minimised experimental variability and enabled clear interpretation of treatment effects under controlled conditions. Although these findings are not intended to directly represent field behaviour, they provide a mechanistic, stage-specific understanding of hemp responses to transient rootzone saturation. This information is relevant to controlled environment and nursery production systems commonly used in the cultivation of hemp and closely related crops, such as medicinal cannabis, where irrigation management is critical and preventing substrate saturation can help mitigate growth penalties.

Conclusion

This study demonstrates that hemp is highly susceptible to waterlogging, with both the duration of saturation and the developmental stage at which it occurs exerting strong effects on physiological function, growth, and yield. Even short periods of waterlogging early in the crop cycle caused lasting reductions in photosynthesis, biomass accumulation, and seed yield, while prolonged waterlogging resulted in complete plant mortality. Compared with many cereals, hemp displayed markedly lower tolerance to waterlogging, with yield losses exceeding 50% after only 6 days of waterlogging.

To optimise fibre and seed yield, irrigation must be managed to avoid rootzone saturation for more than 3 days, particularly in during early vegetative growth. Successful cultivation requires well-aerated, free-draining soil or growing media, and careful water management to minimise periods of hypoxia. Because the physiological responses observed here were driven primarily by the duration and timing of saturation rather than substrate, these findings are relevant to a range of production systems where transient waterlogging may occur.

This work provides a mechanistic, stage-specific understanding of hemp responses to waterlogging stress and offers a foundation for improving management strategies in both field and controlled-environment production. It also highlights the need for future breeding and agronomic research aimed at enhancing waterlogging tolerance in hemp.

Abbreviations

ANOVA	Analysis of variance
DAS	Days after sowing
DW	Dry weight
FI	Flower Initiation
F_v/F_m	Chlorophyll fluorescence
Gs	Stomatal conductance
IRGA	Infrared gas analyser
PAM	Phase amplitude modulated
PAR	Photosynthetic active radiation
Pn	Net CO_2 assimilation rate
PWUE	Photosynthetic water use efficiency
SE	Standard error
SLA	Specific leaf area
TADW	Total above ground dry weight
Tr	Transpiration rate
TSW	Thousand seed weight

VE Vegetative stage

Declarations

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Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution

All authors contributed to the study conception and design. T.B.A, S.L and M.H supervised the project. I.V.K prepared the original draft. All authors reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

