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Root growth, turnover and soil microbial biomass in Bt and non-Bt cotton (*Gossypium hirsutum* L.)

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Abstract

The introduction of transgenic *Bacillus thuringiensis* (Bt) cotton (*Gossypium hirsutum* L.) has had a substantial impact on pest management in the cotton industry. While there has been substantial research done on the impact of Bt on the above-ground parts of the cotton plant, less is known about the effect of Bt genes on below ground growth of cotton. The aim of this research was to test the hypothesis that Bt [Sicot 80 BRF (Bollgard II Roundup Ready Flex)] and non-Bt [Sicot 80 RRF (Roundup Ready Flex)] transgenic cotton varieties differ in root growth and root turnover, carbon indices and microbial biomass. A field experiment was conducted in Narrabri, north-western NSW. The experimental layout was a randomised block design and used minirhizotron and core break and root washing methods to measure cotton root growth and turnover during the 2008/09 season. Root growth in the surface 0-0.1 m of the soil was measured using the core break and root washing methods, and that in the 0.1 to 1 m depth was measured with a minirhizotron and an I-CAP image capture system. These measurements were used to calculate root length per unit area, root carbon added to the soil through intra-seasonal root death, carbon in

roots remaining at the end of the season and root carbon potentially added to the soil. Microbial biomass was also measured using the ninhydrin reactive N method. Fruit retention and plant height was recorded over the growing period. Root length densities and length per unit area of non-Bt cotton were greater than Bt cotton. Bt cotton had greater fruit retention and greater plant heights than non-Bt over the growing period. There were no differences in root turnover between Bt and non-Bt cotton at 0-1 m soil depth, indicating that soil organic carbon stocks may not be affected by cotton variety. Cotton variety did not have an effect on soil microbial biomass. The results indicate that while there are differences in root morphology between Bt and non-Bt cotton, these do not change the carbon turnover dynamics in the soil.

Keywords: Bacillus thuringiensis, Bollgard II, genetic modification, minirhizotron, root length density, soil biota,

Introduction

In Australia, cotton (*Gossypium hirsutum* L.) crops account for less than 1% of the total agricultural area. Worldwide, Australia is the third largest cotton exporter (Knox *et al.* 2006). Advances in genetic engineering and technology have enabled the introduction and expression of genes in plants to produce agronomically useful traits such as insect and disease resistance. Several species of crop plants (including cotton, corn and potato) have been genetically modified to express genes of various subspecies of *Bacillus thuringiensis* (Bt) encoding insecticidal proteins (δ-endotoxins) (Head *et al.* 2002). Genetically modified Bollgard II® (GM) cotton contains the *Cry1Ab* and *Cry2Ac* genes, which are derived from *Bacillus thuringiensis* subsp. *kurstaki*. This allows the plant to produce its own insecticide. Bollgard II cotton has increased insect protection

compared with conventional non-Bollgard cultivars, and thus retains a higher number of bolls (Richards 2005, Paytas *et al.*, 2008). Despite the ongoing public debate surrounding the use and commercialisation of genetically modified crops, the total area of agricultural land used to grow such crops is increasing (Baumgarte and Tebbe 2005). The development of Bt cotton has led to improved pest management in cotton farming systems. The introduction of commercial GM cotton varieties has reduced environmental pollution from synthetic insecticides, increased worker safety, and improved grower profitability (Chen *et al.* 2005).

Previous studies comparing Bt and non-Bt cotton have focused on above ground measurements of cotton plants including boll retention and yield (Hofs *et al.* 2006). It is possible that the introduction of the Bt gene and expression of insecticidal proteins may have altered metabolic processes related to both vegetative and reproductive growth (Chen *et al.* 2005). There are differences between Bt and non-Bt cotton varieties including changed vegetative and reproductive growth characteristics, which affect the expression of lint yield potential, fibre quality and agronomic practices. Bt transgenic cotton varieties have shown increased plant height, higher relative growth rate and biomass in vegetative growth, smaller bolls, and reduced fibre micronaire and lint percentage. Chen *et al.* (2005) highlighted that Bt cotton cultivars have a more intense leaf nitrogen metabolism than their parents during reproductive development.

The introduction of Bt transgenic cotton (Bollgard II) allowed for higher levels of early season fruit retention as it is less prone than conventional varieties to suffer tip damage and fruit losses due to *Helicoverpa* larvae damage. Its high boll load may lead to increased water stress post-flowering. It is possible that potentially higher early fruit load on Bollgard II cotton makes it

more sensitive to water stress (Richards 2005). While there has been considerable research on above-ground characteristics, knowledge of below-ground effects are limited. Higher fruit retention may lead to a reduction in the resources available for root system development (Richards 2005). Early season fruit retention may potentially affect cotton plant development and modify plant structure, changing plant water requirements and uptake through effects on vegetative and root growth. The rationale for this is that crops with high fruit retention put more resources into fruit and less into vegetative growth. Hence, they may have a smaller root system that is less able to explore the soil for water; thus affecting the capacity to extract moisture from soil (Neilsen and Roberts 2006; Richards 2005). These observations were supported by Yeates *et al.* (2009) who reported that Bt cotton was more susceptible to water stress than non-Bt cotton at peak flower to cut-out. However, measurements of soil water extraction by Richards *et al.* (2008) showed that there were no differences in soil moisture extraction between Bollgard II and the conventional non-Bt variety under fully irrigated conditions.

Cotton roots play a critical role in water and nutrient uptake. As water and nutrient uptake are determined by the root system, they may be affected by plant genotype. Root systems are dynamic and develop according to biologically-determined patterns. However, the quantity of roots produced depends on the interaction between biological patterns (genetics) and other factors such as general plant vigour and local soil conditions (Klepper 1991). The root distribution, while governed by its genome, is also affected by the availability of nutrients, soil moisture and penetration resistance of the soil (Andren *et al.* 1991). However, there have been few studies comparing the roots of Bt and non-Bt cotton. One study by Zhang *et al.* (2005) found that there were some differences in root characteristics between different plant genotypes of cotton. An

insect resistant Bt hybrid cultivar ('CRI29') had a thicker root diameter, while an early maturing Bt cultivar ('CRI37') had the thinnest root diameter. Recent studies by Hulugalle *et al.* (2009) suggested that Bollgard II cultivars have reduced root mass and turnover compared with non-Bollgard conventional cultivars, but these experiments were done over different years and climatic factors may have influenced the results. Root carbon turnover is defined as the net change in mass of root carbon due to losses caused by root death and gains due to the initiation of new roots (Hulugalle *et al.* 2009). These findings imply that a smaller proportion of photosynthesised carbon may be translocated into the roots of the Bollgard II cotton. In contrast, Sarkar *et al.* (2008) showed that Bt cotton produced comparatively more root volume than non-Bt cotton in pot experiments.

