Waterlogging effects during cotton boll development on P and K dynamics in the soil and plant

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Abstract
A growth chamber study was undertaken with a Vertosol soil to investigate the effect of a 7-day waterlogging (WL) period on soil redox, soil solution P, K, Na and Mn concentrations and ^{33}P and ^{86}Rb applied immediately prior to flooding used to trace uptake into the plant. Soil redox potentials fell from 370 mV to 30 mV throughout the waterlogging period. Waterlogging increased the soil solution concentration of P, K, Na and Mn with the greatest increase in Mn (145 fold). Uptake of ^{33}P into the tops was reduced by 90% and ^{86}Rb by 63% during the waterlogging period compared to the –WL treatment. In the waterlogging treatment the majority of the P taken up was translocated to the developing fruit (61.5%) compared to 21.4% of the ^{86}Rb. The majority of ^{86}Rb (57.1%) accumulated in the stems of the plants. It is not possible to conclude what effect that the substantial increase in soil solution Mn concentration had on ion accumulation. Waterlogging during boll fill has been shown to have marked effects on the P and K dynamics, the two nutrients implicated in "premature senescence" and suggests both nutrients are involved in the process.

Keywords
Phosphorus, potassium, premature senescence, isotopes.

Introduction
Premature senescence of cotton was first reported in California in the early 1960's (Ashworth et al. 1982, cited in Oosterhuis 1995) and in Australia in the early 1980's in the Emerald Irrigation Area of Queensland and many debates over the causes have ensued for a number of years as researchers have endeavoured to understand the processes involved. Although many hypotheses on the cause of premature senescence have been put forward, the common belief is that potassium is fundamentally involved, particularly during the boll filling stage (Wright 1999). More recent studies (Rochester and Constable 2003; Milroy et al. 2009) have shown changes in leaf nutrient concentrations during waterlogging. Underground, premature senescence caused by K deficiency is characterised by negative root growth (Li et al. 2012) and lower root vigour (Yu et al. 1996). According to Oosterhuis (1995) the explanation for the deficiencies were unclear at that time and the current literature is still ambiguous.

Flood irrigation, as widely practiced in the Australian cotton industry on heavy textured Vertosol soils, can result in transient waterlogging which can potentially affect nutrient uptake and maybe one reason for premature senescence. The objective of this experiment was to investigate the effect of waterlogging on the dynamics of P, K and Na in cotton grown on a Vertosol soil.

Methods
Experimental Design and treatments
The treatments included were plus and minus waterlogging (WL) and 2 harvest times (immediately prior to and after waterlogging) with three replicates. Each replicate was placed in separate growth cabinets set at 30°C daytime temperature and 20°C night time temperature with 14 hrs of total day length. The cabinets were set to allow a gradual increase of light and temperature for one hour to full light and temperature, then 12 hours maximum light and temperature before one hour of gradual light and temperature reduction to night time. Treatments were randomised within each replicate. Pots were re-randomised within each growth cabinet daily after weighing and watering. The soil used was a cracking clay (Vertosol) collected from field 44 (0-30 cm) of Togo Station (lat. 30° 10' S., long. 149° 35' E.), a large commercial cotton farm in the Namoi Valley of NSW. Basal nutrients were applied prior to potting. Three seeds of cotton (*Gossypium hirsutum* L.) cv. Siokra L22 were planted per pot and pots were then watered to field capacity (FC). After 10 days the pots were thinned to 2 plants and then thinned to 1 plant 14 DAS. This allowed for selection of the most homogenous plants.

The pots containing a single plant in all treatments were watered to field capacity daily until waterlogging was imposed at 76 days after sowing (DAS). Immediately prior to waterlogging 3 pots were harvested to obtain starting yield and nutrient concentrations. Carrier free ^{33}P and ^{86}Rb (a surrogate tracer for K) was added at a depth of 10 cm to the remainder of the pots by injecting a 10 mL dose solution containing 4.985 MBq of ^{33}P and 8.020 MBq of ^{86}Rb at 5 equally spaced locations around the plant before the pots were watered to weight. This allowed the isotope to be delivered close to the active root system, and for the isotopes to diffuse into the soil solution. The pots in the +WL treatment were then waterlogged with distilled water for 7 days before being allowed to dry back to field capacity. Control pots continued to be watered to field capacity daily, or twice daily as required.