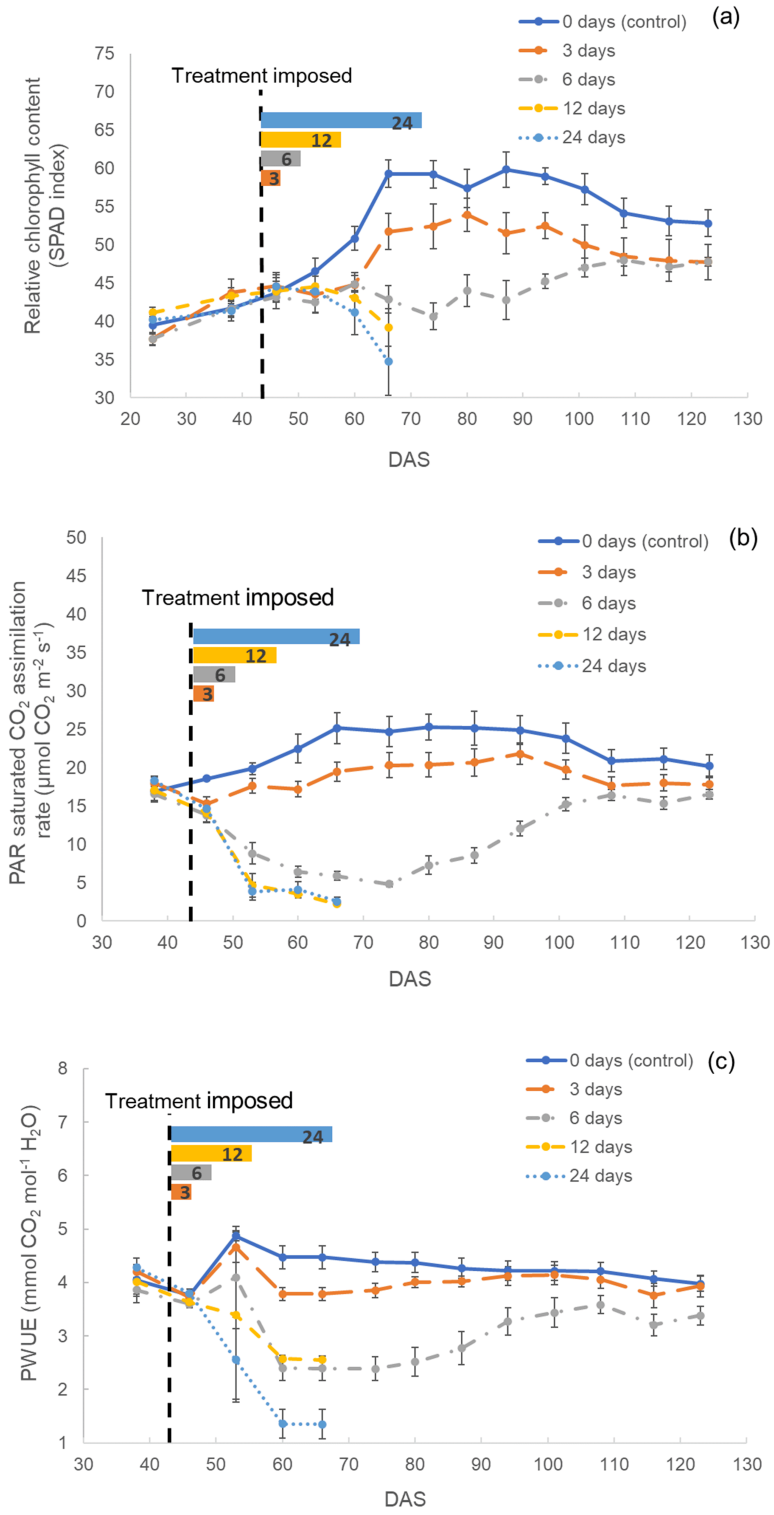

Figure 1

Relative chlorophyll content (SPAD index, a), PAR-saturated CO_2 assimilation rate (A_{sat}, b), and photosynthetic water use efficiency (PWUE, c) of hemp leaves in Experiment 1. Each value represents the mean ± S.E. (n = 6). Horizontal coloured bars at the top of each graph indicate the duration of waterlogging events for each treatment. Solid blue lines represent the 0-day waterlogging or control treatment; orange long-dash lines represent 3-day waterlogging; grey dash-dot lines represent 6-day

waterlogging; yellow dash lines represent 12-day waterlogging; and light blue dotted lines represent 24-day waterlogging.