As well as differences in root architecture, soil microbes may be affected by the Bt proteins. The below ground implications of the presence of transgenic proteins are largely unknown due to the complexity of soil ecosystems and limited information of their accumulation and persistence in the soil (Knox and Vadakattu 2007). Concerns have been expressed that Bt plants could release toxins into the soil, affecting the soil microbes and hence, soil microbial biomass. It is therefore important to understand the relationship between microbes and plants in the cotton farming system.

Soil microbial biomass responds quickly to changing soil conditions. Laboratory studies have shown that Bt proteins bind to clay minerals and humic acids (Saxena *et al.* 1999). In a study by Sarkar *et al.* (2008), soil under Bt cotton had significantly higher microbial biomass carbon than soil under non-Bt cotton. However, Baumgarte and Tebbe (2005) found that the presence of *CryIAb* and *CryIF* proteins in maize plants did not have a significant effect on soil microbial

communities. Head *et al.* (2002) also showed that Bt proteins were not detected in the soil after repeated cropping using transgenic Bt cotton cultivars.

There is conflicting information in the literature on the effect of Bt genes on the growth and turnover of roots, and microbial biomass in Bt cotton. The aim of this study was to test the hypothesis that there are differences in the root growth, turnover, carbon indices and soil microbial biomass between Bt and non-Bt transgenic cotton.

Materials and Methods

Site description

The experimental site (Field 18) was located at the Australian Cotton Research Institute (ACRI) (149°47'E, 30°13'S) 20 km west of Narrabri, NSW. The site has a semi-arid climate with summer dominant rainfall. The average annual rainfall is 642.1 mm, and the average annual temperature is 19.2°C (Bureau of Meteorology 2009). The soil at the site has been classified as Vertosol (Isbell 1996). The particle size distribution of the 0-1 m soil depth was 61% clay, 11% silt and 28% sand.

Experiment

The experiment was conducted during the 2008-2009 growing season. Two transgenic cotton cultivars, Bt [BRF, Sicot 80 (Bollgard II Roundup Ready Flex)] and non-Bt [RRF, Sicot 80 (Roundup Ready Flex)] were sown after conventional tillage (disc ploughing and incorporation of cotton stalks to 0.2 m, chisel ploughing to 0.3 m, followed by bed construction) in a cotton-wheat rotation. Both cultivars contain the Roundup Ready Flex genes and are tolerant to glyphosate herbicide. However, the Bollgard II cultivar expresses two Bt toxins, *Cry1Ac* and *Cry2Ab*. The rows were spaced at 1 m intervals with vehicular traffic being restricted to the furrows.

The experiment was sown on 13th October 2008 after good spring rains. 100 mm of water was applied via surface irrigation when rainfall was insufficient to meet evaporative demand. Cotton was picked using a 4-row picker during early June after defoliation in May. After picking, the cotton residue was slashed.

The experimental layout was a randomised complete block design and analysed as a split plot with the two factors; cultivar (Bt and non-Bt) as the main-plot and location within the field [divided into 3 sections: top (head ditch), middle and bottom (tail drain)] as the sub-plot (Figure 1). There were 2 replicates. The individual plots were 200 m long and 4 rows wide.

