

Cotton growth and yield dynamics across canopy layers in response to soil waterlogging

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Abstract

Due to an indeterminate growth habit, we hypothesised that different canopy layers of cotton might be variably influenced by soil waterlogging. The field-grown cotton cultivar (Sicot 71BRF) was waterlogged at early (WL_{early}, 77 days after planting [DAP]) and late reproductive phases (WL_{late}, 101 DAP) for 120 h. Data from different canopy layers e.g. bottom eight (MSN₁₋₈), middle five (MSN₉₋₁₃), and upper five main stem nodes (MSN₁₄₊) were collected 1 d (post-WL) and 7 d after termination of waterlogging (post-recovery). Both waterlogging events significantly reduced post-WL dry biomass, leaf N concentration and fruit development on MSN₁₋₈. In addition, WL_{early} significantly reduced photosynthesis and increased total soluble sugars (TSS) in the MSN₁₋₈ and MSN₁₄₊ leaves, although MSN₁₄₊ leaves restored photosynthesis, N levels and TSS at recovery. It suggested that WL plants maintained photosynthesis of the upper leaves possibly by transporting N from the lower canopy leaves. Reduction (22%) in seed cotton yield under WL_{early} was the result of fruit loss from first position fruits of the upper and lower sympodial fruiting branches (FB₁₋₅ and FB₁₁₊). Despite restoring the growth through improved photosynthesis and N supply, no yield recovery on FB₁₁₊ suggested that the plants used these assimilates for growth of the established fruits. No significant yield reduction in response to WL_{late} suggested that the established cotton bolls were less sensitive to abscission across all canopy layers.

Keywords: Canopy layers, lint yield, growth phase, leaf development, nitrogen re-mobilisation, photosynthesis.

Abbreviation: DAP_days after planting; DW_dry weight; FR_fruit retention; FB_{1-5_lower} 5 fruiting nodes; FB_{5-10_middle} 5 fruiting nodes; FB_{11-15_top} 5 fruiting nodes; GB_green bolls; LAI_leaf area index; MS_{N1-8_lower} 1-8 main stem nodes; MS_{N9-13_middle} 9-13 main stem nodes; MS_{N14-18_top} 14-18 main stem nodes; NWL_non-waterlogged; P_n_photosynthesis; post-recovery_7 days after termination of waterlogging; post-WL_day after termination of waterlogging; pre-WL_pre-waterlogging; SLA_specific leaf area; SLN_specific leaf nitrogen; TSS_total soluble sugars; WL_{early}_waterlogging at early reproductive phase; WL_{late}_waterlogging at late reproductive phase; WL_waterlogged.

Introduction

Soil waterlogging is an important abiotic factor that influences the worldwide distribution and production of plant species. According to FAO (2007), 20-30 Mha land area has been affected by soil waterlogging as a result of improper irrigation practices. Intensive irrigation on poorly drained soils coupled with erratic heavy rainfall events can induce soil waterlogging, reducing O₂ diffusion to rooting zones. Waterlogging and subsequent soil O₂ deficiency influences bioavailability of many essential nutrients in the rhizosphere, making them unavailable for uptake (Steffens et al., 2005). In addition, inhibited ATP synthesis and plasma membrane H⁺-ATPase in hypoxic roots (Jackson et al., 2003) suppress the active uptake of nutrients from O₂ deficient soils. Nutrient deficiency during reproductive growth stage can negatively affect plant growth and fruit development (Milroy et al., 2009). Cotton (*Gossypium hirsutum* L.) is an important fibre and oilseed crop grown over 30 million hectares worldwide (USDA 2012). Limited capacity to develop functional root aerenchyma (Conaty et al., 2008) makes cotton a relatively waterlogging sensitive crop. Yield reduction in a waterlogged cotton crop is associated with the duration; the crop remained exposed to root zone O₂ deficiency. For example, Hodgson and Chan (1982) observed 8% - 18% lint yield reduction in

cotton under a short term waterlogging (16-32 h), while the yield reduction could reach up to 30% under 9 d of waterlogging (Wu et al., 2012). In Australia, where cotton is cultivated on poorly drained soils, the crop often experiences soil O₂ deficiency after furrow irrigation or heavy summer rainfall. Current improvements in cotton production systems and breeding programs have substantially increased per hectare yield, but limited work has been reported on specifically improving our understanding of waterlogging tolerance mechanisms in cotton. Waterlogging-induced yield reduction is often associated with inhibited nutrient uptake, photosynthesis and consequently fruit production. As the developing reproductive organs require an ample supply of nutrients, stressed cotton plants with restricted nutrient supply from roots may obtain these nutrients from leaves. This can induce leaf growth reduction, premature senescence and consequently impair overall biomass production (McLeod, 2001). As the developing fruits in cotton rely heavily on subtending leaves for carbohydrate supply (Constable and Rawson, 1980), waterlogging-induced changes in nutrient status of leaves throughout the canopy may differentially influence growth and lint yield. Investigations into the physiology of waterlogging damage to

cotton growth have been obtained by measuring changes in leaf N concentrations from the youngest fully expanded leaves of top of the canopy (Ashraf et al., 2011, Milroy et al., 2009) or assessing changes in growth of the whole plant (crop yield) (Bange et al., 2004). However, to meet the demand of actively developing bolls, the plants may remobilise N from older leaves to the upper canopy and maintain photosynthesis, affecting interpretation of the impacts of waterlogging. Limited information is available on nutrient re-distribution, biomass, and fruit development across various layers of the cotton canopy. To understand the mechanisms of waterlogging damage, it is essential to study the growth and nutrient dynamics across different canopy layers. These experiments aimed to (1) study the growth and yield losses in cotton crop exposed to waterlogging at different reproductive phases and to (2) understand the waterlogging-induced changes in leaf N dynamics and photosynthesis within the canopy and relate these to fruit growth and final yield.

Results

Changes in volumetric soil water

After 120 h waterlogging at any crop growth phase, there was a significant increase in volumetric soil water content. The gap between soil water content of *WL* and *NWL* soils further grew at post-recovery (Fig 1). *WL* plots contained 13% and 15% higher volumetric soil water contents at post-*WL* and post-recovery, respectively, (averaged across the two treatments) compared with *NWL* plots.

Effect of waterlogging on cotton growth

Both waterlogging treatments primarily reduced the post-recovery dry weight (*DW*) of cotton plants. Waterlogging at early reproductive phase (*WL*_{early}) caused 13% and 22% reduction in post-recovery *DW* of leaves and green bolls, respectively, compared with *NWL* control but effect of waterlogging at late reproductive phase (*WL*_{late}) was significant on only the stem *DW* (13% reduction). No significant change in square *DW* was recorded under any waterlogging treatment (Table 1).