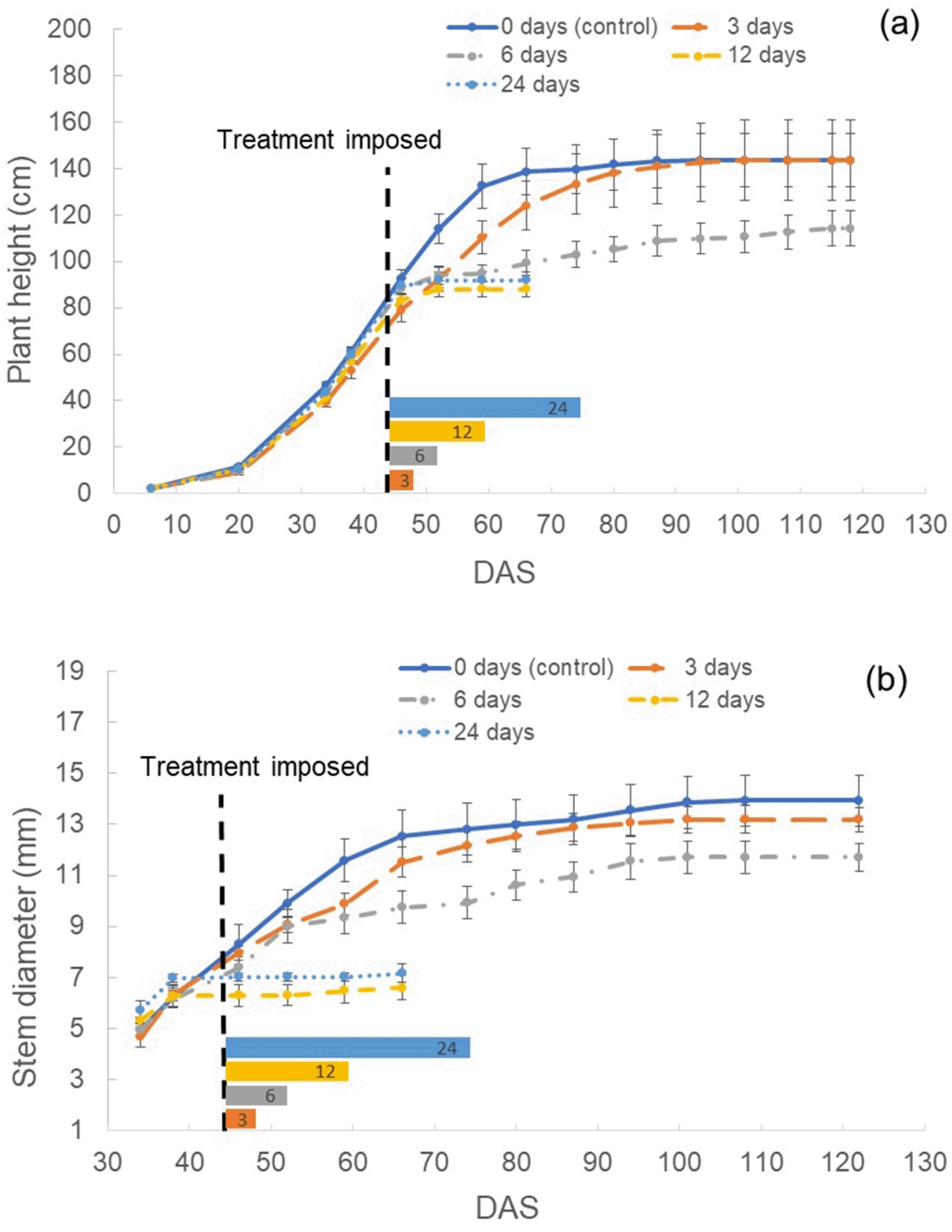

Figure 2

Plant height (a) and stem diameter (b) in Experiment 1. Each value represents the mean ± S.E. (n = 6). Horizontal coloured bars at the base of each graphs indicate durations of waterlogging events for each treatment. Solid blue lines represent the 0-day waterlogging or control treatment; orange long-dash lines represent 3-day waterlogging; grey dash-dot lines represent 6-day waterlogging; yellow dash lines represent 12-day waterlogging; and light blue dotted lines represent 24-day waterlogging.

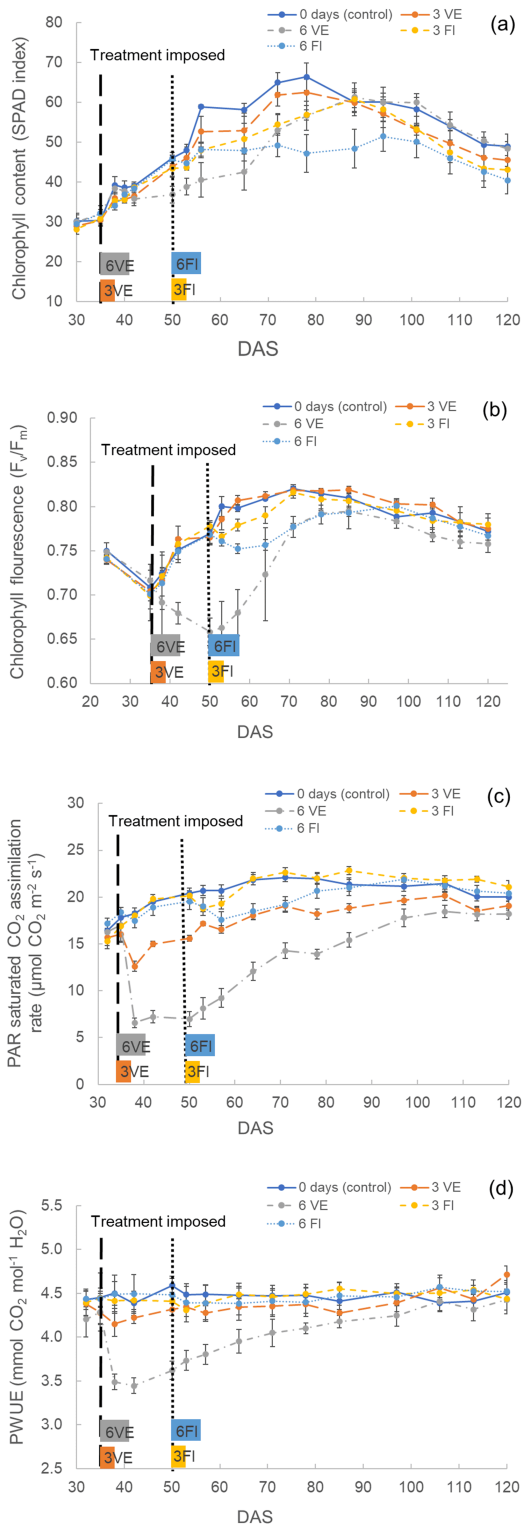

Figure 3

Relative chlorophyll content (SPAD index, a), Chlorophyll fluorescence (b), PAR-saturated CO_2 assimilation rate ($A_{sat,}$ c) and photosynthetic water use efficiency (PWUE, d) of hemp leaves in Experiment 2. Each value is the mean ± S.E. (n = 5). Horizontal coloured bars at the base of each graph indicate durations of waterlogging events for each treatment. Solid blue lines represent the 0-day waterlogging or control treatment; orange long-dash lines represent 3-day waterlogging at vegetative

stage (3 VE); grey dash-dot lines represent 6-day waterlogging at vegetative stage (6 VE); yellow dash lines represent 3-day waterlogging at flower initiation stage (3 FI); and light blue dotted lines represent 6-day waterlogging at flower initiation stage (6 FI).