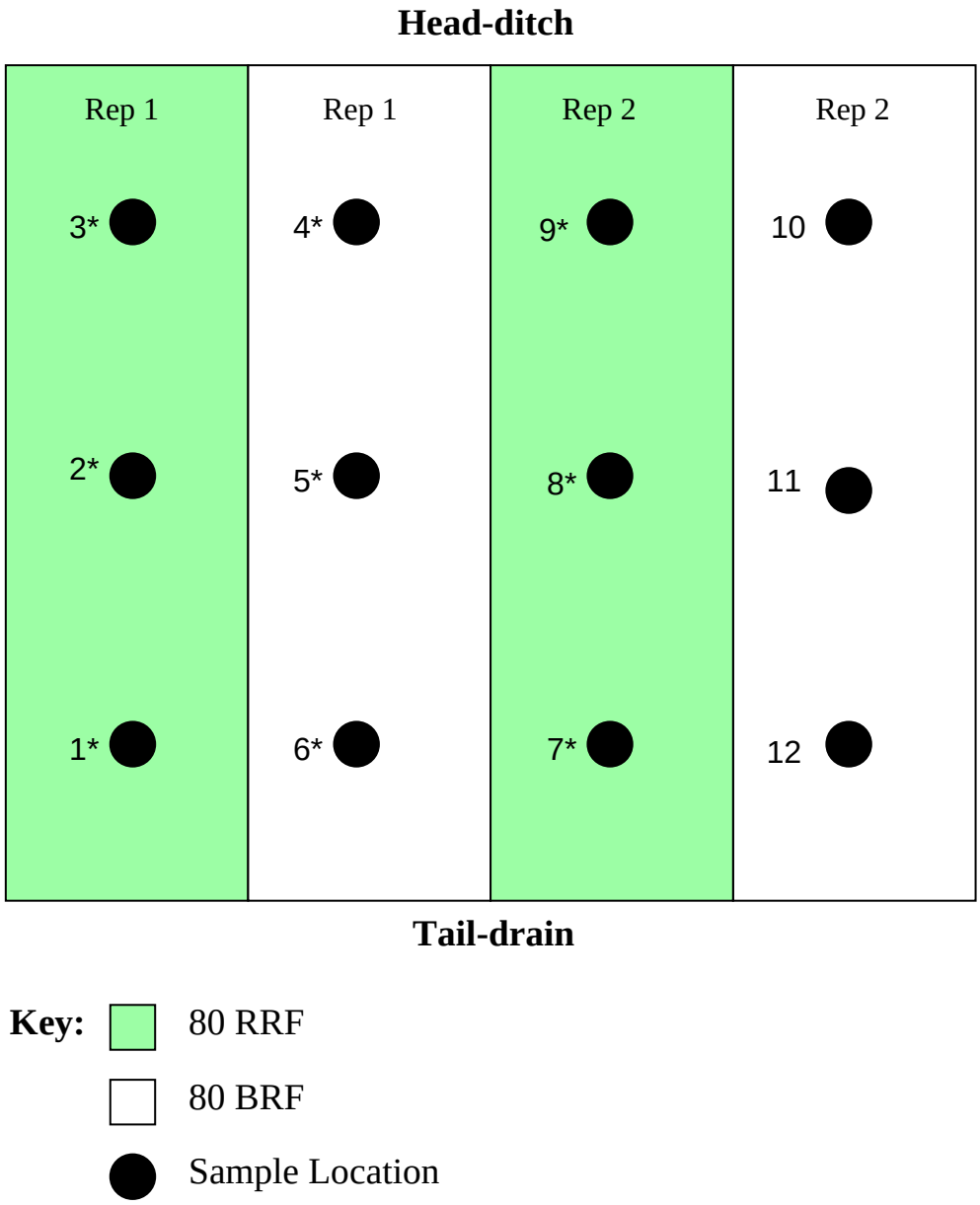

Figure 1: Experimental layout using a randomised complete block design

(* Tube number)

Data collection

Core break method

As the minirhizotron does not accurately capture images in the top 10 cm of the soil, the core break method (Drew and Saker 1980) was used to determine the number of roots at the surface. The 10 cm diameter core was manually inserted using a mallet to a depth of 10 cm into the soil. One core was taken close to each minirhizotron point. In the field, each core was broken in half and the numbers of roots on each surface counted (Figure 2). One core (a subsample) was taken to the laboratory in a sealed, labelled plastic bag for root washing and root biomass analysis. Core break samples were taken on 16 December 2008, 5 January, 19 January, 2 February and 18 March 2009.

Figure 2: Core break method. The core is split in half and visible roots are counted.

Root washing method

10% sodium hexametaphosphate and 1 M sodium hydroxide were dissolved in warm water to give a 2:1 solution. The soil samples from the core break were soaked in the solution for 4 hrs in order to disperse the soil. Once dispersed, the samples were washed through a 0.2 mm sieve to collect the

roots. The roots were then stained in a 0.1% Congo red solution, which stains live roots a red colour, in order to differentiate live from dead roots (Bohm 1979). The roots were placed on a 0.2 mm sieve and rinsed to remove the Congo red stain, with the stain set by washing roots in absolute alcohol for approximately 2 min. The absolute alcohol was rinsed off using water. Roots were sorted using tweezers after floating in a shallow white tray with water. Live roots were placed in a beaker containing deionised water. A sub-sample of roots was obtained, and the number of intersections were counted using Newman's line intersection method with a 1 cm x 1 cm grid (Smit *et al.* 2000), and placed into another beaker containing deionised water (Figure 3). All beakers were dried in the oven at 105^{o}C and root dry weights were obtained.

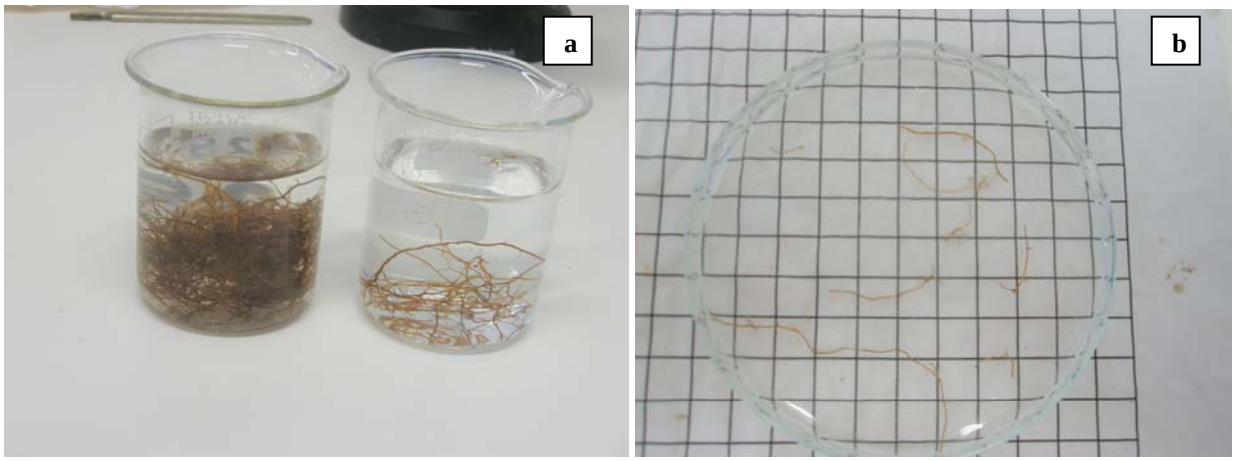

Figure 3(a) Sub-samples of live roots and **(b)** the numbers of intersections of live roots counted using the Newman's line intersection method and a 1 cm^2 grid.

Minirhizotron measurements

A minirhizotron was used to measure root growth and turnover non-destructively. Six clear plexiglass (50 mm diameter) minirhizotron access tubes were installed to a depth of 1 m, at 30^{o} from the vertical, for each cotton cultivar prior to plant growth (Figure 4). Root growth in the surface 0.1 to 1 m depth was measured at 0.1 m intervals with a "Bartz" BTC-2 video microscope

and I-CAP image capture system ("minirhizotron"). Images of root growth were recorded in two orientations, left and right side of each tube at each time, once every 2-5 weeks to coincide with vegetative growth, flowering, boll-initiation/filling and boll-filling/opening and exported to Rootracker software for analysis to estimate cotton root growth indices. The data for each orientation at each depth throughout the profile was added together to assess root growth over a 360^{o} plane of vision.