The effect of waterlogging on dry matter production was investigated in more detail by studying the modifications in total dry matter (*TDM*) on different layers of plant canopy. The lower part (*MSN*₁₋₈) of the canopy was relatively more sensitive to waterlogging, which showed a significant reduction (16%) in post-recovery *TDM* in response to *WL*_{early} (Fig 2 A). Similarly, *WL*_{late} caused 18% and 15% reduction in *TDM* of *MSN*₁₋₈ at post-*WL* and post-recovery, respectively (Fig 2 B).

Waterlogging at any reproductive phase had no significant effect on specific leaf area (*SLA*, leaf area per unit dry weight) of the entire canopy (Table 2). However, *WL*_{early} significantly reduced post-*WL* *SLA* of *MSN*₁₋₈ and *WL*_{late} increased post-recovery *SLA* of *MSN*₁₄₊ (Fig 3 A & B). In contrast, both *WL*_{early} and *WL*_{late} significantly reduced post-recovery leaf area index (*LAI*) of the whole canopy (Table 2) and the effect was significant on lower canopy layer, *MSN*₁₋₈ (Fig 3 C & D).

Effect of waterlogging on fruit production in cotton

Production of new squares in both *WL* and *NWL* plants first increased (termination of *WL*_{early}) and then progressively decreased with the reproductive plant growth. No new

squares were observed in any *WL* or *NWL* plant 7 days after termination of *WL*_{late} (Table 2). *WL*_{early} reduced the post-*WL* number of squares (Table 2) and this loss of squares was significant at the lower canopy (*FB*₁₋₅) (Fig 4 A). In addition, *WL*_{early} significantly reduced post-recovery number of squares at the top of canopy (*FB*₁₁₊) possibly by inhibiting development of new squares (Fig 4 A) although the effect was not significant in the whole canopy (Table 2). *WL*_{early} also caused 23% and 27% reduction in the number of green bolls (*GB*) at post-*WL* and post-recovery, respectively, compared with the *NWL*_{early} control. Reduction in *GB* was recorded on *FB*₁₋₅ at post-*WL* and on *FB*₁₋₅ and *FB*₆₋₁₀ at post-recovery (Fig 4 C). Significantly reduced post-recovery *GB* at the lower canopy could be attributed to post-*WL* loss of squares in response to *WL*_{early}.

Due to fewer squares at the time of treatment, *WL*_{late} had no significant effect on the number of squares (Fig 4 B) but it significantly reduced (17%) number of *GB* at post-*WL* (Table 2). This loss of *GB* was also observed on lower and mid of canopy (*FB*₁₋₅ and *FB*₆₋₁₀). The effect of *WL*_{late} was non-significant on the entire canopy *GB* at post-recovery (Table 2), due to development of additional bolls on *FB*₁₁₊ (Fig 4 D).

Leaf nitrogen dynamics in response to soil waterlogging

Waterlogging at the early reproductive phase caused 23% reduction in post-*WL* leaf N (%) concentration of the whole canopy (Table 3). This reduction was mainly recorded in *MSN*₁₋₈ and *MSN*₉₋₁₃ leaves, but N (%) concentration in *MSN*₁₄₊ of *WL* leaves was similar to *NWL* leaves at post-recovery (Fig 5 A). Similarly, *WL*_{early} significantly reduced post-*WL* and post-recovery specific leaf N (*SLN*) of *MSN*₁₋₈ leaves (Fig 5 C), although no significant change in *SLN* of the entire canopy was observed (Table 3).

*WL*_{late} caused 22% and 13% reduction in the entire canopy N (%) concentration at post-*WL* and post-recovery, respectively (Table 3). The post-*WL* reduction in N (%) was observed in the lower canopy leaves (*MSN*₁₋₈), but the effect on any specific canopy layer was not significant at recovery (Fig 5 B). In addition, *WL*_{late} significantly reduced post-*WL* and post-recovery *SLN* in *MSN*₁₋₈ leaves (Fig 5 D) and the reduction in *SLN* of whole canopy was significant at post-*WL* only (Table 3).

Effect of waterlogging on leaf gas exchange and total soluble sugars

Data collected from cotton leaves at various canopy positions showed that rate of photosynthesis (*P_n*) of cotton leaves were in the order of *MSN*₁₄₊ > *MSN*₉₋₁₃ > *MSN*₁₋₈ (Fig 6 A). Waterlogging had no significant effect on *P_n* of middle canopy (*MSN*₉₋₁₃) leaves but it significantly reduced post-*WL* *P_n* in *MSN*₁₋₈ and *MSN*₁₄₊ leaves (Fig 6 A). *WL* leaves on the upper canopy layer (*MSN*₁₄₊) restored *P_n* to the level of *NWL* leaves at post-recovery. No significant effect of waterlogging was recorded on other gas exchange parameters such as stomatal conductance, intercellular CO₂ concentrations and transpiration rate at any canopy position (data not shown). *WL*_{early} significantly increased post-*WL* total soluble sugar (*TSS*) contents in the leaves at various canopy positions (Fig 6 B). For example, *WL* leaves at top (*MS*_{N14+}), middle (*MS*_{N9-13}) and bottom (*MS*_{N1-8}) positions contained 21%, 42% and 59% higher *TSS*, respectively, compared with their respective *NWL* leaves (Fig 6 B). *TSS* contents of *WL* leaves recovered to the level of *NWL* at post-recovery, except in *NS*_{N1-8} leaves, which still contained significantly higher *TSS* (25%) than *NWL* leaves (Fig 6 B).

Table 1. Changes in shoot dry biomass of cotton in response to 120 h of waterlogging at early (77 days after planting, DAP) and late reproductive phase (101 DAP). Data are presented on a ground area basis (m^{-2}).

| Treatment | Stem DW (g m^{-2}) | | | Leaf DW (g m^{-2}) | | | Square DW (g m^{-2}) | | | Green bolls DW (g m^{-2}) | | |
|----------------------|-------------------------------|---------|---------------|-------------------------------|---------|---------------|---------------------------------|---------|---------------|--------------------------------------|---------|---------------|
| | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery |
| WL _{early} | 44.22 | 173.14 | 300.49 | 66.79 | 140.76 | 140.11 | 12.79 | 16.72 | 7.46 | 5.27 | 85.95 | 129.41 |
| NWL _{early} | 39.27 | 157.87 | 284.14 | 64.72 | 151.26 | 161.61 | 12.66 | 18.68 | 7.13 | 3.12 | 97.06 | 165.82 |
| WL _{late} | 152.76 | 223.60 | 460.66 | 108.46 | 204.88 | 236.70 | 2.91 | 0.30 | 0.66 | 173.13 | 377.09 | 520.28 |
| NWL _{late} | 163.97 | 236.86 | 528.78 | 148.14 | 213.53 | 267.36 | 7.20 | 2.26 | 1.25 | 161.3 | 383.23 | 548.82 |

ANOVA (F test *P* values were calculated by comparing the means of waterlogged (WL) and non-waterlogged (NWL) plants, separately, for each year and treatment time)

| | Early waterlogging | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery |
|--------------------|--------------------|---------|---------------|--------|---------|---------------|--------|---------|---------------|--------|---------|---------------|
| Early waterlogging | NS | NS | NS | NS | NS | * | NS | NS | NS | NS | NS | * |
| Late waterlogging | NS | NS | * | NS | NS | NS | NS | NS | NS | NS | NS | NS |

Values are the means of four individual replications. * Means are significantly different at $P \leq 0.05$; NS, means are not significantly different at $P \leq 0.05$.