Dry matter production and soil solution measurement
At each harvest the plants were divided into leaves, petioles, stem (monopodial and sympodial branches) and fruit. These parts were then weighed, dried and reweighed. After drying, the lint was removed from the seed by a six-saw experimental gin. Boll walls and immature bolls (containing no fibre), fuzzy seed, and lint were then weighed separately. Because the soil contained radioactive material, thorough removal of roots was not possible. Instead the bulk of the roots were carefully removed from the soil by hand, washed, weighed, dried and re-weighed. A modified tensiometer fitted with a ceramic cup was inserted to a depth of 10 cm in each pot of each treatment. A suction pressure was used to extract sufficient soil solution into the tensiometer (usually 7-10 mL) for analysis and the solution removed using a syringe.

Nutrient and isotope analyses
The dried plant samples were ground, and a sub-sample taken and digested in a sealed chamber using perchloric acid and hydrogen peroxide (Anderson and Henderson 1986) and analysed for P and K, using an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP). A 3 mL aliquot of the plant digest or a 3 mL sample of the soil solution was mixed with a scintillation fluid and ^{86}Rb and ^{33}P counted in a liquid scintillation counter (LSC).

Redox Potential of Soil
Three platinum redox electrodes were placed in each treatment to a depth of 10 cm. Soil redox potential (Eh) was measured at the same time as soil solution was extracted. Measurements were taken weekly from 56 DAS until waterlogging commenced. During the waterlogging period, soil redox potential was measured daily.

Statistical Analyses
All data were statistically analysed using Genstat 5. Analysis of variance was performed separately for each harvest and means were separated at $P<0.05$ using least significant differences (Genstat 5 1993). Repeated measurement analysis was performed using the 'arepmeasures' procedure of Genstat 5 on redox potential and soil solution measurements (Genstat 5 1997). Residuals were examined for homogeneity and data were transformed when necessary.

Results
Soil Redox Potential
Soil redox potentials in the –WL and +WL treatments were similar before waterlogging was imposed. Eh values of between 300 and 370 mV were recorded on both treatments. A sharp decline in Eh was recorded after 24 hours of waterlogging with Eh dropping from in excess of 350 mV to just over 50 mV. Redox potential declined further over the next 24 hours to around 30 mV. Eh in the +WL treatment remained steady at around 30 mV throughout the waterlogging period. Once the waterlogging treatment had been removed the Eh rose sharply within the first 24 hours, but was significantly lower than that of the –WL treatment. By 89 DAS the Eh had returned to levels similar to that prior to the waterlogging treatment being imposed.

Soil Solution Nutrient Analyses
Waterlogging tended to increase the soil solution concentration of the major ions. Phosphorus concentrations were similar to the control in the +WL treatment (0.12 µg/mL) for the first three days of waterlogging, but by day four P concentrations rose significantly higher than in the –WL treatment (Figure 1). Phosphorus concentration remained higher in the +WL treatment for the next four days. As the waterlogging effect subsided the soil solution P concentration declined to levels that were similar to the –WL treatment.

Soil solution K concentrations rose rapidly from an initial value of 3.4 µg/mL after the waterlogging treatment was imposed (Figure 1). A significant increase in K concentrations was observed 2 days after waterlogging and by day 3 the K concentration had declined to a level similar to the –WL treatment. Soil solution Na concentrations showed a similar pattern to those for K (Figure 1). Na concentrations rose rapidly after the waterlogging treatment was imposed and a significant increase was observed 2 days after waterlogging had commenced. By day 3 Na concentrations had declined to a level similar to the –WL treatment.

Soil solution Mn concentrations were by far the most sensitive to waterlogged conditions. Manganese concentrations increased sharply from $3.71*10^{-3}$ µg/mL 2 days after waterlogging commenced, and continued to rise for another 3 days. Manganese concentrations peaked five days after flooding was imposed, when Mn concentrations had increased by over 145 fold (54 µg/mL). Concentrations declined as waterlogging progressed, and once the waterlogging treatment was removed the soil solution Mn concentration declined rapidly.