Figure 4(a) Clear plastic minirhizotron access tube, installed 30^{o} from the vertical **(b)** collecting data using the minirhizotron

Root growth and carbon index calculations

Relationships were derived between root number in the cores and root weight, and root weights in each core were estimated. Relative root length (mg/m) (root length/root weight) was also calculated. Carbon concentration in the oven-dried root material was measured by combustion with a LECO CHN-2000 analyser. The measurements included the length and number of live roots at each time of measurement, changes in root length, number and length of roots that died, new roots that developed between measurements and net root change in root numbers and length. These measurements were used to calculate indices including root length per unit area to a depth of 1 m

(L_A), root C turnover, root C added to soil as a result of root death throughout the season, C in roots remaining at end of season and total root C potentially available for addition to soil C stocks using the following equations (Hulugalle *et al.* 2009):

- Net change in root carbon (g/m^2) = net change in root length × relative root length (mg/m) × root carbon concentration

- Root carbon added to the soil (g/m^2) = length of roots which died × relative root length (mg/m) × root carbon concentration

- Root carbon at the end of the season = sum of net changes in root carbon added between times of measurement in all depths (1)

- Root carbon added to the soil during the season = sum of root carbon added to soil due to root death between times of measurement in all depths (2)

- Root carbon which could be potentially added to soil organic carbon (3) = root carbon at the end of the season (1) + root carbon added to the soil during the season

Plant mapping

Cotton plants within a 1 m row were tagged. Squares (flower buds) and bolls (fruits) were counted and average height of plants in the 1 m row was measured to compare the above ground and reproductive parts of the plants with the root data. These measurements were taken on 18th

December 2008 (66 DAS), 6th January (85 DAS), 15th January (94 DAS), 21st January (100 DAS), 29th January (108 DAS) and 5 February 2009 (115 DAS).

Microbial biomass

Microbial biomass was measured on soil samples taken on the 19th January 2009, using the ninhydrin reactive N method (Martens 1995).

Preparation of soil extracts

The gravimetric water content of approximately 5 g soil was measured by weighing and recording pre and post oven dried (105^{o}C) soil. A desiccator was used to cool the soil post drying.

Fumigation of soil samples

Two samples of approximately 1 g of soil per sample were weighed from the original soil collected, and weights were recorded with one sample being placed into a 10 mL sample tube and the other into a 20 ml glass jar. Three ml of 0.5M K_2SO_4 (potassium sulphate, pH 6.8) was added to one of the samples and extract by shaking using an orbital shaker at 180 rpm for 60 minutes. The soil extract was separated by centrifuging at 2000 g or 2500 rpm for 5 minutes. The supernatant was recovered and placed into a fresh 10 mL tube.

Column and chloroform preparation

The end of a 40 cm glass column was blocked with a glass-wool plug and filled with aluminium oxide to a height of 30 cm. The column was dried overnight at 100^{o}C. The column was set up in a fume hood, with a conical flask underneath. A funnel was sealed to the top of the column using

Parafilm. Approximately 60 ml of chloroform was passed through the column and collected in the conical flask. The filtered chloroform was transferred to a beaker with anti-bumping beads and placed at the bottom of the desiccator.

Preparation of desiccator and soil samples

Two wet paper towels were placed in the bottom of a glass desiccator to prevent the soil from drying out during fumigation. Several glass jars were labelled using a pencil to identify the samples. Approximately 1 g of soil was weighed into glass jars and recorded. The chloroform beaker containing anti-bumping beads was placed into the desiccator with the soil samples placed around it (Figure 5). A vacuum was applied to the desiccator using a water pump. The vacuum was maintained until the chloroform boiled. The samples were incubated under vacuum in the desiccator in the dark for 7 days at 22^{o}C. After incubation the desiccator was flushed in the fume hood by opening the valve slowly. The chloroform was removed and the air was exchanged in the desiccator by applying the vacuum twice before the samples were removed. The fumigated soil was transferred to a 10 mL tube and 3 mL K_2SO_4 was added to the extract as above (orbital shaking, centrifugation and transfer to fresh tube), and samples were stored at -20^{o}C prior to NDH-N analysis.

Figure 5: A desiccator was used to fumigate the soil samples.

Analysis of NDH-N

20 ml tubes were numbered (2 for each sample: unfumigated and fumigated plus 14 for blanks and standards). 1 ml of unfumigated soil extract was added to specific tubes. 0.5 ml of fumigated soil extract and 0.5 ml of K_2SO_4 was added to their corresponding sample tubes. A series of both leucine and ammonium sulphate standards were included to provide 0.35, 0.875, 1.75, 2.625 and 3.5 μg N ml^{-1} standard. Two distilled water blanks with about 4 ml of water were also included. To the 1 ml of test samples, 2.5 ml of citric acid buffer was added and mixed by vortex. 1.75 ml of ninhydrin reagent was added and mixed by vortex. The tubes were placed in the boiling water bath for 27 minutes. The rack of tubes was removed from boiling water bath, placed in ice cooled water, the lids were removed and 4 ml of ethanol diluent was added. Samples were read and recorded using a spectrophotometer at OD_{570} within 30 minutes.

Statistical analysis

Data was collected over the 2008-2009 cotton season and analysed using Genstat release 9.2. Correlation was tested between the rows and columns of the field design. The best model was the simple "Identity" model, which meant either analysis of variance (ANOVA) or residual maximum likelihood (REML) tests could be used. The data was analysed using ANOVA at the significance level 0.05. The assumptions of ANOVA tests (i.e. normally and equal variances) were checked. Data was Log_e transformed where variances were not constant.

Results

Core break and root washing

Root length density (Lv mm/cm^3) in the top 10 cm of the soil profile was higher (P<0.05) in non-Bt cotton (RRF) than Bt cotton (BRF) at 64 days after sowing (DAS), 98 DAS and 112 DAS (Figure 6). On the dates when sampling record a Lv that was not significant between treatments, there remained a trend for non-Bt cotton to have a higher Lv than Bt cotton.

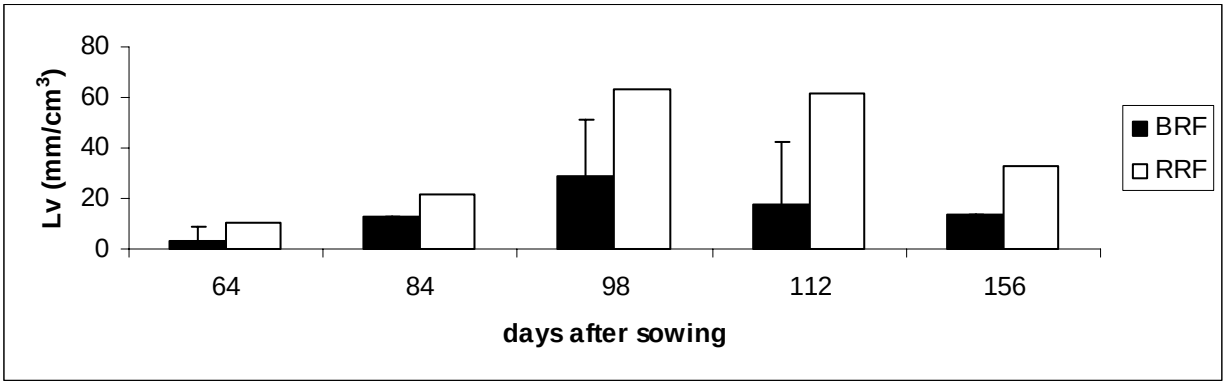

Figure 6: Root length density (Lv mm/cm^3) of core samples in the top 10 cm of soil profile for non-Bt cotton (RRF) and Bt-cotton (BRF) at 64 DAS, 98 DAS and 112 DAS. Vertical bars are LSDs at P=0.05.