WL_{early}= waterlogging at the early reproductive growth phase of cotton (77 DAP) and WL_{late}= waterlogging at late reproductive phase (101 DAP)

Non-pollinated young fruits were defined as squares and pollinated fruits as green bolls; DW, dry weight.

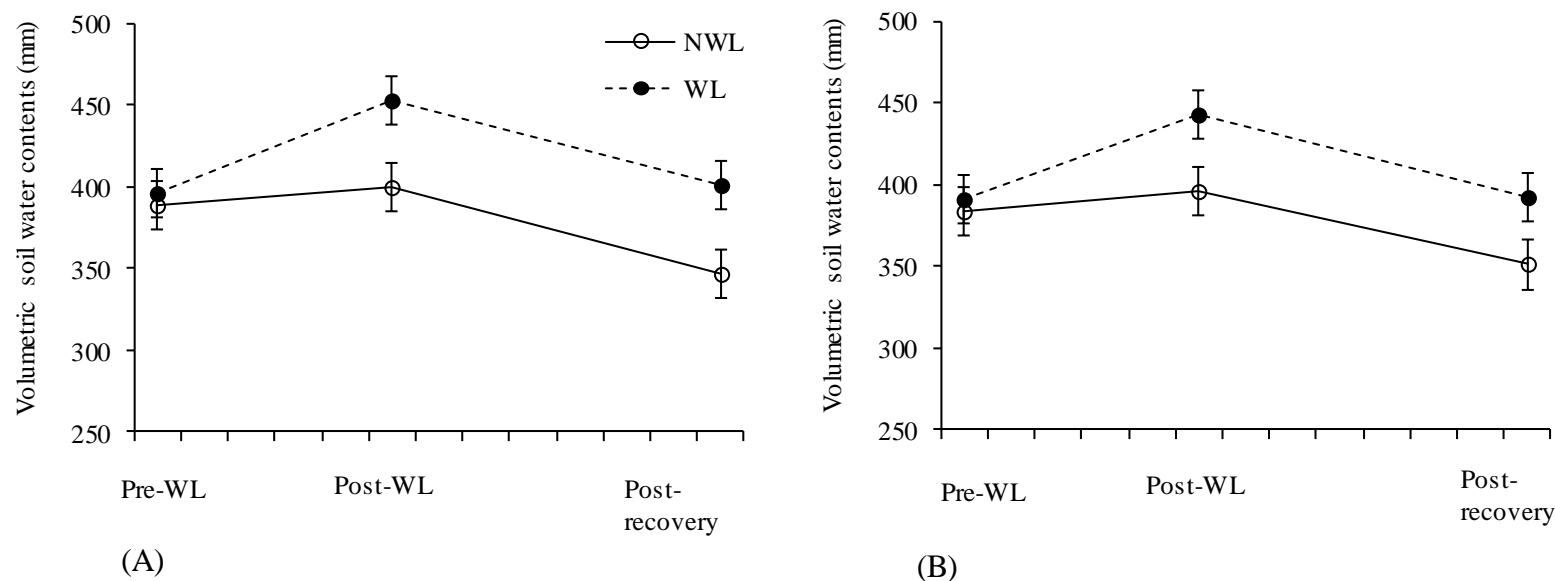


Fig 1. Changes in water content (mm) of the soil in response to 120 h of waterlogging (A) at early and (B) late reproductive growth phase of cotton. Data were collected 1 day before waterlogging (Pre-WL), one day (Post-WL) and 7 d after termination of waterlogging (Post-recovery). Values are the mean of four independent replications with (\pm) standard error; * = means are significantly different at ($P \leq 0.05$). Values presented in the figure are means of the four independent replications with (\pm) standard error. WL = waterlogged; NWL = non-waterlogged

Table 2. Changes in fruit production and leaf development of cotton in response to 120 h of waterlogging at early (77 days after planting, DAP) and late reproductive phase (101 DAP). Data are presented on a ground area basis (m^{-2}).

| Treatment | Number of squares (m^{-2}) | | | Number of green bolls (m^{-2}) | | | Specific leaf area ($\text{m}^2 \text{g}^{-1}$) | | | Leaf area index | | |
|--|---------------------------------------|---------|---------------|---|---------|---------------|---|---------|---------------|-----------------|---------|---------------|
| | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery |
| WL _{early} | 131.00 | 139.69 | 73.63 | 90.9 | 82.4 | 99.2 | 0.012 | 0.014 | 0.016 | 0.81 | 1.3 | 2.25 |
| NWL _{early} | 128.50 | 154.88 | 77.44 | 89.7 | 106.5 | 136.5 | 0.013 | 0.015 | 0.016 | 0.93 | 1.35 | 2.91 |
| WL _{late} | 21.31 | 2.5 | 0 | 59.37 | 134.3 | 149.41 | 0.014 | 0.014 | 0.018 | 2.41 | 2.89 | 2.61 |
| NWL _{late} | 31.25 | 9.2 | 0 | 59.17 | 162.5 | 158.61 | 0.012 | 0.014 | 0.018 | 2.48 | 3.01 | 3.07 |
| ANOVA (F test <i>P</i> values were calculated by comparing the means of waterlogged (WL) and non-waterlogged (NWL) plants, separately, for each year and treatment time) | | | | | | | | | | | | |
| Early waterlogging | NS | * | NS | NS | * | * | NS | NS | NS | NS | NS | * |
| Late waterlogging | NS | NS | NS | NS | * | NS | NS | NS | NS | NS | NS | NS |

Values are the means of four individual replications. * Means are significantly different at $P \leq 0.05$; NS, means are not significantly different at $P \leq 0.05$. WL_{early} = waterlogging at the early reproductive growth phase of cotton (77 DAP) and WL_{late} = waterlogging at late reproductive phase (101 DAP). Specific leaf area = leaf area per unit dry weight, non-pollinated young fruits were defined as squares and pollinated fruits as green bolls.