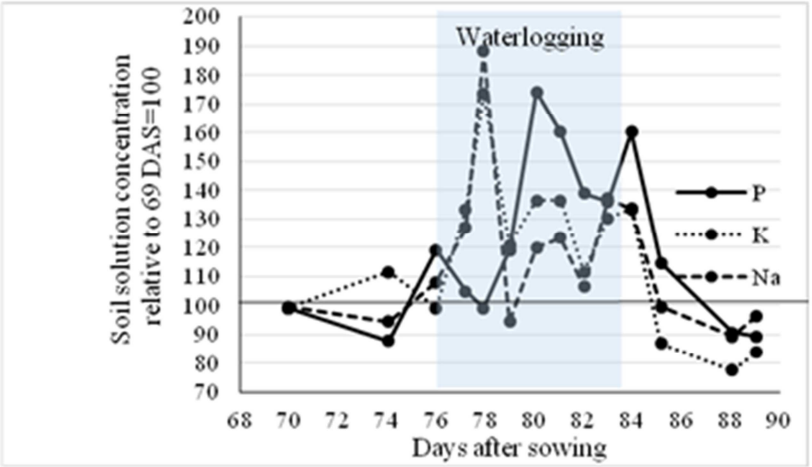

Figure 1. The effect of waterlogging on relative P, K, and Na concentration of the soil solution relative to the average soil solution concentration of the –WL treatment prior to waterlogging (100%).

^{33}P uptake and distribution

Uptake of ^{33}P was reduced by 90% during the waterlogging period compared to the –WL treatment (Table 1). Waterlogging significantly reduced the ^{33}P content of the fruit, leaves, stems and roots compared to the non-waterlogged treatment. In the waterlogging treatment the majority of the P was translocated to the developing fruit (61.5%), with similar quantities found in the stems and leaves (16.7%), with the remaining 5.6% contained within the roots.

Table 1. The effect of waterlogging on ^{33}P and ^{86}Rb content (KBq) of cotton tops and distribution in the plant.

Isotope	Soil water	Plant content (KBq) after 7 days	% recovery of isotope	Root	Stem	Leaf	Fruit
				% distribution			
^{33}P	-WL	671a	13.5	8.8	26.4	28.4	36.4
	+WL	67b	1.3	5.6	16.7	16.7	61.1
	% change	90		-37	-37	-41	+68
^{86}Rb	-WL	57a	0.7	5.3	31.6	31.6	31.6
	+WL	21b	0.3	14.3	57.1	7.1	21.4
	% change	63		+171	+81	-77	-32

[A] Average of 3 replicates. Means followed by the same letter within columns are not significantly different (P<0.05).

^{86}Rb uptake and distribution

During the 7 d waterlogging period ^{86}Rb accumulation was 63% lower in the +WL compared to the -WL treatment. The highest accumulation of the ^{86}Rb (57.1%) was in the stem and lowest in the leaf (7.1%)

(Table 1). By contrast ^{86}Rb accumulated equally in the stem, leaf and fruit (31.6%) in the non-waterlogged treatment. The % recovery of ^{86}Rb was less than P during the waterlogging period (Table 1) and ^{86}Rb uptake during the waterlogging period was low for both the -WL and the +WL treatments. It is not possible to conclude what effect that the substantial increase in soil solution Mn concentration had on ion accumulation.

Conclusion

The use of the radioisotopes applied immediately prior to waterlogging to trace the uptake and distribution of phosphorus (^{33}P) and potassium (^{86}Rb) supported the results of earlier total nutrient uptake studies and demonstrated that the uptake of these ions during the waterlogging period was significantly reduced as was their distribution within the plant. This supports the results of Hocking et al. (1987) who found that the most consistent and dramatic differences between severely waterlogged plants and those with well aerated root systems occurred with P and K. They found that the concentrations of these ions were always reduced in waterlogged plants, whereas changes in concentrations of other nutrients were less consistent.

The use of radioisotopes also demonstrated that at the stage of plant development when waterlogging was imposed the majority of the demand was to the developing fruit. When the supply from the roots was restricted these ions were relocated from other parts of the plant to meet the fruits' nutrient demands. That waterlogging reduces P uptake to a greater extent than K may be the reason that foliar symptoms during a "premature senescence" event appear as reddening of the leaves, which is indicative of P deficiency.

Acknowledgements

The financial support of the Cooperative Research Centre for Sustainable Cotton Production and the Potash and Phosphate Institute enabled this work to be undertaken.

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