Root growth

Non-Bt (RRF) cotton had greater (P<0.05) root area per unit length (L_A) than Bt (BRF) cotton to a depth of 1 m when averaged over the whole season. Values of L_A (cm/cm^2) were 61 cm/cm^2 for Bt and 102 cm/cm^2 for non-Bt (Figure 7a). L_A of non-Bt cotton was consistently (but not always significantly) higher than Bt cotton throughout the growing season (Figure 7b).

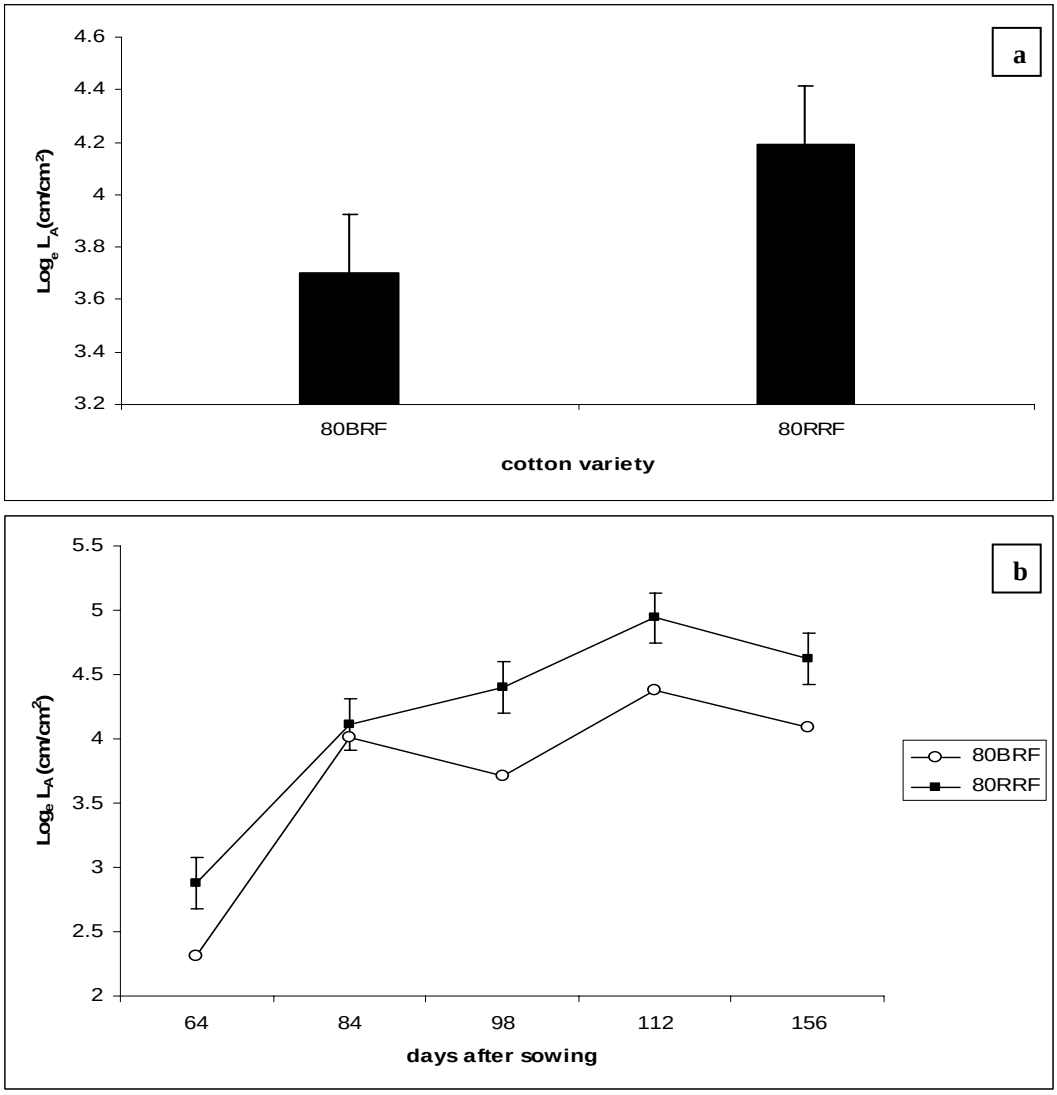

Figure 7 (a) Effect of cotton variety on root length per unit area (L_A cm/cm^2) to a depth of 1 m. Vertical bars are LSDs at P=0.05 and **(b)** Effect of cotton variety on root length per unit area, L_A, to a depth of 1 m over the growing period. Vertical bars are SEM's.

Root turnover

There were no differences in any of the carbon indices between Bt and non-Bt cotton ($P>0.05$; Table 1). There was no difference in net carbon turnover between Bt (BRF) and non-Bt (RRF) cotton Net carbon was 36 g/m^2 (0.3 t/ha) for Bt cotton and 149 g/m^2 (1.5 t/ha) for non-Bt cotton. There were also no differences ($P>0.05$) between root weight (104 g/m^2 for Bt and 149 g/m^2 for non-Bt cotton), the amount of carbon loss (41 g/m^2 for Bt and 45 g/m^2 for non-Bt cotton) and the amount of carbon potentially added to the soil (77 g/m^2 for Bt and 102 g/m^2 for non-Bt cotton). There was a general trend for non-Bt cotton (RRF) to be consistently higher than Bt cotton (BRF), however these differences were not statistically significant.

Table 1: Log_e transformed root turnover.