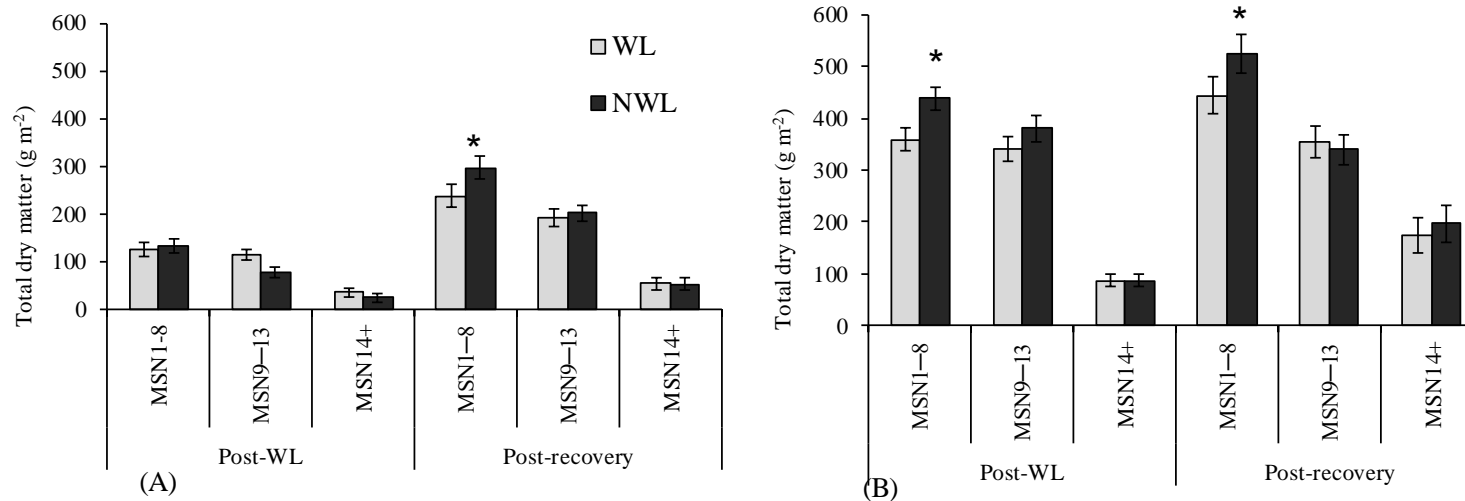


Fig 2. Changes in total dry matter in different layers of cotton canopy in response to 120 h of waterlogging at (A) early and (B) late reproductive phase. Values presented in the figure are means of the four independent replications with (\pm) standard error. Means of waterlogged and non-waterlogged plants were separately compared for each segment and each treatment time. * = means are significantly different at ($P \leq 0.05$). MSN1-8 = bottom 8 main stem nodes; MSN9-13 = middle 5 main stem nodes; MSN14+ = main stem nodes above 13. Post-WL = data collected one day after waterlogging; Post-recovery = data collected 7 days after termination of waterlogging. WL = waterlogged; NWL = non-waterlogged

Table 3. Changes in leaf nitrogen concentrations in response to 120 h of waterlogging at early (77 days after planting, DAP) and late reproductive phase (101 DAP). Values are the means of four individual replications. * Means are significantly different at $P \leq 0.05$; NS, means are not significantly different at $P \leq 0.05$. Specific leaf nitrogen=nitrogen concentrations per unit leaf area, WL_{early}= waterlogging at the early reproductive growth phase of cotton (77 DAP) and WL_{late} = waterlogging at late reproductive phase (101 DAP). Values are the means of four individual replications. * Means are significantly different at $P \leq 0.05$; NS, means are not significantly different at $P \leq 0.05$.

| Treatment | | Leaf N (%) | | | Specific leaf nitrogen (mg cm ⁻²) | | |
|---|-----|------------|---------|---------------|---|---------|---------------|
| | | Pre-WL | Post-WL | Post-recovery | Pre-WL | Post-WL | Post-recovery |
| WL _{early} | WL | 3.85 | 3.11 | 3.26 | 3.12 | 2.25 | 2.31 |
| NWL _{early} | NWL | 3.91 | 3.73 | 3.57 | 3.11 | 2.41 | 2.42 |
| WL _{late} | WL | 3.41 | 2.49 | 2.65 | 2.24 | 1.63 | 2.00 |
| NWL _{late} | NWL | 3.64 | 3.20 | 3.04 | 2.27 | 2.11 | 2.19 |
| ANOVA (F test P values were calculated by comparing the means of waterlogged (WL) and non-waterlogged (NWL) plants, separately, for each year and treatment time) | | | | | | | |
| Early waterlogging | | NS | * | NS | NS | NS | NS |
| Late waterlogging | | NS | * | * | NS | * | NS |

Specific leaf nitrogen=nitrogen concentrations per unit leaf area, WL_{early}= waterlogging at the early reproductive growth phase of cotton (77 DAP) and WL_{late} = waterlogging at late reproductive phase (101 DAP).

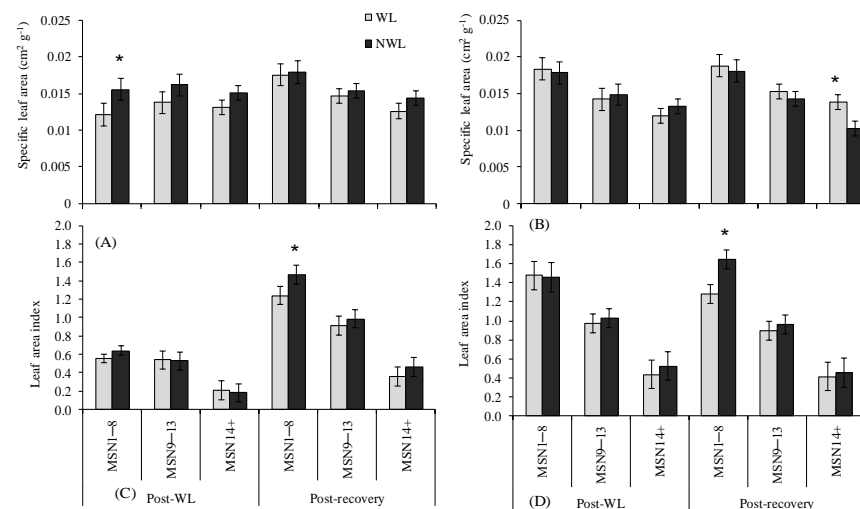


Fig 3. Changes in specific leaf area [(A) at early and (B) late reproductive phase] and leaf area index [(C) at early and (D) late reproductive phase] in different layers of cotton canopy in response to 120 h of waterlogging. Values presented in the figure are means of the four independent replications with (\pm) standard error. Means of waterlogged and non-waterlogged plants were separately compared for each segment and each treatment time. * = means are significantly different at ($P \leq 0.05$). MSN1-8 = bottom 8 main stem nodes; MSN9-13 = middle 5 main stem nodes; MSN14+ = main stem nodes above 13. Post-WL = data collected one day after waterlogging; Post-recovery = data collected 7 days after termination of waterlogging. WL = waterlogged; NWL = non-waterlogged; Specific leaf area = leaf area per unit dry weight.