	Mean BRF (g/m^2)	**Mean RRF(g/m^2)**	**P-value**
Net carbon	3.42	3.93	0.172
Root weight	4.45	4.95	0.175
Carbon lost	3.48	3.51	0.945
Carbon potential	4.16	4.49	0.398

Plant mapping

Bt cotton had a higher ($P<0.05$) number of squares than non-Bt cotton at the first three sampling dates (Figure 8a). At 115 DAS, non-Bt cotton had a higher ($P<0.05$) number of squares than Bt cotton. Bt cotton (BRF) had a higher ($P<0.05$) number of bolls than non-Bt cotton (RRF) at each of the five sampling times (Figure 8b). Bt cotton had greater ($P<0.05$) plant height than non-Bt cotton during most of the growing season (Figure 8c). There was no difference ($P>0.05$) in plant height at the beginning (66 DAS) or the end (115 DAS) of the sampling period.

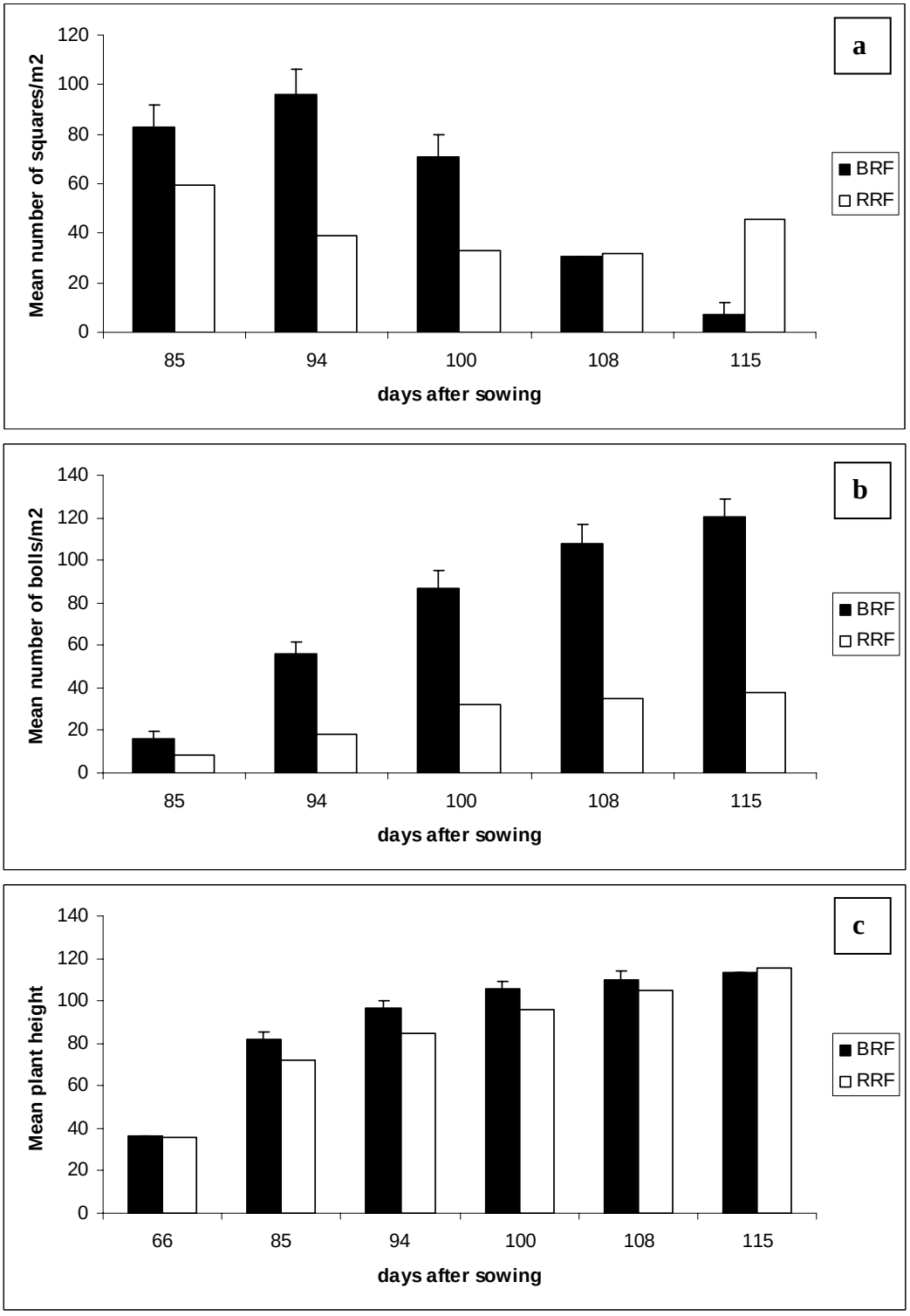

Figure 8: The effect of BRF (Bt) and RRF (non-Bt) on **(a)** mean number of squares/m^2 **(b)** mean number of bolls/m^2 and **(c)** mean plant height over the sampling period. Vertical bars are LSDs at P=0.05.

Microbial biomass

There were no differences in microbial biomass (P>0.05) of soil where Bt and non-Bt cotton were grown (Table 2).

Table 2: Mean microbial biomass content (μg C g^{-1} soil) of soil from Bt and non-Bt cotton

	80 BRF	**80 RRF**	***P*-value**
MB-C (μg C g^{-1} soil)	39.6	37.8	0.643

Discussion

Root growth is determined both by plant genetic components and by soil physical and chemical properties. Root growth is influenced by a number of factors such as soil impedance, soil water content, soil temperature, oxygen and nutrient supply (Muñoz-Romero *et al.* 2009). The root system is important for anchorage of the plant, as well as for the uptake of nutrients and water.

This study showed root length densities (RLD) and length per unit area (L_A) of non-Bt cotton to be greater than Bt cotton. The RLD in the surface 0-10 cm of soil of non-Bt cotton was also higher than the RLD of Bt cotton at three of the five sampling times. Non-Bt cotton consistently had higher levels of root growth than Bt cotton. These data showed that genotype plays an important role in the growth of cotton roots and are consistent with those reported by Hulugalle *et al.* (2009) who used the same sampling methods under similar growing conditions. In contrast, these findings differed from those reported by Sarkar *et al.* (2008), who showed that Bt cotton had a greater root volume than non-Bt cotton, however that study was done as a pot experiment and may not be representative of field conditions. Considering that the taproot of a plant may reach a depth of 25 cm by the time that the cotyledons unfold (Oosterhuis 1990) and that the dimensions of the pots

used in the experiment conducted by Sarkar *et al.* (2008) were 25 cm in diameter and 35 cm high, it is likely that the root systems were restricted particularly as Hulugalle *et al.* (2009) reported that cotton roots grow to a depth of at least 1 m. In addition, total root volume was measured using the combination of minirhizotron, core break and root washing methods, in this study as well as in Hulugalle *et al.* (2009), rather than a water displacement method as by Sarkar *et al.* (2008). The combination of destructive and *in-situ* sampling methods allowed repeated measures over the entire growing season of cotton plants. Zhang *et al.* (2006; 2005) demonstrated that plant genotype affected root growth of cotton and that genotype had an effect on root diameter. A mid-season Bt variety ('CRI29') had the thickest root diameter while an early maturing Bt variety ('CRI37') had the thinnest diameter. This experiment used a combination of root auger and image analysis methods for root sampling and measurements. However RLD was measured using a ruler, which may not be as accurate as the combination of core break, root washing and minirhizotron methods used in the current study. Minirhizotron tubes have been reported as an effective means of monitoring root development and root length density (Bland and Dugas 1988).