Table 4. Changes in seed cotton yield and yield components measured at final harvest in response to 120 h of waterlogging at early (77 days after planting, DAP) and late reproductive phase (101 DAP).

| Treatment | Seed cotton yield (plant ⁻¹) | Number of bolls (plant ⁻¹) | Boll weight (g) | Plant height (cm) | Nodes (plant ⁻¹) | FR (%) |
|---|--|--|-----------------|-------------------|------------------------------|--------|
| WL _{early} | 48.68 | 11.35 | 4.13 | 81.50 | 19.75 | 42.45 |
| NWL _{early} | 62.25 | 13.32 | 4.45 | 89.15 | 22.25 | 52.64 |
| WL _{late} | 56.52 | 12.95 | 4.02 | 93.15 | 21.50 | 45.91 |
| NWL _{late} | 61.87 | 13.31 | 4.43 | 91.51 | 22.50 | 51.25 |
| ANOVA (F test <i>P</i> values were calculated by comparing the means of waterlogged (WL) and non-waterlogged (NWL) plants, separately, for each treatment time) | | | | | | |
| Early waterlogging | * | * | NS | * | * | * |
| Late waterlogging | NS | NS | NS | NS | NS | NS |

Values are the means of four individual replications. * Means are significantly different at $P \leq 0.05$; NS, means are not significantly different at $P \leq 0.05$. FR= fruit retention, WL_{early}= waterlogging at the early reproductive growth phase of cotton (77 DAP) and WL_{late} = waterlogging at late reproductive phase (101 DAP).

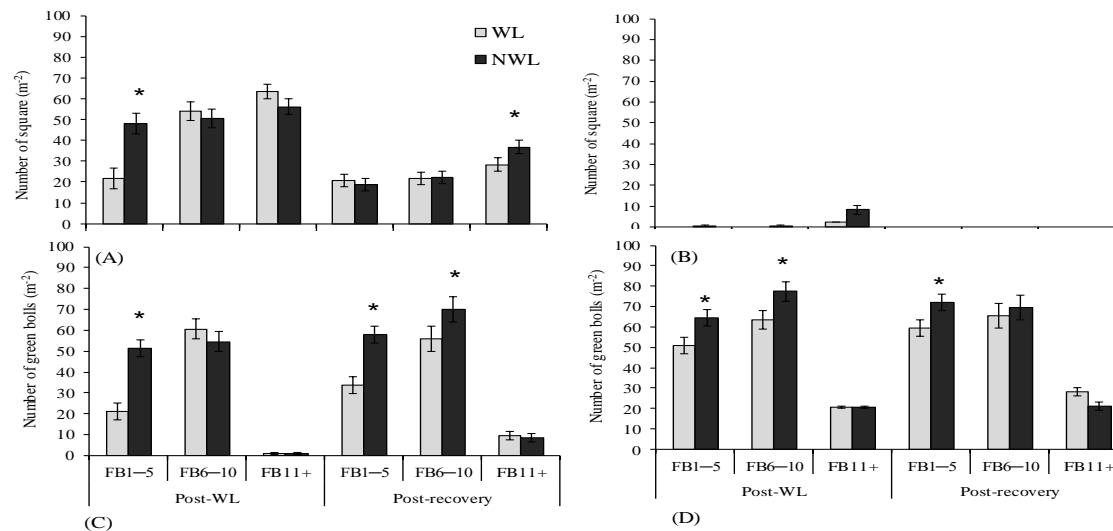


Fig 4. Changes in number of squares [(A) at early and (B) late reproductive phase] and green bolls [(C) at early and (D) late reproductive phase] in different layers of cotton canopy in response to 120 h of waterlogging. Values presented in the figure are means of the four independent replications with (\pm) standard error. Means of waterlogged and non-waterlogged plants were separately compared for each segment and each treatment time. * = means are significantly different at ($P \leq 0.05$). FB₁₋₅ = bottom 5 fruiting branches on main stem; FB₆₋₁₀ = middle 5 fruiting branches on main stem; FB₁₁₊ = fruiting branches on main stem above FB₁₀. Post-WL = data collected one day after waterlogging; Post-recovery = data collected 7 days after termination of waterlogging; WL = waterlogged; NWL = non-waterlogged. Non-pollinated young fruits were classified as squares and pollinated flowers+bolls as green bolls.

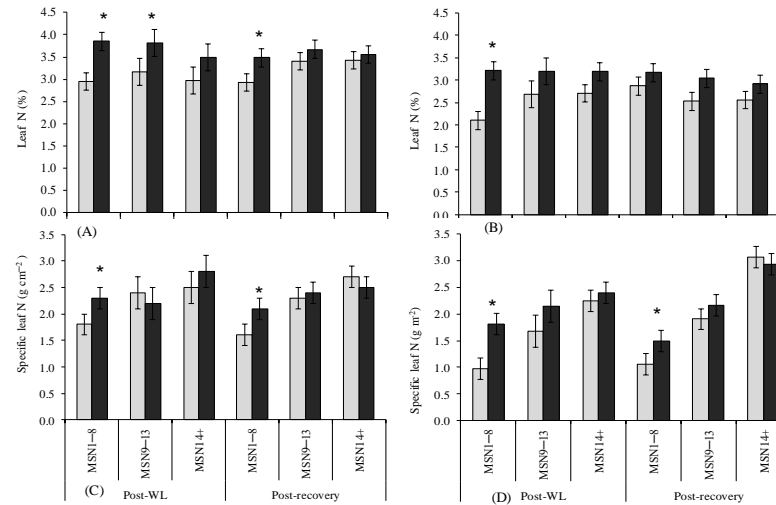


Fig 5. Changes in leaf N (%) concentration [(A) at early and (B) late reproductive phase] and specific leaf N [(C) at early and (D) late reproductive phase] in different layers of cotton canopy in response to 120 h of waterlogging. Values presented in the figure are means of the four independent replications with (\pm) standard error. Means of waterlogged and non-waterlogged plants were separately compared for each segment and each treatment time. * = means are significantly different at ($P \leq 0.05$). MSN1-8 = bottom 8 main stem nodes; MSN9-13 = middle 5 main stem nodes; MSN14+ = main stem nodes above 13. Post-WL = data collected one day after waterlogging; Post-recovery = data collected 7 days after termination of waterlogging. WL = waterlogged; NWL = non-waterlogged; Specific leaf nitrogen = nitrogen concentrations per unit leaf area.

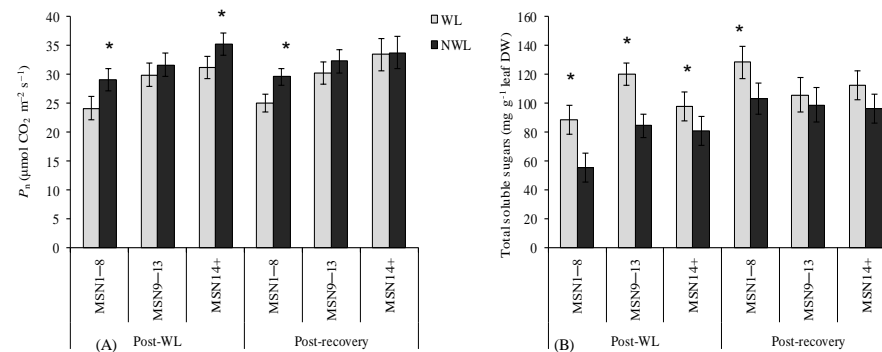


Fig 6. Changes in (A) rate of photosynthesis and (B) total soluble sugar contents of cotton leaves at different position of canopy in response to 120 h of waterlogging at early reproductive phase. Values presented in the figure are means of the four independent replications with (\pm) standard error. Means of waterlogged and non-waterlogged plants were separately compared for each segment and each treatment time. * = means are significantly different at ($P \leq 0.05$). MSN1-8 = bottom 8 main stem nodes; MSN9-13 = middle 5 main stem nodes; MSN14+ = main stem nodes above 13. Post-WL = data collected one day after waterlogging; Post-recovery = data collected 7 days after termination of waterlogging. WL = waterlogged; NWL = non-waterlogged; DW = dry weight; P_n = rate of photosynthesis.

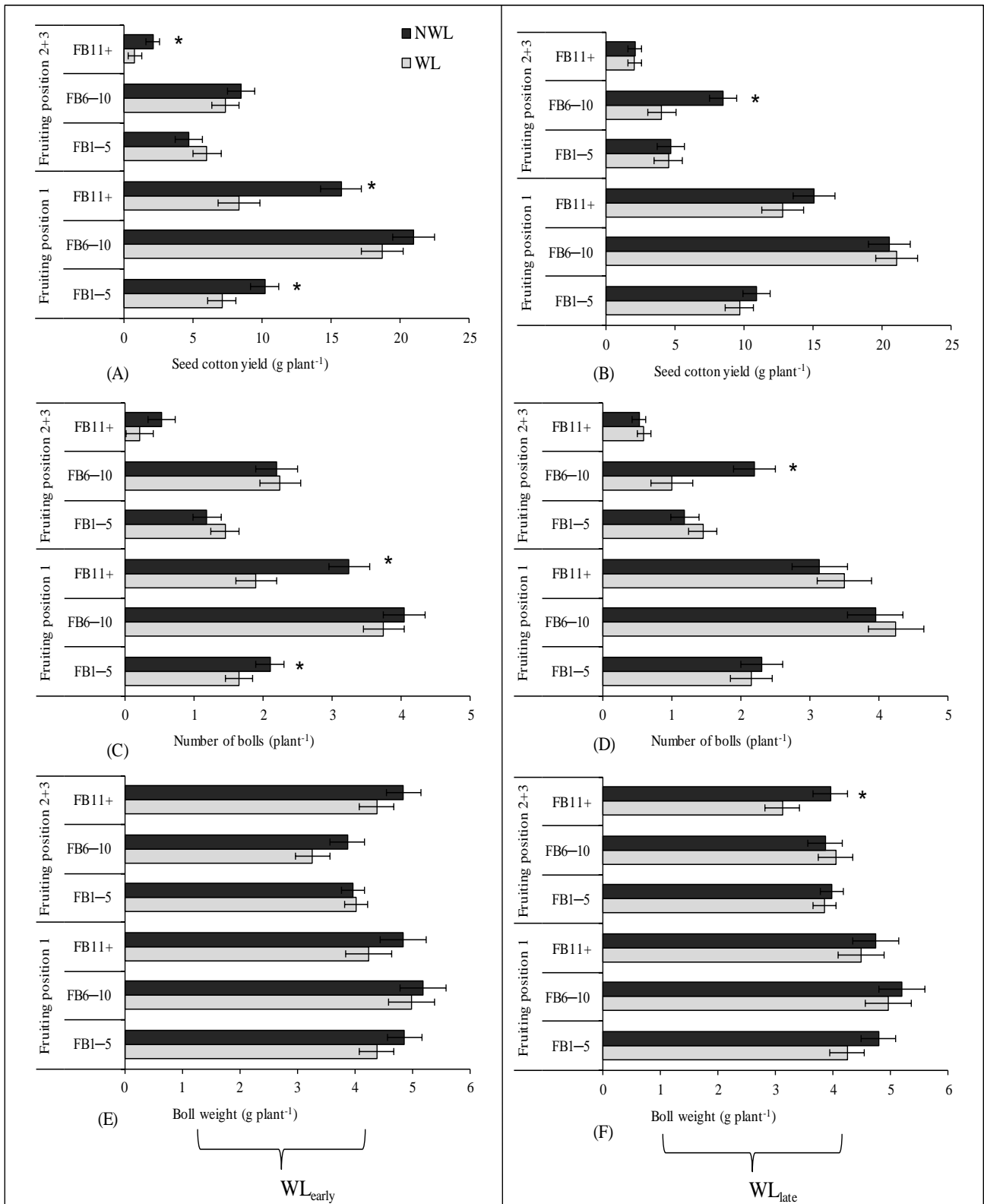


Fig 7. Changes in cotton yield and yield components on different fruiting branches and fruiting positions in response to 120 h of waterlogging. Seed cotton yield under waterlogging at (A) early and (B) late reproductive phase, Total number of bolls per plant under waterlogging at (C) early and (D) late reproductive phase. Weight of individual boll under waterlogging at (E) early and (F) late reproductive phase. Data were collected at crop maturity and presented as means of four independent replications with (\pm) standard error. Means of waterlogged and non-waterlogged plants were separately compared for each segment and each treatment time. * = means are significantly different at ($P \leq 0.05$). FB₁₋₅ = bottom 5 fruiting branches on main stem; FB₆₋₁₀ = middle 5 fruiting branches on main stem; FB₁₁₊ = fruiting branches on main stem above FB₁₀; Fruiting position 1 = a fruit on the fruiting branches closest to the main stem; Fruiting position 2+3 = fruits on the fruiting branches next to fruit on fruiting position 1.