Fruit retention of Bt cotton was higher than non-Bt cotton with the Bt cotton variety having greater numbers of both squares and bolls and greater plant heights over the growing period. The increased boll load in this study was due to higher fruit retention possibly as a result of reduced pressure from *Helicoverpa* larvae in the Bt variety. In other studies, Bollgard II cotton varieties had higher early fruit retention, faster accumulation of boll weight and lower leaf area than equivalent conventional varieties (Paytas *et al.* 2008; Richards 2005). Higher boll load may require high carbon inputs, possibly at the expense of the root system. This study showed reduced root growth in Bt cotton that may be related to higher boll retention in Bt cotton, creating a greater

carbon demand in above-ground organs. It has been suggested that a reduced root system combined with higher early fruit loads may make Bollgard II cotton more sensitive to water stress than conventional varieties (Richards 2005), and this was also evident in other field experiments (Yeates *et al*. 2009). Root length density is important for soil water extraction equations (Bland and Dugas 1988). Water and nutrient uptake models rely on precise measurements of root length densities and length per unit area (Pierret *et al.* 2000). Perhaps a better understanding of the development and changes in root length densities and lengths per unit area for Australian cotton cultivars will result in improved cotton models.

The root systems of plants are subject to fast cycles of renewal and decay within the growing season. Root turnover represents a normal part of the lifecycle. Root mortality and turnover are important aspects of the development and function of root systems (Smit *et al.* 2000), however root dynamics are poorly understood. Although there were differences in root growth and boll retention between Bt and non-Bt cotton varieties in this study, there were no differences in carbon indices in the 1 m deep soil profile, indicating that soil organic carbon stock may not be affected by cotton variety. Similarly, Sarkar *et al.* (2008) reported no difference in root carbon between Bt and non-Bt cotton. Hulugalle *et al.* (2009) also speculated that it is unlikely that cotton roots will significantly contribute to soil carbon stocks in irrigated cotton farming systems. Knowledge of root turnover is still largely unknown as there have been few studies, particularly in cotton farming systems. However in this experiment root turnover did not contribute substantially to soil carbon stocks. Other studies reported that root turnover and decomposition is important in below-ground carbon and nitrogen cycling as it provides a carbon source for microbial populations (Huang *et al.* 2008; Pregitzer *et al.* 1997; Read *et al.* 2003).

Soil microbial biomass plays a key role in the turnover of soil organic matter (Chotte *et al.* 1998). This study showed that there were no differences in microbial biomass between soils where Bt and non-Bt cotton was grown. Knox *et al.* (2008a) also found that GM cotton did not affect arbuscular mycorrhizal colonisation of roots. In contrast, Sarkar *et al.* (2008) demonstrated that Bt cotton has a positive impact on most microbial and biochemical indicators including microbial biomass C, N and P and soil enzyme activities, despite using a similar chloroform-fumigation-extraction method to that carried out in the current study. However, these results were based on pot-experimental data and there is a need to confirm these results in the field. Sarkar *et al.* (2008) conducted their experiment in sandy loam soils, whereas this study as well as experiments conducted by Knox *et al.* (2008a; 2008b) were done on Vertosols, which may account for the observed differences. Knox *et al.* (2008b) showed that different cotton varieties can influence microbial populations and functions, although there were no differences in microbial biomass between Bt and non-Bt cotton. There is still conflicting evidence as to the effect of growing Bt crops on soil microbes. Research on interactions between plants and soil microbes is still limited due to the inability to grow the majority of soil micro-organisms in the laboratory.

Soil health and carbon sequestration are currently topical issues. The need to quantify both root biomass and turnover has become more important as a consequence of the potential inclusion of agriculture in carbon trading schemes and the use of soils for carbon sequestration. Plant root systems represent one of the principal means for the movement of carbon from both the atmosphere and the terrestrial biosphere into the soil (Smit *et al.* 2000). This experiment provides useful information about root growth and turnover and the impact of Bt and non-Bt cotton on root

growth and turnover.

Future research should focus on experiments using Bt and non-Bt cotton varieties under controlled environments (e.g. growth chambers) and on different soil types in the field as well as over several years and climatic conditions. This field study was undertaken on Vertosols, however, it is well known that soil characteristics play an important role in root growth and development throughout the profile (Karamanos *et al.* 2004). More work is needed to investigate root systems of cotton, as knowledge of these root systems is important to our understanding of plant-water and plant-nutrient uptake relationships. There is also the potential for these studies to be applied to other GM crops, to discover the effects that either the modified genes or the plants physiological responses to the benefits of the GM trait have on root growth and development and how these may contribute to the addition of carbon to the soil. More studies are required on the roots of cotton in order to improve our understanding of underground organs. This research will improve our management practices and improve soil health.

Conclusions

Root length densities and length per unit area of non-Bt cotton were greater than Bt cotton. This may be related to higher boll retention in Bt cotton, creating a higher carbon demand in above-ground organs at the expense of root growth. There were no differences in root turnover between Bt and non-Bt cotton in the 1 m deep soil profile, indicating that soil organic carbon stock was not affected by cotton variety. Cotton variety (Bt and non-Bt) did not have an effect on soil microbial biomass.

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References

Andren O, Kalman R, Katterer R (1991) A non-destructive technique for studies of root distribution in relation to soil moisture. *Agriculture, Ecosystems and Environment* **24**, 269-278.

Baumgarte S, Tebbe CC (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. *Molecular Ecology* **14**, 2539-2551.