Effect of waterlogging on seed cotton yield

Waterlogging at early reproductive phase significantly reduced the seed cotton yield, which was associated with the production of fewer fruiting nodes and lower fruit retention (Table 4). WL_{early} -induced reduction in seed cotton yield (22%) of the entire canopy at harvest was attributed to fewer bolls (15% lower than NWL) produced (Table 4). This yield loss was observed on lower (FB_{1-5}) and upper fruiting branches (FB_{14+}) (Fig 7A), and was the result of lower number of bolls on FP_1 (Fig 7 C). Boll weight across different canopy layers remained unchanged under WL_{early} (Fig 7 E). Similarly, seed cotton yield and yield components on FP_{2+3} remained significantly unaffected by WL_{early} (Fig 7 C). Waterlogging at late reproductive phase had no significant effect on seed cotton yield and yield components at harvest (Table 4). In addition, the number of FP_1 bolls on different fruiting branches remained unaffected by WL_{late} , although, significantly lower FP_{2+3} bolls and consequently seed cotton yield were recorded on FB_{6-10} (Fig 7 B & D). WL_{late} also significantly reduced the weight of FP_{2+3} bolls on FB_{11+} (Fig 7 F).

Discussion

Waterlogging-induced yield losses in cotton have already been reported (Najeeb et al., 2015, Zhang et al., 2015), where increased fruit loss was the major cause of yield reduction. This study investigated the effect of waterlogging on the fruiting pattern of cotton across the canopy. Due to an indeterminate growth habit, developing fruits on different main-stem nodes of a cotton plant responded variably to soil waterlogging. Our data have affirmed the hypothesis that waterlogging-induced changes were different across canopy layers. Significantly higher inhibition of growth and yield under WL_{early} compared with WL_{late} suggested the sensitivity of cotton to soil waterlogging at early reproductive phase, confirming the earlier data of Bange et al. (2004).

Yield reduction from WL cotton was the result of fewer bolls produced at the upper and lower parts of the canopy. WL_{early} primarily inhibited production of new fruits in the upper canopy, which was evident from a significantly fewer squares of WL plants at post-recovery. As the development of new squares had almost ceased at WL_{late} , it caused no significant yield loss in cotton. Fruit reduction in the lower canopy of WL cotton was most likely a result of abscission of young fruits caused by increased ethylene biosynthesis in cotton tissues (Christianson et al., 2010, Najeeb et al., 2015). As the WL plants contained significantly fewer bolls on FP_1 , the main contributor to lint yield, significant yield loss was observed in WL_{early} plants in this study. Kuai et al. (2015) also reported significant lint yield reduction in the lower canopy of WL cotton due to loss of bolls from FP_1 . In contrast to Kuai et al. (2015) who observed new growth on upper FB , yield reduction in our study was the result of fewer bolls produced both at upper and lower canopy. This discrepancy is most likely due to the fact that WL crop in this study was not able to support further growth of new fruiting sites. This is plausible given the overall reduction in crop leaf area.

No significant yield loss in response to WL_{late} affirmed the earlier studies, which proposed that cotton bolls become less sensitive to ethylene-induced shedding a few weeks after pollination (Guinn, 1982). However, a degree of yield reduction in the middle of the canopy (developing bolls at the time of treatment) in response to WL_{late} could be the result of impaired N acquisition. As the WL plants retained relatively

more FP_1 bolls on FB_{1-10} (Fig 7 D), seed cotton yield in the entire canopy remained unaffected by WL_{late} (Table 4).

Another potential mechanism of waterlogging-induced yield loss in cotton is through inhibition of photosynthesis and photo-assimilate supply to developing bolls, which can instigate fruit abscission (Guinn, 1974). In addition, impaired carbon metabolism can induce yield losses in WL plants by arresting the development of new fruiting branches at the top of canopy (Guinn, 1985). Significantly reduced P_n and concomitant abscission of GB at post-recovery in the lower canopy highlighted the role of sugar supply for developing fruits (Kuai et al., 2015). In the present study, an immediate reduction in (post- WL) leaf N (%) concentration in the lower canopy leaves indicated that P_n and yield reduction could be a consequence of impaired N acquisition. In WL soils, inhibited root growth (Huck, 1970) and impaired N supply can suppress P_n by arresting leaf expansion (Milroy and Bange, 2013) or accelerating leaf senescence (McLeod, 2001). Delayed recovery of LAI , SLN and P_n only in lower canopy leaves (MS_{N1-8}), suggested potential re-mobilisation of N to upper leaves, which restored P_n and fruit growth at the top of canopy. Modification in leaf size and SLN are the major adaptive responses in many plant species, experiencing N deficiency (Vos and Van Der Putten, 1998). Plants can adapt to N deficiency either through one or a combination of strategies e.g. maintain leaf growth by reducing SLN (strategy I), restrict expansion of new leaves and maintain SLN (strategy II) or senesce older leaves and re-mobilise N from old to new leaves (strategy III) (Massignam et al., 2012). No changes in SLN in the upper canopy layers (MS_{N9-13} and MS_{N14+}) indicated that WL cotton plants adapted strategy II and exhibited an immediate P_n inhibition. On the other hand, changes in SLN in the lower part of canopy could be result of mobilisation of N from older to new leaves (strategy III). In contrast to Milroy et al. (2009) and McLeod (2001) who observed recovery of the nutrients in youngest fully expanded and entire canopy leaves, respectively, after termination of waterlogging, we observed no recovery in N concentrations in lower canopy leaves. This variable behaviour of leaves on different canopy positions suggested that re-distribution of N from lower canopy leaves may have masked effects of waterlogging in the upper leaves. Earlier, McLeod (2001) reported that WL cotton plants transport nutrients (N, P and K) from leaves to meet the nutrient requirement of developing bolls, and consequently alter their distribution within the plant. Studying the relationship between leaf area, leaf N and gas exchange in 22 plant species, Meziane and Shipley (2001) proposed that SLA is the major variable that directly influences both leaf N and P_n . Changes in leaf dry biomass per unit area influenced the activity of carboxylation enzyme (Oren et al., 1986), and thus P_n in cotton leaves. Accumulation of significantly higher TSS in leaf tissues of WL cotton in our study could be associated with the inhibited carbohydrate demand from hypoxic root tissues, which may have suppressed photosynthesis through feedback metabolite regulation (Martin et al., 2002). Significant reduction in SLN , SLA , P_n along with increased TSS in MS_{N1-8} leaves at post-recovery indicated the lower capacity of these tissues to recover from WL -induced stress. As developing bolls obtain 60–87% of their dry matter from the leaves present on the same branches (Constable and Rawson, 1980), this could explain the greater loss of fruits from lower canopy. Contrarily, WL plants could retain FP_1 fruits on FB_{11+} through sustained P_n and carbohydrate supply to developing fruits, yield compensation on these nodes was prevented by restricted production of new fruits. Inhibited P_n and leaf development of cotton under WL_{early} might have initiated an

early cut-out (Bange and Milroy, 2000), and increased photo-assimilates supply at recovery was used for growth of the established fruits instead of initiating production of new fruits.

Materials and Methods

Experimental site

Field experiments were conducted at the Australian Cotton Research Institute, Narrabri (30.12°S, 149.35°E), a major cotton-producing region in Australia. The soil type of the region is classified as endocalcareous, medium grey Vertosol (Isbell, 1996) with 60–65% clay fraction, 8.0–8.8 pH, and low in organic matter content.

Plant material

A commercial cotton cultivar Sicot 71BRF ([Bollgard II® Roundup Ready Flex®], CSIRO Australia) was used (Stiller, 2008). Seeds were sown on laser-levelled field using a commercial planter, and a high input management and insect control was practiced during the cropping season (Hearn and Fitt, 1992).

Waterlogging treatments

The field experiment had three treatment areas, waterlogged at early reproductive phase and waterlogged late reproductive phase and non-waterlogged control. The experiment was conducted in a randomised complete block design with four replicates of each treatment. The crop was allowed to develop until early reproductive growth phase (77 days after planting, *DAP*), and then exposed to waterlogging (WL_{early}) by extending irrigation in the central four rows of each WL plot for 120 h. Plants in a separate area were waterlogged at late reproductive growth phase (101 *DAP*) for 120 h. A similar treatment (120 h of continuous irrigation) had previously been used for inducing soil waterlogging in these soils (Bange et al., 2004). *NWL* treatment (for both waterlogging events) received the normal 8 h irrigation at the same time of the WL_{early} and WL_{late} treatments. To assess the effects of WL_{early} and WL_{late} , data from WL_{early} and WL_{late} and their respective controls were collected at different times and were treated as separate experiments.

Volumetric soil water

A calibrated neutron moisture meter (503DR Hydroprobe, CPN International, Martinez, CA) was used to measure volumetric soil water (mm) throughout the soil profile from 20 cm to a depth of 120 cm. Probe tubes were located in the central row of each treatment plot.

Biomass harvest

Cotton plants were harvested from 1 m² ground-surface area (taken from below the cotyledon) from each treatment block. The harvested plants were divided into three parts on the basis of node position on main stem, i.e. MS_{N1-8} , MS_{N9-13} and MS_{N14+} . Each part was further subdivided into leaves, stem and fruits. Non-pollinated young fruits were classified as squares and pollinated fruits as green bolls (*GB*). Number of squares and *GB* were counted and leaf area was measured from fresh leaves using the LICOR 6100 LA-3100. Plant parts (leaves, stem and fruits) were dried at 70°C for at least 72 h, and dry weights were used to calculate dry biomass,

specific leaf area (*SLA*) and leaf area index (*LAI*) for each canopy layer.

Leaf nitrogen

The dried leaf samples from each specific canopy layer were separately ground using a sample mill (Foss Tecator Cyclotec 1093) fitted with 1.0 mm screen. Part of the sample (100 mg) was used for analysing leaf N concentration using a CHN analyser (Model CHN 900, LECO, St. Joseph, MI). The leaf N concentrations were expressed on leaf N concentration (N %), and leaf area basis (specific leaf N, mg cm⁻²). Data on plant *DW* and leaf N contents were also presented separately on an entire canopy and for each canopy layer basis.

Leaf gas exchange and total soluble sugar

Before the start of waterlogging, leaves on different main stem nodes (MS_{N1-8} , MS_{N9-13} and MS_{N14+}) were tagged on different canopy layers. Four individual plants per replicate were selected and three individual leaves per layer (each on a different node) on each plant were tagged. Rate of photosynthesis was measured from the tagged leaves using a Li-6400 portable photosynthesis system (Li-Cor Ltd, Lincoln, NE, USA). Light intensity of the leaf chamber was fixed as 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature of the sensor head was set at optimal day temperature range for photosynthesis i.e. 30°C cf. (Burke et al., 1988). Measurements were taken during 1000 and 1230 h (Eastern Summer Time – Australia).

A subsample from the dried ground leaves (25 mg) of each canopy layer was used for measuring total soluble sugars by anthrone assay (Yemm and Willis, 1954). The samples were extracted by 80% ethanol and the supernatants were used for determining soluble sugars. A reaction mixture containing 100 μL of the supernatant + 3 mL anthrone reagent was placed in boiling water bath for 10 min and then immediately cooled on ice. The absorbance of the mixture was measured at 630 nm. Sugar content in leaf tissues was extrapolated from the standard glucose curve and presented in mg g⁻¹ leaf *DW*. Previous experiments indicated that waterlogging at late reproductive phase had no significant effect on cotton lint yield (Bange et al., 2004); therefore, data on leaf gas exchange and total soluble sugars were collected only under WL_{early} in this study. The data were collected one day before (pre- WL), one day (post- WL) and seven days after termination of waterlogging (post-recovery). As the *WL* and *NWL* plants showed no significant variation in growth and physiological components at pre- WL , only post- WL and post-recovery data of different canopy layers are presented.

Cotton yield

Plants from 1 m² of the central row of each plot were harvested at crop maturity and data on seed cotton yield and yield components were separately collected from different canopy layers. Cotton bolls from the lower five fruiting branches (FB_{1-5}), middle five fruiting branches (FB_{6-10}) and fruiting branches above 10 (FB_{11+}) were separately collected and weighed. In addition, bolls present on different fruiting positions (1st fruiting position and 2nd+3rd fruiting position) within each layer were also separately collected and weighed. The fruiting position closest to the main stem was defined as 1st fruiting position (FP_1), followed by 2nd and 3rd fruiting positions (FP_{2+3}). Fruit retention was calculated as the

percentage of final retained fruits to total fruiting sites on sympodial fruiting branches.

Data analysis

The effect of each treatment event (early and late waterlogging) was considered each as an independent experiment. One-way ANOVA was performed to identify the significant changes ($P < 0.05$) in growth and yield components. Data for different growth and yield components were separately analysed for each canopy layer and treatment time using the SAS JMP v. 9 (SAS Institute, Cary, NC, USA) statistical program. Respective means were compared using the Tukey's HSD test.

Conclusions

Changes in the response of crop across various canopy layers of cotton plants under waterlogging suggest the importance of considering stress damage on a whole canopy basis. Remobilisation of nutrient towards top of the canopy indicated the tendency of cotton plants to maximise utilisation of available radiation and at this point in the canopy could mask the overall impacts of waterlogging on the canopy. Waterlogging at the early reproductive phase significantly suppressed yield and yield components of cotton. No significant yield losses were observed when plants were waterlogged at the late reproductive phase, indicating higher sensitivity of newly developing fruits to abscission. These fruits were potentially abscised through waterlogging-induced ethylene production and inhibited photo-assimilate supply in the lower canopy. Delayed or complete inhibition of leaf growth and P_n in the lower canopy during recovery from waterlogging caused higher fruit losses on these nodes. In contrast, WL plants maintained FP₁ bolls at the upper canopy by restoring leaf N and photosynthesis after termination of waterlogging, although the WL plants could not support new fruit growth. This study elucidated that waterlogging-induced carbon and N dynamics across the cotton canopy layers and established the importance of protecting early fruits from waterlogging damage.

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