Bland WL, Dugas WA (1988) Root length density from minirhizotron observations. *Agronomy Journal* **80**, 271-275.

Bohm W (1979) 'Methods of studying Root Systems.' (Springer-Verlag: Germany).

Bureau of Meteorology (2009) 'Climate statistics for Australian locations.' (Australian Government (http://www.bom.gov.au/climate/averages/tables/cw_054120.shtml accessed 21 October 2009).

Chen DH, Ye GY, Yang CQ, Chen Y, Wu YK (2005) Effect of introducing Bacillus thuringiensis gene on nitrogen metabolism in cotton. *Field Crops Research* **92**, 1-9.

Chotte JL, Ladd JN, Amato M (1998) Measurement of biomass C, N and 14C of a soil at different water contents using a fumigation-extraction assay. *Soil Biology & Biochemistry* **30**, 1221-1224.

Drew MC, Saker LR (1980) Assessment of a rapid method, using soil cores, for estimating the amount and distribution of crop roots in the field. *Plant and Soil* **55**, 297-305.

Head G, Surber JB, Watson JA, Martin JW, Duan JJ (2002) No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollgard) use. *Environmental Entomology* **31**, 30-36.

Hofs JL, Hau B, Marais D (2006) Boll distribution patterns in Bt and non-Bt cotton cultivars I. Study on commercial irrigated farming systems in South Africa. *Field Crops Research* **98**, 203-209.

Huang G, Zhao X, Su Y, Zhao H, Zhang T (2008) Vertical distribution, biomass, production and turnover of fine roots along a topographical gradient in a sandy shrubland. *Plant and Soil* **308**, 201-212.

Hulugalle NR, Weaver TB, Finlay LA, Luelf NW, Tan DKY (2009) Potential contribution by cotton roots to soil carbon stocks in irrigated Vertosols. *Australian Journal of Soil Research* **47**, 243-252.

Isbell RF (1996) 'The Australian Soil Classification.' (CSIRO Publishing: Collingwood, Vic).

Karamanos AJ, Bilalis D, Sidiras N (2004) Effects of reduced tillage and fertilization practices on soil characteristics, plant water status, growth and yield of upland cotton. *Journal of Agronomy and Crop Science* **190**, 262-276.

Klepper B (1991) Crop root- system response to irrigation. *Irrigation Science* **12**, 105-108.

Knox OGG, Constable GA, Pyke B, Gupta VVSR (2006) Environmental impact of conventional and Bt insecticidal cotton expressing one and two Cry genes in Australia. *Australian Journal of Agricultural Research* **57**, 501-509.

Knox OGG, Nehl DB, Mor T, Roberts GN, Gupta VVSR (2008a) Genetically modified cotton has no effect on arbuscular mycorrhizal colonisation of roots. *Field Crops Research* **109**, 57-60.

Knox OGG, Vadakattu GVSR (2007) Evaluation of border cell number and Cry protein expression from root tips of *Gossypium hirsutum*. In 'Proceedings of the 6th Pacific Rim

Conference on the biotechnology of *Bacillus thuringiensis* and its environmental impact, Victoria, BC, Canada, 30 October - 3 November, 2005.' pp. 139-140.

Knox OGG, Vadakattu GVSR, Gordon K, Lardner R, Hicks M (2008b) Varietal differences in cotton- belowground. In '(14th Australian Cotton Conference 'Broadbeach, Gold Coast, 12-14 August).

Martens R (1995) Current methods for measuring microbial biomass C in soil: potentials and limitations. *Biological Fertility and Soils* **19**, 87-99.

Muñoz-Romero V, Benítez-Vega J, López-Bellido RJ, Fontán JM, López-Bellido L (2009) Effect of tillage system on the root growth of spring wheat *Plant and Soil*.

Neilsen J, Roberts G (2006) Water extraction of high retention cotton crops. (Australian Society of Agronomy http://www.regional.org.au/au/asa/2006/concurrent/water/4615_neilsenj.htm accessed 13/1/09).

Oosterhuis DM (1990) 'Nitrogen Nutrition of Cotton: Practical Issues.' (American Society of Agronomy, Inc: Madison, Wisconsin).

Paytas M, Yeates S, Fukai S, Huang L (2008) Effect of early moisture deficit on growth, development and yield in high retention Bt cotton. In '14th Australian Society of Agronomy Conference'Adelaide).

Pierret A, Moran CJ, McLachlan CB, Kirby JM (2000) Measurement of root length density in intact samples usisng X-radiography and image analysis. *Image Analysis and Stereology* **19**, 145-149.

Pregitzer KS, Kubiske ME, Chui K, Hendrick RL (1997) Relationships among root branch order, carbon, and nitrogen in four temperate species. *Oecologia* **111**, 302-308.

Read DB, Bengough AG, Gregory PJ, Crawford JW, Robinson D, Scrimgeour CM, Young IM, Zhang K, Zhang X (2003) Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. *New Phytologist* **157**, 315-326.

Richards D (2005) What's happening in water this season? *The Australian Cottongrower* **26**, 48-53.

Richards D, Yeates S, Roberts J, Gregory R (2008) Does Bollgard II cotton use more water? In '14th Australian Cotton Conference '. Broadbeach, Gold Coast pp. 621-626.

Sarkar B, Patra A, Purakayastha TJ, Megharaj M (2008) Assessment of biological and biochemical indicators in soil under transgenic Bt and non-Bt cotton crop in a sub-tropical environment. *Environmental Monitoring and Assessment* **156**, 595-604.

Saxena D, Flores S, Stotzky G (1999) Insecticidal toxin in root exudates from Bt corn. *Nature (London)* **402**, 480.

Smit AL, Bengough AG, Engels C, Van Noedwijk M, Pellerin S, Van De Geijn SC (2000) 'Root Methods: A handbook.' (Springer: Germany).

Yeates S, Roberts J, Neilsen J, Richards D (2009) Toward better water management of Bollgard II cotton. *The Australian Cottongrower* **30**, 22-27.

Zhang L, Li B, Yan G, Werf Wvd, Spiertz JHJ, Zhang S (2006) Genotype and planting density effects on rooting traits and yield in cotton (Gossypium hirsutum L.). *Journal of Integrative Plant Biology* **48**, 1287-1293.

Zhang LZ, Cao WX, Zhang SP, Zhou ZG (2005) Characterizing root growth and spatial distribution in cotton. *Acta Phytoecologica Sinica* **29**, 266-273.